The Effect of Administering Jeruju (*Acanthus ilicifolius*) Leaf Extract on Blood Parameters of Tilapia (*Oreochromis niloticus*)

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Abstract. This study was aimed to investigate the effect of various doses of jeruju leaf extract administered through feed on the blood parameters of tilapia, including erythrocyte count, leukocyte count, and differential leukocyte count and to determine the dose effectively affected the blood parameters of tilapia. Jeruju is a type of herbaceous plant or phytopharmaca which has the potential to enhance fish immunity. This plant is mostly found in coastal areas. The study was conducted from July to August 2018 in the Laboratory of Tanjung Balai Polytechnics (extract preparation), Blood Laboratory of Kisaran (blood examination) and Dusun IV in Pahang Village, Talawi Subdistrict, Batu Bara Regency (research location). The research method applied was the experimental method with Completely Randomized Design (CRD) consisted of four (4) treatments and six (6) replications, namely A (0 g/kg), B (8 g/kg), C (10 g/kg), and D (12 g/kg). The blood parameters of tilapia which included leukocyte count, erythrocyte count, and the percentage of lymphocytes, neutrophils, and monocytes were examined on the 0th, 7th and 14th day of the study. The administration of jeruju leaf extract resulted in a significant effect on leukocyte count, erythrocyte count, and lymphocyte percentage (P<0.05), but did not significantly affect the percentage of neutrophils and monocytes.

1. Introduction

Tilapia (*Oreochromis niloticus*) is easy to cultivate and has an important economic value with a relatively stable selling price compared to other farmed fish [1]. Tilapia is one of fishery commodities that enters both local and global markets. High market demand for tilapia motivates fish farmers to perform tilapia farming. Moreover, tilapia is also able to be cultivated in the backyard, it grows fast, its culture technology can be easily applied by the community, the product is easily marketed, and its farming business requires lower cost [2]. However, tilapia culture also faces serious problems since tilapia is easy to get stressed or sick which could lead to fish death. Hence, a quick and serious solution is necessary to prevent higher losses suffered by fish farmer.

Effort to control and prevent fish disease is done by improving fish immune system [3]. Enhancing fish immune system is able to be achieved through various methods, such as the addition of probiotics in fish diet or rearing water. Many experts found that mangrove leaves contain bioactive compounds that can be used as antibacterial agents [4, 5, 6]. A material is categorized as bacteriostatic agent if it can inhibit bacterial growth or to kill bacteria [7].
Several materials are expected to have the ability to increase fish immune system, namely vaccine, immunostimulant, probiotics, and fitoharmaka [8]. Jeruju is type of mangrove which contains a bioactive compound that is potential to be used as immunostimulant (antibacterial) [9]. Extract of jeruju leaves contains active compounds, such as polyphenols, alkaloids, and flavonoids [10]. Moreover, alkaloids and flavonoids in jeruju leaves contain great amount of glucosides which leads to increasing leucocyte count in fish, that is one of indicators of improvement of fish immune system [11].

It is expected from this study that jeruju leaf extract will be an alternative ingredient added to fish feed to enhance fish growth and productivity. This study was aimed to investigate the effect of various doses of jeruju extract administered trough feed on the blood parameter of tilapia, which included erythrocyte count, leucocyte count, and differential leucocyte count and to determine the dose effectively affected the blood parameters of tilapia.

2. Materials And Method

2.1 Place and Time
The study was conducted from July to August 2018 in the Laboratory of Tanjung Balai Polytechnics (leaf extract preparation), Blood Laboratory of Kisaran (blood examination) and Dusun IV in Pahang Village, Talawi Subdistrict, Batu Bara Regency (research location). North Sumatera Province.

2.2 Data Analysis
The research method applied in this study was the experimental method with Completely Randomized Design (CRD) consisted of four (4) treatments and six (6) replications, namely A (0 g/kg), B (8 g/kg), C (10 g/kg), and D (12 g/kg). According to [12] Kusningrum (2008), the formula applied to determine the number of replication in CRD: \((r-1) (t-1) \geq 15\), hence number of replication was calculated as follows:

\[(r-1) (4-1) \geq 15\]
\[(r-1) (3) \geq 15\]
\[3r-3 \geq 15\]
\[3r \geq 18\]
\[r \geq 18 / 3 = 6\] replications

2.3 Sampling and Preparation
Jeruju leaves were collected from coastal areas of Batu Bara Regency. Leaves were further sun-dried, blended using a blender to a fine powder. Later, powdered sample was immersed in methanol solvent for 1 x 24 hour at room temperature. Sample that has been immersed was filtered using filter paper. The product of filtration was further collected and put into evaporator to concentrate.

Preparation of medium was done by washing tilapia rearing tank (bucket) and installing blower. A total of 2 tilapia at size of 40 – 70 g/fish that had been acclimatized (for 3 days) were put in each tank.

