

# The effect of *Eleutherine palmifolia* root extract on the hematology of *Oreochromis niloticus* infected with *Pseudomonas fluorescens*

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**Abstract.** *Eleutherine palmifolia* or well-known as Dayakness onion is one of the medicinal plants that can be used as alternative treatment on Nile tilapia *Oreochromis niloticus* infected with bacterium *Pseudomonas fluorescens*. It contains secondary metabolite compounds as antibacterial. This study aims to know the effect of *E. palmifolia* root extract application on the hematology of *O. niloticus* infected with *P. fluorescens* based on erythrocyte, leucocyte, lymphocyte, monocyte, neutrophils, hemoglobin, and hematocrit. The research method was experimental with 3 treatment concentrations, 30 mg L<sup>-1</sup> (A), 50 mg L<sup>-1</sup> (B), and 70 mg L<sup>-1</sup> (C) with 3 replications and negative control. Results showed that *E. palmifolia* root extract had significant effect on the hematology of *O. niloticus* indicated with the amount of erythrocytes, leucocytes, lymphocytes, monocytes, neutrophils, hemoglobin, and hematocrit leading to the normal range. The best concentration was recorded in treatment C as maximum dose that was capable of curing *O. niloticus* infected with *P. fluorescens*. Therefore, *E. palmifolia* root extract could be used as medicine against bacterial infection.

**Key Words:** tilapia, alternative medicine, hematology, antibacterial.

**Introduction.** Nile tilapia *Oreochromis niloticus* is well-known to be able to tolerate various environmental conditions and can survive at wide range of water temperature (Ndiwa et al 2014). Besides, *O. niloticus* is one of the highly economic valuable fishes, because this species is easily cultivated and has relatively fast growth. It makes the cultivation of *O. niloticus* be high in several countries, including Indonesia (Gu et al 2014; Huicab-Pech et al 2017). In Nile tilapia culture, the constraint is disease from bacterial infection, one of bacteria being *Pseudomonas fluorescens*. It makes red skin disease and causes fish mortality (Younes et al 2015; El-Kader & Mousa-Balabel 2017). *P. fluorescens* infects 10.4% of the cultured *O. niloticus* and kill the fish (Wamala et al 2018).

The bacterium-infected fish will also have changes in number of the blood cells. It can result from the function of the blood cells to fight against bacterial infections (Podeti & Benarjee 2017). Therefore, fish blood can be taken as indicator for disease examination based on physiological and pathological changes (Southamani et al 2015). For this, the hematological parameter is very important in fish health status determination. It covers number of erythrocytes, leucocytes, number of leucocyte differential cells (lymphocytes, neutrophils, monocytes), hemoglobin (Hb), and hematocrit (Ht) (Vazquez & Guerrero 2007; Fagbenro et al 2013; Duman & Sahan 2017).

This bacterial infection problem is usually overcome with chemical treatment, such as antibiotic application. However, this practice can leave residues in the organism, or the bacteria can become resistant (Bedasso 2017). However, there is other prevention alternative to use plant extract that may not yield residue and side effect on the organisms (Hardi et al 2017). One of the plants that can be used for treatment is the root of *Eleutherine palmifolia* or well-known as Dayakness onion. It is commonly found living in Kalimantan and has been utilized as traditional medicine for local people (Agustin et al 2016; Atikah et al 2017). In a previous study, Fransira et al (2019a) found that *E.*

*palmifolia* root extract contains the dominance of phenolic, flavonoid, tannin, triterpenoid and saponin compounds that are antibacterial against bacteria *P. fluorescens*. This property is indicated with inhibition zone formed on the disk test.

The secondary metabolite content in *E. palmifolia* root, such as flavonoid and phenol, is known to work as antibacterial through protein denaturation that will stimulate the damage of the bacterial nucleus and cause cell lysis (Trisna et al 2017; Harlita et al 2018). The administration of *E. palmifolia* root extract will increase several parameters of fish hematology due to the ability of the compound to remove the microbial infection that often damages the blood cells so that the fish could produce the types of proportional blood cells capable of fighting against the bacterial infection (Bekeh et al 2016; Maftuch 2017). However, no one has reported the root extract of *E. palmifolia* as treatment of *P. fluorescens*-infected *O. niloticus*. Thus, this study aims to know the effect of *E. palmifolia* root extract administration on the hematology of *P. fluorescens*-infected *O. niloticus* examined from erythrocyte, leucocyte, lymphocyte, monocyte, neutrophils, hemoglobin, and hematocrit.

## Material and Method

**Time and locality.** This study was conducted in February to April 2019 in the Laboratory of Fish Culture, Department of Fish Disease and Health, Brawijaya University Malang, east Java Timur, Indonesia.

***E. palmifolia* bulbs root extract preparation.** Samples of *E. palmifolia* root were obtained from the Technical Implementation Unit of Materia Medica Batu, East Java. The roots were collected, washed, and blended to have fine powder (Ahmad et al 2018). As much as 100 g of *E. palmifolia* was macerated in 600 mL of ethanol solution (PA) for 3 x 24 h at room temperature. The extract was then filtered through Whatman No. 42 filter paper and evaporated in rotary evaporator vacuum at 50°C and 80 rpm (Maftuch et al 2018).

