IMMUNOMODULATORY EFFECT OF ALKALOID BIOACTIVE SUBSTANCES OF JELLYFISH (Bougainvillia sp) ON TIGER GROUPER (Epinephelus fuscoguttatus) INNATE IMMUNE RESPONSE

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Abstract. Immunostimulatory can be used for fish disease prevention as it can enhance fish resistance by increasing the non specific defense mechanisms. The aim of this research was to examine humoral and cellular innate immune responses of Tiger grouper (Epinephelus fuscoguttatus) on alkaloid substance based on phagocyte activity, macrophage, total leukocyte, differential leukocyte (lymphocyte, monocyte, neutrophile) and plasma protein. Jellyfish bioactive substance was determined using UV-Vis, Thin Layer Chromatography (TLC), Irradiation Red (IR) and Nuclear Magnetic Resonance Hydrogen ('H-NMR). Completely Randomized Design was used with 4 treatments i.e: C= 0 ; A=0,5 ; B=0,75 ; C=1;D=1,25 g of alkaloid/kg feed and 3 repetitions. The results showed that bioactive substance in jellyfish extract was identified as alkaloid which is predicted as an N-1 – Benzilalkohol, 4–Oktil Piperidin. There was a significant difference (P<0.05) on leukocytes, macrophage, phagocytes activity and plasma protein. This result indicated that alkaloid substance from jellyfish increased the activity of tiger grouper’s innate immune response.

Keywords: Alkaloid substance, jellyfish, innate immune response, tiger grouper

1. Introduction

The main problem in tiger grouper cultivation is a high number of larval mortality up to 99% [1]. It mainly caused by pathogen bacteria which may increase fish mortality rate to 80%.[2] [3]. Vibrio is a gram negative bacterium which causes the systemic infection on fish called as Vibriosis [4] [5] [6].

Many substances from different sources (bacterial component, chemical agents, animal or plant extracts, etc) have been studied as prospective immunostimulants for fish [7] [8] [9], and some have been reported to give a significant degree of protection against several diseases commonly found in farmed fish, such as Vibrio harveyi [10] [11]. Bioactive substances obtained from natural sources such as jellyfish is considered friendly to our environment. Both poisonous and edible jellyfish produce several kinds of bioactive substances that can used to prevent fish and shrimp diseases. Bioactive substances taken from jellyfish are characterized as alkaloid, fenolate, steroid, and terpenoid groups. It shows the strong activity on fish bacteria and shrimp with the higher inhibitory diameter than using chemical substance and antibiotic [12] [13].

Despite of its potency, research on jellyfish is uncommon. [14], mentioned that jelly fish Pysalia sp could inhibit the growth bacteria found on Tiger prawn larva. Other jelly fish species such as Obelia sp, Bougainvillia sp, Hydra sp, Pennaria sp, Aurelia sp also have potency to act as bactericide and may increase innate immune response (cellular and humoral) of fish [7] [15] [16]. The purpose of this study...
was to examine the influence of alkaloid from jellyfish (*Bougainvillia* *sp*) on humoral and cellular innate immune responses of Tiger grouper (*Epinephelus fuscoguttatus*).

## 2. Materials and Methods

### 2.1. Animal

7.5 cm of Tiger grouper fish larva, obtained from Brackish Water Research Centre Situbondo, were used as tested animals. The culture density was 15 fish/20 liters of water. Acclimatization process was conducted for 2 weeks preceding the experiment. 10-20% of water volume was replaced weekly.

### 2.2. Isolation of alkaloid from jellyfish

Extraction of alkaloid substance from jellyfish *Bougainvillia* *sp* was conducted based on [17]. 250 gram of jellyfish was extracted with petroleum benzene. The process was repeated 4 times. Ethanol, HCL 0.2 N and NaOH 15% were added to filtered extract before it was extracted with chloroform. The extract was then washed with aquadest, dried with Na$_2$SO$_4$, and vacuum evaporated at 30$^\circ$ C. Evaporated extract was then analyzed using Thin Layer Chromatography (TLC), UV-Vis, Irradiation Red (IR) and Nuclear Magnetic Resonance Hydrogen ('H-NMR).

### 2.3. Experimental design

Completely Randomized Design with 4 treatments (alkaloid dose) and 3 repetitions was used. The treatments consists of C = 0 g alkaloid/kg of feed, A = 0.5 g alkaloid/kg feed, B = 0.75 g alkaloid/kg feed, C = 1 g alkaloid/kg feed and D = 1.25 g/kg feed. Treated fish larvas were bathed for 5 days with *V*.harveyi $10^5$ CFU/ml of rearing water. Cellular immune response was measured based on the number of leukocyte, leukocyte differential (monocyte, lymphocyte and neutrophile) [18]. Isolation of macrophages and phagocytes activity calculation based on [19]. Humoral of plasma protein was measured using spectrophotometer and electrophoresis SDS PAGE method [20]. Fish larvae was observed after 28 days of alkaloid treatment and on day 30, 32, 34 days after infected with *Vibrio harveyi*. Data were analyzed using statistical method of one way ANOVA.

## 3. Results

### 3.1. The characterization of alkaloid Bougainvillia sp

Figure 1 showed the molecular structure of alkaloid from *Bougainvillia* *sp* extract based on spectrophotometer H'-NMR measurement which was supported with ultraviolet spectra (UV) and infra-red (IR) spectra.