2.4 Application of Jeruju Leaf Extract Treatment
After being acclimatized, tilapia were given treatment of combinedjeruju extract and feed at dose 0 g/kg, 10 g/kg, 12 g/kg, and 14 g/kg. Feeding ratio at 3% of fish
biomass was applied. Jeruju extract and feed was mixed following the coating method [13]. Administration of jeruju extract though feed was applied for 14 days.

2.5 Observation of Blood Parameter of Tilapia

Blood parameters of tilapia were examined by firstly collecting the blood sample using 3 ml syringe that was injected into the linea lateralis behind the anal fin. Needle was inserted into musculus until it reached spine. Later, the plunger of syringe was pulled back slowly to allow blood entered the syringe. Blood was further collected in the vacuum tube contained anticoagulant EDTA. The blood sample obtained was further examined in the laboratory at day 0th, 7th, and 14th and during the study. Fish blood parameter observed included leucocyte count, erythrocyte count, percentage of neutrophil, percentage of lymphocyte, and percentage of monocyte.

3. Results and Discussion

3.1 Leukocytes

Table 1. Average Leukocyte Count of Each Treatment during the Administration of Jeruju Leaf Extract

| Day    | Treatment A (x10^4 cell/mm³) | Treatment B (x10^4 cell/mm³) | Treatment C (x10^4 cell/mm³) | Treatment D (x10^4 cell/mm³) | Normal (x10^4 cell/mm³) |
|--------|-------------------------------|-------------------------------|-----------------------------|------------------------------|-------------------------|
| Day 0th| 10.78 ±0.39b                  | 11.44 ±0.44b                  | 10.83 ±0.47b                | 11.12 ±0.55b                 | 2 – 15 [14]             |
| Day 7th| 11.99 ±0.31b                  | 12.85 ±0.34b                  | 11.81 ±0.44b                | 12.20 ±0.40b                 |                         |
| Day 14th| 13.15 ±0.24b                  | 13.89 ±0.47b                  | 13.01 ±0.49b                | 13.70 ±0.38b                 |                         |

Description: a: Average leukocyte count
b: Standard deviation

Observation of total leukocyte count in tilapia given different dose of treatment resulted in the highest value of 13.70 x10⁴ cell/mm³ in treatment D. Based on the result of the Analysis of Variance (ANOVA), treatment D and treatment B were not significantly different, indicating that treatment D was not better than treatment B, or both treatments were considered similar. The range of leukocyte count observed was still within the normal range which means that fish was healthy. Normal leukocyte count in fish is between 2 – 15 x10⁴ cell/mm³ [15]. Factor affecting leukocyte count includes fish condition and fish health. It was proven that extract of jeruju leaves significantly increased leukocyte count in brown-marbled grouper [16].
3.2 Erythrocytes

**Table 2. Average Erythrocyte Count of Each Treatment during the Administration of Jeruju Leaf Extract**

| Day    | Treatment A (x10^6 cell/mm^3) | Treatment B (x10^6 cell/mm^3) | Treatment C (x10^6 cell/mm^3) | Treatment D (x10^6 cell/mm^3) | Normal (x10^6 cell/mm^3) |
|--------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------------------|
| Day 0th | 1.33±0.28b                    | 1.32±0.03b                    | 1.33±0.03b                    | 1.35±0.02b                    | 1.05 – 3.0 [17]            |
| Day 7th | 1.34±0.02b                    | 1.34±0.03b                    | 1.35±0.04b                    | 1.36±0.02b                    |                            |
| Day 14th| 1.36±0.03b                    | 1.43±0.03b                    | 1.37±0.04b                    | 1.38±0.02b                    |                            |

**Description:**

- a: Average erythrocyte count
- b: Standard deviation

Observation of total erythrocyte count in tilapia treated with different doses showed that treatment B obtained the highest value of 1.43 x10^6 cell/mm^3. Based on the result of the Analysis of Variance (ANOVA), it was found that treatment B was significantly different from treatment A, C, and D, indicating that treatment B was the best treatment among all. The range of this leukocyte count was still within the normal range, reflecting that fish was healthy. Normal erythrocyte count in fish ranged from 1.05 – 3.00 x10^6 cell/mm^3 [18]. Erythrocytes carry oxygen throughout the body, thus low erythrocyte volume will hinder fish to take oxygen, resulting in oxygen deficiency [19]. Low erythrocyte count indicates anemia, while high erythrocyte count shows that fish is stressed [20]. Found that extract of jeruju leaves could significantly increase erythrocyte count in brown-marbled grouper [21].