***P. fluorescens* bacterium preparation.** A pure culture of *P. fluorescens* rejuvenated on the Pseudomonas Selective Agar media was aseptically taken one dose and put in liquid medium of Trypticase Soy Broth (TSB) for culture. The bacteria-containing solution was then incubated for 24 h at 37°C (Vu et al 2016; Fransira et al 2019a). Afterwards, the bacteria at the density of  $10^7$  CFU mL<sup>-1</sup> were ready to use to infect the fish (Maftuch 2017).

***O. niloticus* fish preparation.** The fish *O. niloticus* were obtained from the market of Kota Batu, east Java, at the size of 7-10 cm long and weight of  $\pm 15$  g, in which each rearing tank was put 10 individuals. The fish were acclimated for 3 days (Hardi et al 2017).

**Toxicity test of *E. palmifolia* root extract on *O. niloticus*.** LC<sub>50</sub> test was done to know the dose of *E. palmifolia* root extract that can cause 50% mortality of the initial fish population. This test was carried out under 24 h-time range (Yuniari et al 2016). In the preliminary study, Fransira et al (2019b) found that Minimum Inhibitory Concentration (MIC) of *E. palmifolia* root extract was 10 mg L<sup>-1</sup>. Thus, the toxicity test employed the following doses: 10 mg L<sup>-1</sup>, 30 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, 70 mg L<sup>-1</sup>, 90 mg L<sup>-1</sup>, and 110 mg L<sup>-1</sup>. These different extract concentrations were set in the aquaria. Six fish were also put in each aquarium. For 24 hours, behavior was observed and the number of fish killed and their time recorded. The results were obtained from concentrations that caused the death of 50% of the initial fish population with the fastest time.

***O. niloticus* fish infection and treatment.** *O. niloticus* was fasted one day before treatment. The fish were then infected with *P. fluorescens* at the density of  $10^7$  CFU mL<sup>-1</sup> through immersion, in which this process was conducted up to the fish collapsed at first and appeared infection symptoms for 24 h (Sari et al 2013). The fish were then transferred to *E. palmifolia* root extract-containing aquaria as the concentrations obtained

in toxicity test. The aquaria of negative control contained bacteria *P. fluorescens* only without extract, each of which was filled with 10 L of water and 10 individuals of fish. After the fish had shown irregular movements, they were taken and moved to the rearing aquaria for several hematological observations.

**Blood sampling of *O. niloticus*.** Fish blood was taken from caudal vein using a 0.5 mL syringe and put into an Eppendorf tube containing anticoagulant EDTA (Ethylene Diamine Tetra Acetic Acid) (Sahan et al 2017). The hematological observations were made 4 times at the time interval of 12 h, 24 h, 36 h, and 48 h after bacterial infection.

**Erythrocyte observation.** The erythrocytes of *O. niloticus* were observed following the method of Kefas et al (2015). The fish blood was then sucked from the Eppendorf tube using erythrocyte pipette, added with Hayem solution up to scale-101 and homogenized. As much as 2 drops of the first blood were removed and the rest was dropped on the Neubauer-typed haemocytometer, then covered with cover glass. Number of erythrocytes was counted under 1000x enlargement microscope.

**Leucocyte observation.** Leucocyte observation was conducted using the method suggested by Payung & Manoppo (2015), in which as much as 50 µL of blood was taken from the Eppendorf tube and mixed with 450 µL of Türk solution, then homogenized through slow shaking. The mixture was then incubated for 5 min at room temperature. The leucocytes were counted using haemocytometer under 1000x enlargement microscope.

**Leucocyte differential observation.** Observations on the differential leucocyte of *O. niloticus* employed the method of Arnold (2005), in which the blood was dropped on the tip of object glass and dragged on the other object glass that the blood could disperse and form a thin blood prepare. Furthermore, the prepare was wind-dried, and then fixed in methanol solution for 5 min. The prepare was then soaked in diluted Giemsa solution (1:20) for 15 min. Afterwards, the prepare was rinsed in distilled water and wind-dried. Prepare is ready to be observed under 1000x enlargement microscope to count the number of lymphocytes, monocytes, and neutrophils.

**Hemoglobin observation.** The hemoglobin observation followed the method of Fagbenro et al (2013), and hemoglobin level was expressed as G% unit. This calculation is based on Sahli method. The blood was inserted into Sahli pipette up to the scale of 0.02 mL by sucking then the pipette was put into the Sahli tube containing 0.1 N HCl. Afterwards, the tube was stirred and left for 5 min and added with distilled water until the color became similar to the standard solution in Sahli tube.

**Hematocrit observation.** Hematocrits of *O. niloticus* were observed following Vazquez & Guerrero (2007), in which the value was determined by standard microhematocrit method and expressed in percent. The blood sample was inserted into a standard capillary tube up to ¾ tube part, and the tube tip was covered with vax (vitrex). Moreover, the microhematocrit tube was centrifuged at 12,000 rpm for 5 min and it was measured on the microcapillary reading.

**Statistical analysis.** Data were analyzed using ANOVA. This analysis was employed to examine the effect of *E. palmifolia* root extract application on the hematology of *O. niloticus* infected with *P. fluorescens*. Then, the data were processed and analyzed using Microsoft Excel 2010 and SPSS (Statistical Package for the Social Sciences) Statistic-25 application.