![Figure 1. The structure alkaloid from Bougainvillia sp.](image)

(N-1 – Benzilalkohol, 4 – Oktil Piperidin).

### 3.2. The effect of alkaloid on innate immune response of tiger grouper

The cellular and humoral immune responses of Tiger grouper, treated with alkaloid substance from jellyfish (*Bougainvillia* *sp*), before and after being infected with *Vibrio harveyi* is shown in Table 1. The result of electrophoresis on tiger grouper’s plasma protein is shown in Fig.2.
Table 1. The effect of jellyfish (*Bougainvillia sp*) alkaloid substance on cellular immune responses of Tiger grouper (*Epinephelus fuscoguttatus*) prior and after infected with *Vibrio harveyi*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pre infection/after given alkaloid</th>
<th>Post infection d 30th</th>
<th>32th</th>
<th>34th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte (10^3 cell/ml)</td>
<td>51.5</td>
<td>70.1</td>
<td>84.2</td>
<td>96.2</td>
<td>104.6</td>
</tr>
<tr>
<td>Neutrophile (%)</td>
<td>6.0</td>
<td>7.3</td>
<td>7.5</td>
<td>8.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2.4</td>
<td>3.2</td>
<td>4.6</td>
<td>6.3</td>
<td>8.45</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>60.95</td>
<td>68.2</td>
<td>70.4</td>
<td>74.8</td>
<td>81.55</td>
</tr>
<tr>
<td>Macrophage (10^5 cell/ml)</td>
<td>1.7</td>
<td>6.2</td>
<td>8.5</td>
<td>10.4</td>
<td>12.9</td>
</tr>
<tr>
<td>Phagocytes activity (%)</td>
<td>18.0</td>
<td>28.1</td>
<td>47.5</td>
<td>62.2</td>
<td>87.5</td>
</tr>
<tr>
<td>Protein plasma (ppm)</td>
<td>400</td>
<td>1050</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig.2. Plasma protein profile of Tiger Grouper: (I) Before infection (II) After infection, control column 6; A=2, B=3, C=4 and D=5 were treatments using different alkaloid dosage; and (M=1) marker.

4. Discussion

Alkaloid treatments were able to increase the number of neutrophils after *Vibrio harveyi* challenge tested. It can be inferred from the result that the number of leukocytes increased to prevent fish from bacteria attack. According [21], immunostimulant may activate leukocyte cells and increase fish resistance toward fungi, virus, parasite and bacteria infections.

Monocytes, with 10 to 15 μm in diameter, contain nuclei and finely granular cytoplasm with lysosomes, phagocytes vacuoles, and cytoskeleton filaments [22]. As fish infected, monocytes will shift from blood cells into tissue and become macrophages.

Observations on macrophages showed that 1g alkaloid/kg of feed was the most effective dose. It could increase the number of macrophages after challenged test. Increasing number of macrophages is very important to fight pathogen bacteria [7] [23]. Macrophages play a major role in both innate and adaptive immune system. In innate immune system, macrophages act as phagocytes cells; produce pro inflammation, ROS (by NADPH oxidase enzyme) and RNS (by nitric oxide (NO) synthase). ROS and RNS are oxidative burst formed by oxidize process. Formed ROS are anion superoxide (O_2^-), H_2O_2, HOCl, singlet oxygen (O) dan hydroxyl radical (OH). In the adaptive immune system, macrophages act as professional APCs (Antigen presenting cells) [23] [24]. The ability of macrophages to kill pathogenic microbes is probably one of the most important mechanisms of protection [25]. The present study demonstrated alkaloid addition on feed increased the production of radical hydroxyl which assumed to be involved in the destruction of *Vibrio harveyi*.

Another indicator for humoral defense system is increasing number of protein plasma in fish blood treated with plant of animal extract [10] [26]. Alkaloid contained protein plasma showed 9 protein bands: 168,85 kDa, 140,68 kDa, 90,78 kDa, 58,47 kDa, 37,75 kDa, 29,97 kDa, 15,73 kDa, 12,64 kDa dan 10,15
kDa. After V. harveyi infection, there were only 7 bands: 170.5 kDa, 86.5 kDa, 65 kDa, 43.88 kDa, 30.87 kDa, 19.3 kDa dan 12.13 kDa. It was assumed that bacterial infection might break or destroy protein bands, while the addition of alkaloid might contribute to the formation of new protein bands which helping fish defense system. [27] [28], stated that protein bands of infected fish was thinner than healthy fish.

5. Conclusion

- Chemical Structure of alkaloid from Bougainvillia sp is 1 – Benzilalkohol, 4 – Oktil Piperidin
- Alkaloid treatments increased phagocytic activity (18% to 87.5 %), macrophage (1.7x10^5 cell/ml to 12.9x10^5 cell/ml), total leukocyte (51.500 cell/ml to 107900 cell/ml), differential leukocyte : lymphocyte (60.95 – 81.55%), monocyte (2.4 to 8.45%), neutrofil (6-9%) and protein in plasma (620 to 1050 ppm).
- The alkaloid of jellyfish increased the activity of the tiger grouper’s innate immune response, and it has potency to be applied as an immunostimulant for fish.

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7. References