3.3 Neutrophils

**Table 3. Average Neutrophil Percentage of Each Treatment during the Administration of Jeruju Leaf Extract**

| Day    | Treatment A (%) | Treatment B (%) | Treatment C (%) | Treatment D (%) | Normal (%) |
|--------|-----------------|-----------------|-----------------|-----------------|------------|
| Day 0th| 11.69±0.28b     | 11.70±0.34b     | 11.83±0.15b     | 11.97±0.24b     | 7.75 – 14.94 [22] |
| Day 7th| 10.48±0.29b     | 10.83±0.62b     | 10.85±0.32b     | 10.96±0.24b     |            |
| Day 14th| 10.89±0.38b    | 11.08±0.54b     | 11.25±0.29b     | 11.28±0.32b     |            |

**Description:**

- a: Average neutrophil percentage
- b: Standard deviation

Based on the observation of average neutrophil percentage in tilapia fish blood, neutrophil percentage tended to decrease on day-14. Treatment dose of 0, 10, 12, and 14 g/kg was found to have a similar declining trend. According this is related to the main function of neutrophil to destroy foreign materials through phagocytosis, called chemotaxis, where cells will migrate to particles to further destroy the particle using lysozyme in phagolysosome [23]. Therefore, neutrophil percentage will not increase in the absence of stimulation by foreign materials including bacteria, virus, and pathogen. This finding was confirmed that increasing number of neutrophil cells indicates increasing number of macrophages at the site of infection, allowing it to easily destroy foreign particles [24].
3.4 Lymphocytes

Table 4. Average Lymphocyte Percentage of Each Treatment during the Administration of Jeruju Leaf Extract

| Day    | Treatment A (%) | Treatment B (%) | Treatment C (%) | Treatment D (%) | Normal (%) |
|--------|-----------------|-----------------|-----------------|-----------------|------------|
| Day 0<sup>th</sup> | 80.70±0.29<sup>a</sup> | 80.56±0.41<sup>b</sup> | 80.45±0.14<sup>b</sup> | 80.82±1.24<sup>b</sup> | 7.75 – 14.94 |
| Day 7<sup>th</sup>  | 81.99±0.62<sup>b</sup> | 81.25±0.54<sup>b</sup> | 81.45±0.51<sup>b</sup> | 81.59±0.27<sup>b</sup> | [25]        |
| Day 14<sup>th</sup> | 82.61±0.83<sup>b</sup> | 83.62±0.63<sup>b</sup> | 81.95±0.71<sup>b</sup> | 82.23±0.39<sup>b</sup> |            |

Description: a : Average lymphocyte percentage  
b : Standard deviation

Observation of lymphocyte percentage in tilapia treated with different doses showed that treatment B obtained the highest value of 83.62%. Based on the result of the Analysis of Variance (ANOVA), it was found that treatment B was significantly different from treatment A, C, and D, indicating that treatment B was the best treatment among all treatments. The range of this leukocyte count was still within the normal range, reflecting that fish was healthy.

Increase in lymphocyte percentage reflected the success of fish immune system to develop immune response in tilapia. Lymphocyte has the ability to transform into plasma cell, namely cell that produces antibody and plays an important role in immunity [26].

3.5 Monocytes

Table 5. Average Monocyte Percentage of Each Treatment during the Administration of Jeruju Leaf Extract

| Day    | Treatment A (%) | Treatment B (%) | Treatment C (%) | Treatment D (%) | Normal (%) |
|--------|-----------------|-----------------|-----------------|-----------------|------------|
| Day 0<sup>th</sup> | 7.60±0.21<sup>b</sup> | 7.72±0.21<sup>b</sup> | 7.71±0.15<sup>b</sup> | 7.70±0.16<sup>b</sup> | 7.75 – 14.94 |
| Day 7<sup>th</sup>  | 7.52±0.72<sup>b</sup> | 7.90±0.63<sup>b</sup> | 7.65±0.59<sup>b</sup> | 7.44±0.38<sup>b</sup> | [27]        |
| Day 14<sup>th</sup> | 6.49±0.94<sup>b</sup> | 5.37±0.84<sup>b</sup> | 6.79±0.72<sup>b</sup> | 6.48±0.55<sup>b</sup> |            |

Description: a : Average monocyte percentage  
b : Standard deviation

Monocyte percentage was observed to have decreasing trend on day-14 which is still related to the function of monocyte as macrophage, that is monocyte is not yet used by the body to perform phagocytosis due to the inexistence of infection in fish body. Revealed a similar finding that low percentage of lymphocyte will be balanced with the high percentage of monocyte and neutrophil, vice versa.

3.6 Water Quality Parameter

The result of water quality parameter measurement obtained during the study showed that temperature ranged of 27ºC – 29ºC, pH ranged between 6.8 – 7.5, and DO ranged from 5.3 – 6.2 ppm. Therefore, it was observed that water quality parameter during study was still within the normal condition and suitable for tilapia culture.
4. Conclusion and Recommendation

4.1 Conclusion
Based on the results of the study, it is concluded that:
1. The administration of jeruju leaf extract through feed had a significant effect on leukocyte count, erythrocyte count, and the percentage of lymphocytes and monocytes, indicating that jeruju leaf affected the blood parameter of tilapia.
2. The dose of 10 g/kg administered for 14 days was found to be the best dose as shown by the increasing erythrocyte count and lymphocyte percentage in tilapia.

4.2 Recommendation
It is recommended to stun fish prior to blood collection to prevent fish stress.

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