**Results.** The hematological parameters, such as erythrocyte, leucocyte, lymphocyte, monocyte, neutrophil, hemoglobin, and hematocrit, of *O. niloticus* infected with *P. fluorescens*, after treatment with *E. palmifolia* root extract at the dose obtained in the toxicity test, showed different hematological range from the negative control treatment.

**Toxicity test of *E. palmifolia* root extract on *O. niloticus*.** In the toxicity test, the concentration range was 20 mg L<sup>-1</sup> based on Wicaksono et al (2018), i.e. 10 mg L<sup>-1</sup>, 30 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup>, 70 mg L<sup>-1</sup>, 90 mg L<sup>-1</sup>, 110 mg L<sup>-1</sup>, respectively. The toxicity test of *E. palmifolia* on *O. niloticus* found different outcomes (Table 1).

Table 1

Toxicity test of *E. palmifolia* root extract on *O. niloticus*

Concentration (mg L <sup>-1</sup> )	Remarks
10	No fish mortality occurs during observations and the fish swim normally.
30	Two fish died in 18 h-observation, in which the fish swim near the surface and start being passive in feeding.
50	One fish died in 12 h-observation, in which the fish gather around the surface, followed with mortality of all fish in 18 h-observation and passive feeding.
70	Three fish died in 11 h-observation, in which the fish gather around the surface, followed with mortality of all fish in 14 h-observation and passive feeding.
90	Three fish died in 10 h-observation, in which the fish swim upside down, and followed with mortality of all fish in 11 h-observation.
110	All fish died in 7 h, in which the fish gather on the surface and swim upside down at the first 3 h.

Table 1 demonstrates that 50% fish mortality (LC<sub>50</sub>) occurs at the dose of 90 mg L<sup>-1</sup> of *E. Palmifolia* root extract. Therefore, this study used 3 concentrations, 30 mg L<sup>-1</sup> (A), 50 mg L<sup>-1</sup> (B) and 70 mg L<sup>-1</sup> (C).

**Erythrocyte analysis of *O. niloticus*.** Erythrocyte analysis on *P. fluorescens*-infected *O. niloticus* then treated with *E. palmifolia* root extract yielded different mean number of erythrocytes (Figure 1). The highest number of erythrocytes was recorded in treatment C (70 mg L<sup>-1</sup>), in which total erythrocytes at 12 h, 24 h, 36 h, and 48 h increased from 1.71±0.02 x 10<sup>6</sup> cells mm<sup>-3</sup> to 1.87±0.05 x 10<sup>6</sup> cells mm<sup>-3</sup> to 2.04±0.03 x 10<sup>6</sup> cells mm<sup>-3</sup> to 2.17±0.04 x 10<sup>6</sup> cells mm<sup>-3</sup> respectively. The erythrocytes in K- treatment (fish without extract immersion) had drastic decline. ANOVA revealed that the administration of *E. palmifolia* root extract highly significantly influenced the number of erythrocytes in *P. fluorescens*-infected *O. niloticus* (p < 0.01).

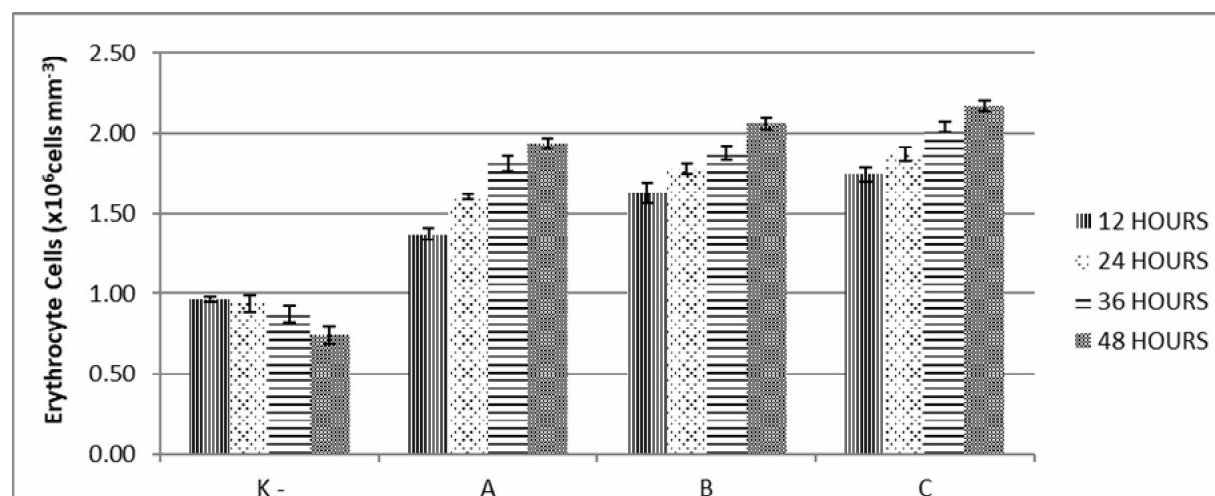


Figure 1. Mean number of erythrocytes of *O. niloticus*. K- infected fish, A - 30 mg L<sup>-1</sup>, B - 50 mg L<sup>-1</sup>, and C - 70 mg L<sup>-1</sup>.

