Effect of alkaloids derived from jellyfish (*Aeginura* sp.) on the intestinal histopathology and relative percentage survival (RPS) of tiger grouper (*Epinephelus fuscoguttatus*) infected by *Vibrio harveyi*

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Abstract. The purposes of this research were to determine the effect of alkaloid jellyfish compounds on intestinal histopathology of tiger grouper and to determine the best doses to the relative percent survival (RPS) of tiger grouper. The method of this research was descriptive with completely randomized design. The treatment of active alkaloid compound on feed was investigated for 28 days. The fish were then challenged with *Vibrio harveyi* at 10⁵ CFU/cell for 7 days. Alkaloids were added to the feed with the doses (g alkaloid/kg feed) of 0 (control); A = 0.5; B = 0.75; C = 1.0; and D = 1.25. The intestinal histopathology and RPS were observed. The best RPS was found at a treatment of C with the value of 100%.

1. Introduction
The intensification of grouper culture has led to a number of disease outbreaks with an increasing range of pathogens causing them. *Vibriosis* is a common disease caused by *Vibrio carchariae, Vibrio alginolyticus, Vibrio parahaemolyticus* and *Vibrio harveyi* and is one of the most serious problems in various stages of grouper culture. The mortality rate of the seeds in farming can reach up to 99%, and this is mainly caused by infections by pathogenic bacteria [1].

The rate of *Vibrio harveyi* outbreaks in the hatchery of tiger grouper can be calculated within a few hours. *Vibrio* sp. attack can lead to the destruction of organs in fish and wounds on the skin [2]. To control this disease, particularly a bacterial disease, various types of antibiotics such as chloramphenicol, erythromycin and oxytracycline have been used. But apparently, a lot of antibiotics raise the resistance of new bacterial strains in response to the disease [3, 4]. Thus, it is necessary to control the disease using natural materials which are still limited to saponin and rotenon. Consequently, a breakthrough in the utilization of jellyfish that are environmentally friendly as immunostimulants should be made [1, 5].
Jellyfish, from phylum *Coelenterata*, is both poisonous and edible and has bioactive compounds that prevent diseases in fish and shrimp. Bioactive compounds derived from jellyfish such as alkaloids, phenolic, steroids and terpenoid show a strong activity against bacteria in fish with a bigger diameter of resistance compared to chemicals and antibiotics [6]. Research on jellyfish is uncommon in spite of its potential utilization. Yahya *et al.* [7] states that *Pysalia* sp can inhibit the growth of bacteria in shrimp larvae. In addition to *Pysalia* sp., there are other jellyfish species with potential as immunostimulants such as *Gonionemus* sp., *Obelia* sp., *Hydra* sp., *Pennaria*, *Aurelia* sp., *Aeginura* sp., *Bougainvillia* sp. etc. [8].

Based on previous findings, it is necessary to explore unconventional natural resources in order to get the benefits of bioactive compounds from jellyfish as immunostimulants, particularly for tiger grouper seeds. Our study evaluated the effect of different fish feeds in the alkaloid substances of jellyfish on intestinal changes pathologies and its impact on the increase in the Relative Percentage of Survival.

2. Methodology

The extraction and isolation of the alkaloids in jellyfish (*Aeginura* sp.) was done using the modified method by Maldoni [9]. The filtered jellyfish (250 grams) was extracted with petroleum benzene. This process was repeated four times. Four days later, the extract was evaporated in vacuum at a temperature of 40°C. The resulting extract was filtered and evaporated into 10 mL of chloroform extract. After settling, the sediment of chloroform extract that contained alkaloids was separated using Thin Layer Chromatography (TLC), UV spectroscopy, infrared (IR) spectroscopy and Nuclear Magnetic Resonance Hydrogen (‘H-NMR).

The methods used in this research were descriptive and experimental methods with Completely Randomized Design (CRD). The alkaloid substances of *Aeginura* sp. were used in the feed given at certain doses as follows: control = 0 g alkaloid/kg feed; A = 0.5 g alkaloid/kg feed; B = 0.75 g alkaloid/kg feed; C = 1.0 g alkaloid/kg feed; and D = 1.25 g alkaloid/kg feed. They were tested with *Vibrio harveyi* in 10^5 cfu/mL for seven days. The fish tested was tiger grouper (*Epinephelus fuscoguttatus*), 7-8 cm in size, at the age of D 90. The fish were kept in a glass tank with a volume of 15 liters (five levels of treatment) at a density of 10 fish per tank. The intestinal sample for the histopathological analysis was taken after 28 days of the administration of immune-stimulant and after the bacterial infection on Day 35. The preparation of the histological analysis of intestine used method by Lightner [10]. The RPS of grouper fish was recorded at the beginning (day 1) and the end (day 38) of the study.

3. Results and discussion

3.1. Characterization of alkaloids *Aeginura* sp.

3.1.1 Characterization of spectroscopy UV

For preparative TLC analysis, the ratio of chloroform to methanol was 2:8. This dilution was used for the preparative TLC analysis, UV spectroscopy, infrared spectroscopy and H’NMR.

The characterization of molecule by UV yielded the results of spectra, absorbance data and maximum wavelength of jellyfish, which are presented in table 1 and figure 1.