**Leucocyte analysis of *O. niloticus*.** After *E. palmifolia* root extract application, the leucocytes of *P. Fluorescens*-infected *O. niloticus* varied (Figure 2). The lowest number of leucocytes occurred in treatment C (70 mg L<sup>-1</sup>), in which total leucocytes in 12 h, 24 h, 36 h, and 48 h declined from 0.83±0.08 x 10<sup>4</sup> cells mm<sup>-3</sup> to 11.75±0.10 x 10<sup>4</sup> cells mm<sup>-3</sup> to 8.68±0.33 x 10<sup>4</sup> cells mm<sup>-3</sup> to 6.93±0.10 x 10<sup>4</sup> cells mm<sup>-3</sup> respectively, while the leucocytes rose in treatment K- (infected fish without extract immersion). ANOVA revealed that *E. palmifolia* root extract highly affected the amount of leucocytes in *P. fluorescens*-infected *O. niloticus*.

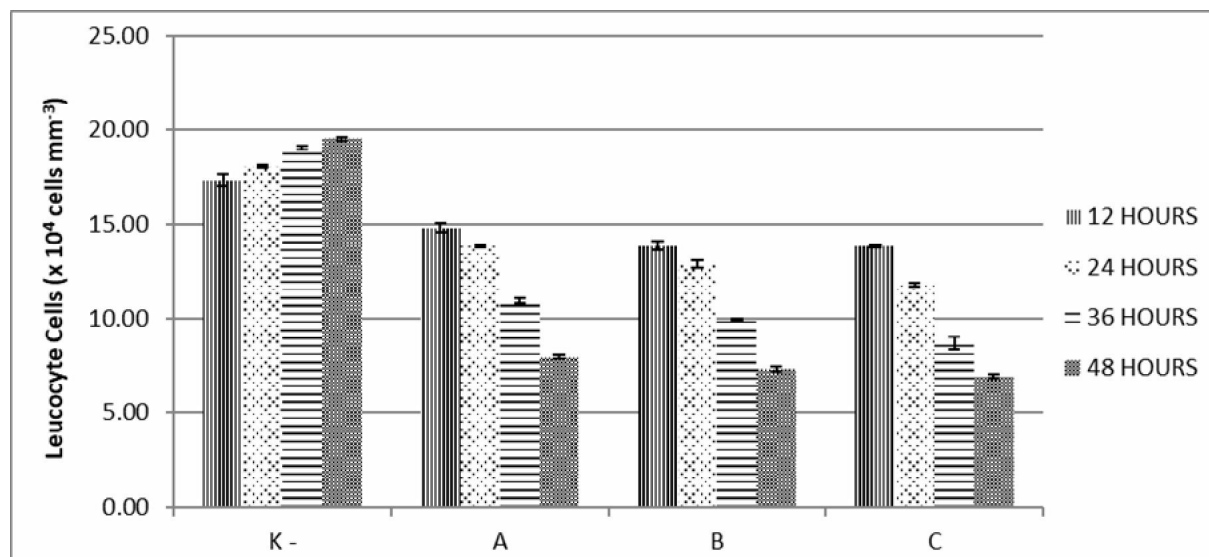


Figure 2. Mean number of leucocytes in *O. niloticus* (K- infected fish, at the concentration of 30 mg L<sup>-1</sup>(A), 50 mg L<sup>-1</sup> (B), and 70 mg L<sup>-1</sup> (C).

**Lymphocyte analysis of *O. niloticus*.** The lymphocytes of *O. niloticus* infected with *P. fluorescens*, after *E. palmifolia* root extract application, yielded different number of lymphocytes (Figure 3). The highest was recorded in treatment C at the concentration of 70 mg L<sup>-1</sup>, in which total lymphocytes at 12 h, 24 h, 36 h, and 48 h rose from 68.00±1.00% to 71.00±1.00% becoming 78.00±1.00% to 79.33±0.58% respectively. Lymphocyte level in treatment K- (infected fish without extract immersion) drastically declined. ANOVA showed that *E. palmifolia* root extract high significantly affected the number of lymphocytes of *O. niloticus* infected with *P. fluorescens* (p < 0.01).

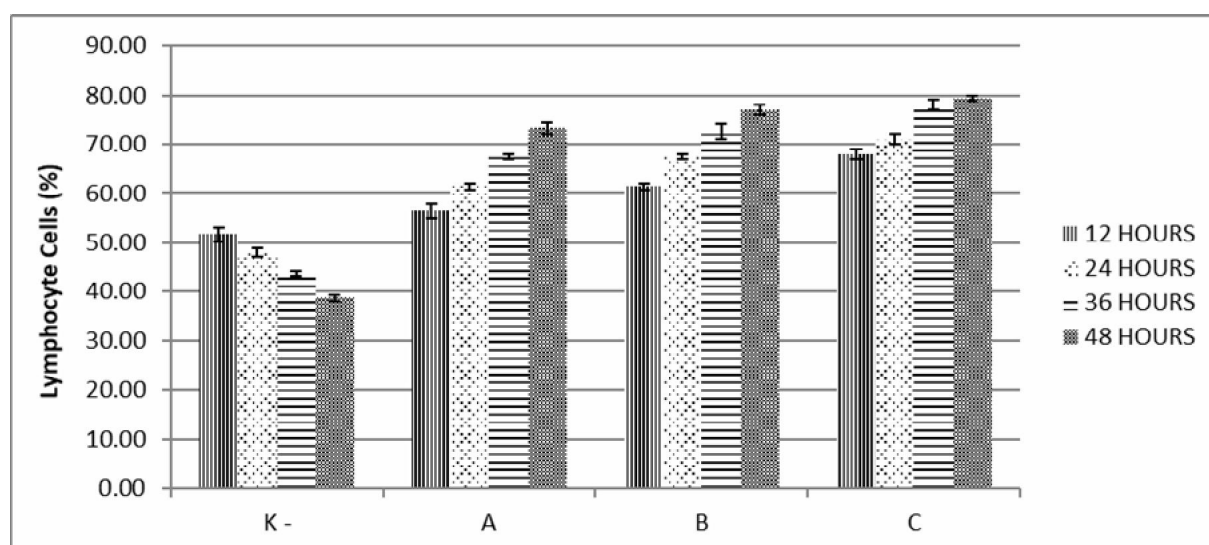


Figure 3. Mean number of lymphocytes of *O. niloticus* (K- infected fish A with the dose of 30 mg L<sup>-1</sup>, B with 50 mg L<sup>-1</sup>, and C with 70 mg L<sup>-1</sup>).

**Monocyte analysis of *O. niloticus*.** The results of monocyte analysis showed a decreased value after *E. palmifolia* root extract application. Figure 4 shows the lowest number of monocytes occurs at the dose of 70 mg L<sup>-1</sup> (treatment C). Treatment C showed monocytes at 12 h is 10.33±0.58%, 24 h is 9.00±0.00%, 36 h is 7.67±0.58%, and 48 h is 5.00±1.00%. Number of monocytes in treatment K- (infected fish without extract immersion) drastically increases. ANOVA revealed that *E. palmifolia* root extract highly significantly influenced the number of monocytes of *O. niloticus* infected with *P. fluorescens* ( $p < 0.01$ ).

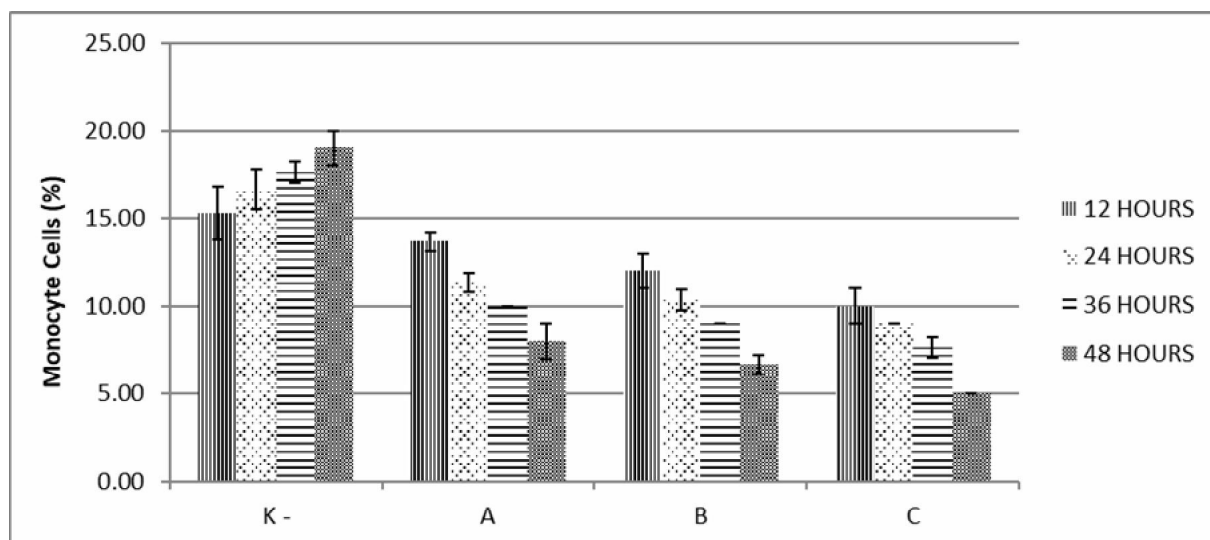


Figure 4. Mean number of monocytes of *O. niloticus* (K- infected fish A with the dose of 30 mg L<sup>-1</sup>, B with 50 mg L<sup>-1</sup>, and C with 70 mg L<sup>-1</sup>).

**Neutrophil analysis of *O. niloticus*.** The neutrophil analysis showed a decreased value after *E. palmifolia* root extract application. Figure 5 demonstrates that the lowest number of neutrophils occurs at the dose of 70 mg L<sup>-1</sup> (C), compared to other treatments. Neutrophils in treatment C showed at 12 h is 23.67±0.58%, 24 h is 20.67±0.58%, 36 h is 17.07±0.58%, and 48 h is 14.33±0.58%. In treatment K- (infected fish without extract immersion), number of neutrophils drastically increases. ANOVA indicates that *E. palmifolia* root extract highly significantly influences the number of neutrophils of *P. fluorescens*-infected *O. niloticus* ( $p < 0.01$ ).