According to the qualitative results, the absorption of chloroform extract showed strong wavelengths of about 239.4 nm and 284.8 nm.

| Species        | λ_max (nm) | A_max |
|----------------|------------|-------|
| *Aeginura* sp  | λ1 = 239.4 | A1 = 0.2002 |
|                | λ2 = 284.8 | A2 = 0.0406 |
The transition could relate to carbonyl bond (C=O) or C=N and N-C=C with alkaloid characteristics. Based on the molecular analysis of the chloroform extract, there were unsaturated molecules containing aromatic nucleus and heteroatom, carbonyl bond C=N and characteristic alkaloid.

3.1.2 Characterization with infrared spectroscopy

Infrared spectroscopy was used to support the H'-NMR spectra data in determining the chemical structure of alkaloid extract. The results of the identification using infrared spectroscopy indicated that the molecules analyzed contained some functional chains, each of which had an absorption band at specific wavenumber as shown in figure 2.
The occurrence of absorption band at a wavenumber of 3.441.32 cm⁻¹ shows the existence of stretching vibrations in the functional bond O-H. The N-H stretching vibrations of primary and secondary amine showed an absorption band at a wavenumber of 3.750—3.000 cm⁻¹. There is a possibility that the molecular functional chain of alkena in aromatic system supported by the occurrence of absorption band at a wavenumber of 3.022.73 cm⁻¹, arose out from C-H stretching vibrations. The aromatic system in the molecular analysis was also supported at a wavenumber 1.714.87 cm⁻¹. According to Silverstein et al. [11], aromatic hydrocarbon is at a wavenumber of 2.000—1.650 cm⁻¹.

The pyrimidine ring is a molecule supported by absorbance at a wavenumber of 1.643.50 cm⁻¹. C-N chain is at a wavenumber of 1.280.85 cm⁻¹ and 1.360.10 cm⁻¹. The C=N stretching vibrations will show an absorption band at a wavenumber of 1.689—1.471 cm⁻¹.

3.2 Intestine histopathology
The results of the study on the condition of the tiger grouper's intestine after being given an alkaloid immune stimulant showed a normal histological form with the length of villi, width of lamina propria, width of villi, thickness of muscle layers and number of goblet cells per segment being measured (figure 3). The samples were taken from all three parts of the intestine (proximal, medial and distal) [12].

![Figure 3](image)

**Figure 3.** (A) Normal cross section of the intestine after an alkaloid immune stimulant consisting of (1) muscularis external (2) Villi (3) goblet cells, 400x HE (bar = 100 μm). (B) After tested challenge with *Vibrio* liver damage such as (1) fusion 400 x HE, (bar = 100 μm).

Figure 3 shows the intestinal bacterial villi controlled with fusion-attached bacteria, and there was no tissue damage in the fish in treatments A, B, C and D. As a result, the bacterial attack of *V. harveyi* in the gut does not necessarily cause anatomical changes.

*V. harveyi* causes death from decreased appetite or abnormal movement of the fish. As suggested by Karthigayani *et al.* [13], some illnesses appear to involve functional impairment (e.g.: decreased appetite or abnormal movement) which actually reaches clinical symptoms even though at that time no anatomical abnormality was found.
3.3 Relative percentage of survival

The results showed that after the immune stimulation was given, the RPS value was 100 % for all treatments. But after the administration of immune stimulation, the best RPS was gained by the tiger grouper in treatment C with 1 gr alkaloids/kg of feed (100 %), followed by treatment B (70.59 %) and treatment D (35.29 %), and the smallest RPS value was gained by the fish in treatment A (23.53 %). The average RPS obtained at the end of the study of each treatment with different replicates can be seen in table 2.

**Table 2. Relative Percentage of Survival.**

| Treatment          | Replicates | Total | SR (%)  | Death (%) | RPS (%)  |
|--------------------|------------|-------|---------|-----------|----------|
|                    |            |       | 0 ± SD  | 0 ± SD    | 0 ± SD   |
| 0.5 gr alkaloid    | 30         | 40    | 70      | 35 ±0.7070| 65±0.7071| 23.53±8.3191|
| 0.75 gr alkaloid   | 80         | 70    | 150     | 75±0.7070 | 25±0.7071| 70.59±8.3198 |
| 1 gr alkaloid      | 100        | 100   | 200     | 100 ± 0   | 0        | 100a0      |
| 1.25 gr alkaloid   | 50         | 50    | 100     | 55±0.7071 | 45±0.701 | 35.29±8.3184|
| Control            | 10         | 20    | 30      | 15±0.7071 | 85±0.7071|           |

The lowest RPS value was 23.53 %. According to Sakai [5], the main factors determining the effect of a compound are the dose and the concentration of the compound. The concentration of alkaloids of *Aeginura* sp. as an immune stimulant ingredient fed to the tiger grouper did not provide any immune response. The dose was too high that the immune stimulant effect could not increase the immunity. Because the fish body was unable to respond to the mechanisms of cellular and humoral response, antibodies were not formed and toxic effects may be resulted. *Aeginura* sp. has cytotoxic bioactive compounds. These toxins can disrupt or destroy cell membranes, which can disrupt the transport of compounds in and out of cells. However, the results of this study were better than those of Burrels *et al.* [14], which used 2 % β-glucan + 2% immune stimulant nucleolide for 21 days with RPS maintained at 37 % in Rainbow Trout after *Vibrio anguillarum* infection.

4. Conclusion

The administration of immune stimulant did not cause damage to tiger grouper intestine after being tested with *Vibrio harveyi*.

The highest Relative Percentage of Survival value was gained in treatment C with 1 gr alkaloid / kg of feed (100 %), followed by treatment B (70.59 %) and treatment D (35.29 %), and the smallest RPS value was gained in treatment A (23.53 %).

5. References

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