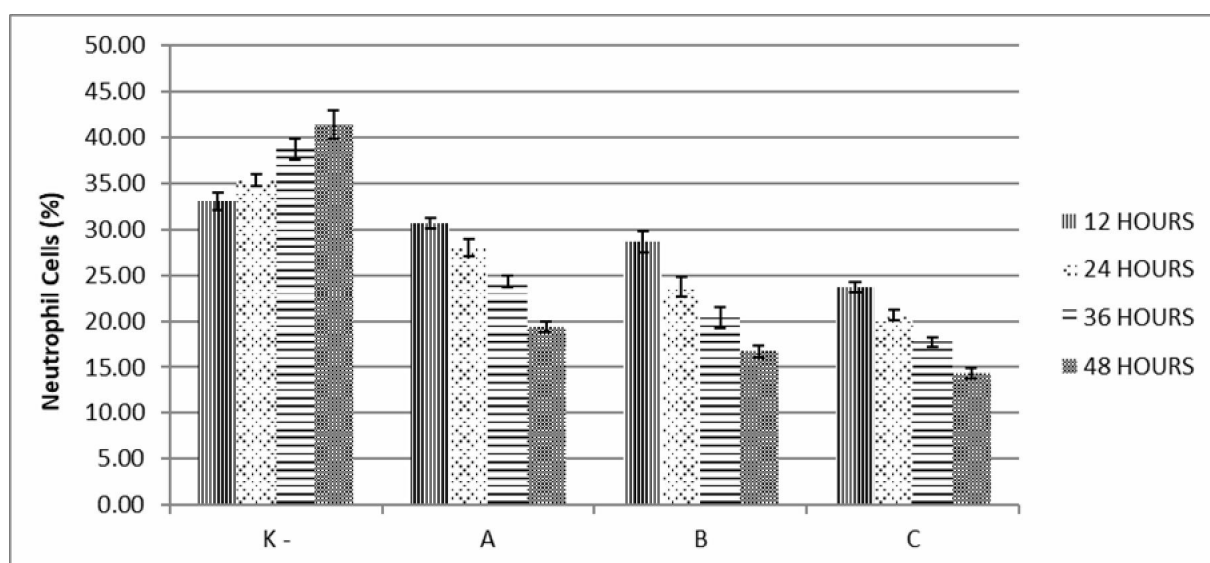


Figure 5. Mean number of neutrophils of *O. niloticus* (K- infected fish A with the dose of 30 mg L<sup>-1</sup>, B with 50 mg L<sup>-1</sup>, and C with 70 mg L<sup>-1</sup>).

**Hemoglobin analysis of *O. niloticus*.** After *E. palmifolia* root extract application, hemoglobin showed an increased value. The highest number of hemoglobin was recorded at the dose of 70 mg L<sup>-1</sup> (C), compared to other dose (Figure 6). Total hemoglobin in treatment C at 12 h is 5.00±0.20 G%, 24 h is 6.00±0.10 G%, 36 h is 6.53±0.12 G%, and 48 h is 6.70±0.10 G%, while total hemoglobin in treatment K- (infected fish without extract immersion) drastically declines. ANOVA indicated that *E. palmifolia* root extract highly significantly affected the number of hemoglobin of *P. fluorescens*-infected *O. niloticus*.

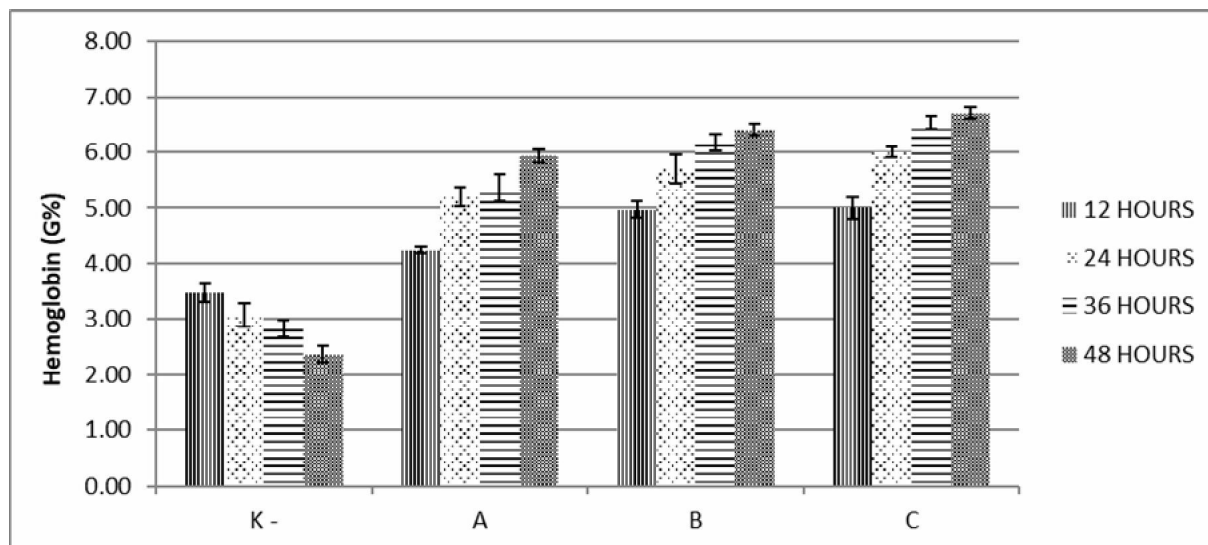


Figure 6. Mean number of hemoglobin of *O. niloticus* (K- infected fish A with the dose of 30 mg L<sup>-1</sup>, B with 50 mg L<sup>-1</sup>, and C with 70 mg L<sup>-1</sup>).

**Hematocrit analysis of *O. niloticus*.** Figure 7 shows that the highest hematocrit occurs at the treatment concentration of 70 mg L<sup>-1</sup> (C), compared to other concentration. Total hematocrits in treatment C at 12 h, 24 h, 36 h, and 48 h rose from 23.67±0.58% to 27.67±0.58% becoming 29.67±0.58% to 32.67±0.58% respectively. In treatment K- (infected fish without extract immersion) the hematocrit has drastic declined in this research. ANOVA indicated that *E. palmifolia* root extract highly significantly influenced the number of hematocrits of *O. niloticus* infected with *P. fluorescens* ( $p < 0.05$ ).

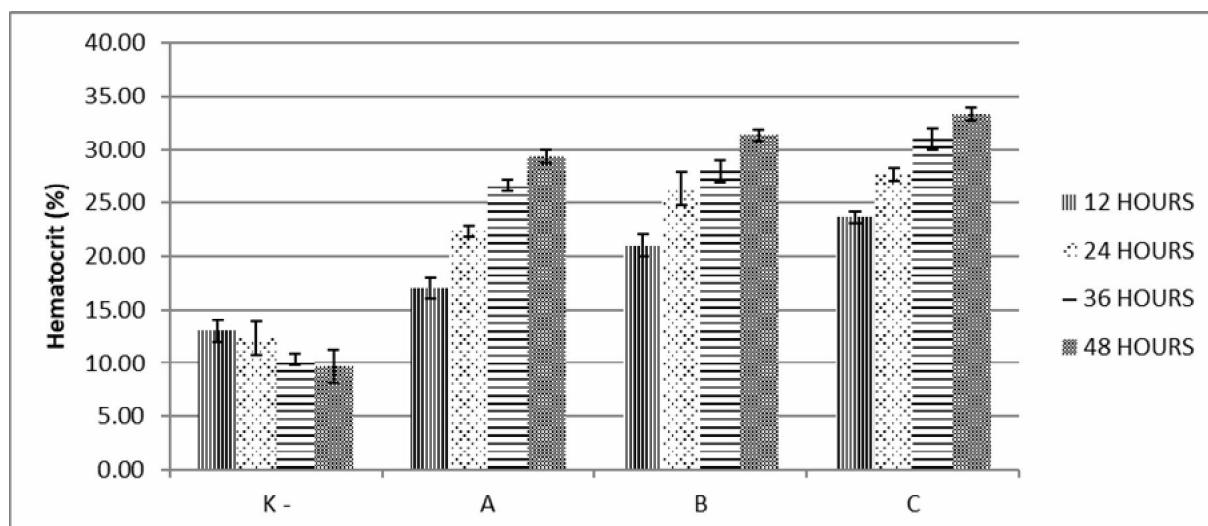


Figure 7. Mean number of hematocrits of *O. niloticus* (K- infected fish A with the dose of 30 mg L<sup>-1</sup>, B with 50 mg L<sup>-1</sup>, and C with 70 mg L<sup>-1</sup>).

**Discussion.** *E. palmifolia* root extract is known containing phenols, flavonoid, triterpenoid, saponin, and tanin (Subramaniam et al 2012; Harlita et al 2018). In the present toxicity test, fish mortality occurred due to excessive secondary metabolite content of the extract, such as phenol and saponin. It is in agreement with Moraes et al (2015) that excessive phenol compounds absorbed in the fish blood could spread to other body parts and cause biological disturbance, such as metabolism inequilibrium. It could also show mucus hyperproduction on the skin and gills. The mucus looks clear in the water that turned turbid. According to Marrelli et al (2016), saponin hemolyzes the erythrocytes through interaction with the cell membrane that leads to increase in membrane permeability, and as a result, loss of hemoglobin occurs.

Values in different hematology will significantly affect the fish physiological condition, health, and survival. This blood analysis is beneficial for diagnostic purposes, to evaluate the health conditions of fish and compliance with the environmental conditions (Jeronimo et al 2014; Kulkarni 2015). Blood is known to have a role in specific and non-specific defense system of fish, since blood can work to fight infection of the pathogenic microbes (Etim et al 2014; Docan et al 2017). The present study found that the administration of *E. palmifolia* root extract gave the hematological conditions of *P. fluorescens*-infected *O. niloticus* approaching to normal.

Erythrocytes are the most common blood cells and have as the main function to circulate oxygen to entire body tissues (Witeska 2013; Shen et al 2018). Mean number of erythrocytes in normal fish is  $2.2 \times 10^6$  cells  $\text{mm}^{-3}$ , but in infected fish, it will decline (Madhu et al 2014). The administration of *E. palmifolia* root extract to *P. fluorescens*-infected *O. niloticus* increased the fish erythrocytes. This finding is supported by Hammed et al (2015) that moringa leaf extract application which contains secondary metabolites will yield significant increase in number of erythrocytes of the pathogenic bacteria-infected fish. The increased number of erythrocytes is considered as indication of high carrying capacity of the blood oxygen that is typical for high fish respiration and metabolism. The presence of secondary metabolite compounds could reduce bacterial infection so that the number of erythrocytes rises (Dangeubun & Metungun 2017).

Leucocytes function as motile protector to help defend the body against the damage from bacteria, viruses, and parasites. In healthy *O. niloticus* there are approximately 60,000 cells  $\text{mm}^{-3}$  (Masud & Singh 2013; Talpur & Ikhwanuddin 2013; Reyes & Aliasas 2018). Decline in leucocytes of *O. niloticus* infected with *P. fluorescens*, after treated with the extract could result from the antibacterial compound activity. It is supported by Amrevuawho et al (2016) that mean number of leucocytes of the infected fish, after extract immersion, is lower than infected fish without extract application. It could result from that the extract contains the compounds that fight and attack the pathogenic bacteria. The application of phenol-containing extract could kill the bacteria. Phenols in the extract have antibacterial properties through bacterial growth inhibition, causing cell membrane dysfunction, declining the intercellular ATP concentration, and changing the cellular morphology of the bacteria (Tenfen et al 2017).

Lymphocytes are the blood cells that specifically work for immune response (Sharma & Langer 2014). Normal lymphocytes in healthy fish are 80% and will decrease if infected with bacteria. The lymphocytes will firstly decline down to 5.4% and even become 39% (Parvez & Mudarris 2014; Podeti & Benarjee 2017). Increased lymphocytes after extract administration found in the present study is in line with Maryani et al (2018), meaning that the fish humoral immune response is in good condition, so that the resistance to the alien materials occurs, and the antibody is formed. Its increment could reach 83% since phenols could raise the immune system and act as antibacterial (Iman et al 2017).

Furthermore, the monocytes in fish migrate to the tissues and become macrophages. This cell will function to phagocytize bacteria with a longer time compared with other cells in infected fish (Claver & Quaglia 2009; Osman et al 2018). The number of monocytes in the infected fish declines after treated with *E. Palmifolia* root extract. It could result from that the extract itself functions as antibacterial so that monocyte production will be reduced. Flavonoid destroys the cell membrane of the bacteria by constructing complex compounds together with extracellular protein and dissolved



protein, so that the intracellular compound will leak out of bacterial cells. Flavonoid disturbs the cytoplasmic membrane, inhibits the nucleic acid synthesis, and restrains the energy metabolism as well (Xiao et al 2014; Gorniak et al 2019).

Neutrophil is the blood cells that are very important for host defense and firstly recruited to the inflammatory sites and remove the pathogens through various complementary mechanisms (Havixbeck & Barreda 2015; Mortaz et al 2018). It works to defend the body against the bacteria, so that when infection occurs, more of these cells will be produced. Number of neutrophils was found to be directly proportional to the number of monocytes, so that as *E. palmifolia* root extract is given, the percentage of neutrophils will decline. Flavonoid contained in the extract could induce bacterial aggregate formation and thus, highly reduce the number of CFUs (Resende et al 2015). Tanin also could cause inhibition of bacterial attachment and cell membrane penetration (Redondo et al 2014).

Hemoglobin is a protein contained in the erythrocytes with a major function to transfer oxygen and carbon dioxide in the circulatory system and nutrients, and remove the embolism debris. In normal fish, hemoglobin ranges from 5 to 9 G% (Dal'Bo et al 2015; Wang et al 2017). This study found that the range of hemoglobin, after treated with *E. palmifolia* root extract, is directly proportional to the number of erythrocytes. It is in agreement with Alsaïd et al (2015) that hemoglobin level will rise if the number of erythrocytes increase. *E. palmifolia* root extract contained triterpenoid. The cell wall of gram negative bacteria is known to have complex and layered structures that give access to the membrane to be more limited. Thus, this study reveals that triterpenoid compound can become the agent that is capable of penetrating this complex barrier and could address its antimicrobial properties (Amoussa et al 2016).

Hematocrit is the volume of the blood cell compared with blood plasma and expressed in percent. Lower number of hematocrits than 22% indicates that the fish suffered from anemia (Docan et al 2017; Rosidah et al 2018). Hematocrit value is related with number of erythrocytes and hemoglobin (Jeronimo et al 2014). The present study found that treatment with *E. palmifolia* root extract increased the hematocrit of *P. fluorescens*-infected *O. niloticus*. It is supported by Maftuch (2017) that *E. palmifolia* root extract shows inhibition of bacterial growth. Tanin contained in the extract could inhibit the bacterial growth by complexing the enzyme and protein from the outer membrane of the bacteria. Tanin also influences the permeability of the bacterial cell membrane, because it disturbs the absorption of important elements for bacterial growth (Joseph et al 2016).

This study revealed that higher dose application of *E. palmifolia* root extract will give better hematological parameter range. It could result from the secondary metabolite content in *E. palmifolia* root extract, such as flavonoid, tanin and phenol, that causing this extract working as an antibacterial, so that bacterial lysis occurs (Fu et al 2016; Wu et al 2016; Armanda et al 2017).

**Conclusions.** This study revealed that the administration of *E. palmifolia* root extract influenced the hematology of *O. niloticus* infected with bacteria *P. fluorescens*. The effect was indicated with changes in number of erythrocytes, leucocytes, differential leucocytes (lymphocyte, monocyte, neutrophil), hemoglobin, and hematocrit leading to normal level. The treatment C (70 mg L<sup>-1</sup>) was considered the best as maximum concentration.

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