Potency of jellyfish alkaloid (Aeginura sp) on kidney histopathology and relative percent survival (RPS) of tiger grouper (Epinephelus fuscoguttatus) against Vibrio harveyi

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Abstract. The purpose of this research were to determine the effect of alkaloid jellyfish compounds on kidney histopathology and to know the best doses to the Relative Percent Survival (RPS) of tiger grouper. The method of this research was descriptive and treatment with Completely randomized design. The treatment of active alkaloid compound on feed was given for 28 days. And then challenge-tested to $10^7$ cells.ml$^{-1}$ of Vibrio harveyi into the media for one day. Using alkaloids compound of Aeginura sp. on the feed with the doses of 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; Alkaloid D = 1.25 g / kg feed. The result of the scoring shows that the 4 treatment with given alkaloid dosage are able to decrease the damage level on the tissues of the fish’ kidneys. The average total damage of treatment C =1gr Alkaloid dosage is found to have the lowest value compared to another treatment with the percentage damages of 13.47% which is categorized low. Those results show better value than the infected fish without any medication with total damage of 42.27% within the medium category. The best RPS was found at a treatment of C with the value of 100%. For further study, it was suggested using a dose of 1 g alkaloid / kg feed for the best result. It was proved by an absent of the damage on kidney and the value of RPS reached 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, a common disease caused by Vibrio car-chariae,Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio harveyi and is one of the most serious problems in various stages of grouper culture. fish farming is the mortality rate of the seeds up to 99% which mainly caused by pathogenic bacteria infection [1].

The Rate of Vibrio harveyi outbreak in tiger grouper hatchery fish can be calculated within a few hours. Vibrio sp attack can lead to the destruction of organs in fish, and the wounds on the skin[2]. To control the disease, particularly bacterial diseases, various types of antibiotics have been used such as
chloramphenicol, and erythromycin and oxytetracycline. But apparently a lot of antibiotics raise the resistance of new bacteria strains in the response to disease [3,4]. Thus it is necessary to control the disease using natural materials, which are still limited to saponins and rotenon, so it needs a new breakthrough in the utilization of jellyfish that are environmentally friendly as immunostimulants [1,5].

The jellyfish is included in Phylum *Colenterata*, both poisonous and edible, possess bioactive compounds to prevent disease in fish and shrimp. Bioactive compounds derived from jellyfish ranges from alkaloids, phenolics, steroids, and terpenoids, showed a strong activity against bacteria in fish with a bigger diameter of resistance compared to chemicals and antibiotics [6]. Research on jellyfish is uncommon despite of its potential utilization. [7] stated that jellyfish *Psalid* sp can inhibit the growth of bacteria on shrimp larvae. There are highly diversity of jellyfish species with potential immunostimulant, not just the jellyfish (*Psalis* sp), but also *Gonionemus* sp, *Obelia* sp, *Hydra* sp, *Pennaria*, *Aurelia* sp., *Aeginura* sp, *Bougainvillia* sp, and others [8].

Based on previous findings, it is necessary to explore unconventional natural resources in order to get the benefits of bioactive compounds from the jellyfish as immunostimulant, particularly for tiger grouper seed. Our studies that evaluated effects of different fish feed on alkaloid jellyfish to kidney changes pathologies and it can increase Relatif Persen Survival.

2. Material and methods

Extraction of jellyfish (*Aeginura* sp) alkaloids using the modified method of Maldoni. Isolation of alkaloid relates to the method that was developed by [9]. The filtered of jellyfish (250 gram) extracted with petroleum benzene. The process was repeated 4 time. Four day later, the extract was evaporated in vacuo at a temperature 40\(^\circ\) C. The resulting extract as melted Na\(_2\)SO\(_4\), filtered and evaporated to 10 ml volume extract klorofom. After settling, the sediment of extract klorofom that contain alkaloid was separated by uses Thin Layer Cromatography (TLC), spectroscopy UV, Infrared spectroscopy (IR) and Nuclear Magnetic Resonance Hydrogen (\(^{1}\)H-NMR).

The method used in this research was description and experimental method with Completely Randomized Design (RAL). Using alkaloid of *Aeginura* sp on the feed given with doses, K= 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 ; D= 1,25 g alkaloid /kg feed. It was challenging test with *Vibrio harveyi* of 10\(^7\) cell.ml\(^{-1}\) bathing for one day. The tested fish used were Tiger grouper (*Epinephelus fuscoguttatus*) 7-8 cm in size at the age of D 90. The fish was kept in a glass tank with a volume of 15 liters (5 levels of treatment), with the density of 10 fish per tank. The kidney sample for histopathology was taken after day 28 of immunostimulant and after infection bacterial on Days 35. The preparation of kidney histology use [10,11] methods. And for RPS to record initial grouper fish (day 1) and at the end of the study (days 35).

3. Results and discussion

3.1. Characterization of alkaloids *Aeginura* sp

3.1.1 Characterization of spectroscopy UV

Base on KLT preparative of kloroform:metanol with comparison 2 : 8. It used this dilution KLT of preparative for the analyses of spectroscopy UV, Infrared and H’NMR. The characterization molecule by UV give result of spectra, absorbance data and maximum wavelength from molecular jellyfish at Table 1 and Figure 1.

The qualitative result that chlorofom extract absorption showed of strong wavelength about 239,4 nm and wavelength at 284,8 nm. Indicate of kromofor with electronic transition n →\(\pi^*\).
Table 1. Maximum wavelength UV

| Species      | \(\lambda_{\text{maks}}\) (nm) | \(A_{\text{maks}}\) |
|--------------|---------------------------------|---------------------|
| *Aeginura sp*| \(\lambda_1 = 239.4\)         | \(A_1 = 0.2002\)   |
|              | \(\lambda_2 = 284.8\)         | \(A_2 = 0.0406\)   |

Figure 1. Spectra UV *Aeginura* sp

Transition can relate to carbonyl bond (C=O) or C=N and N-C=C  alkaloid characteristic. Based on analysis of molecular in chloroform extract were molecule not saturated contain aromatic nucleus and heteroatom, carbonyl of C=N was character alkaloid.

3.1.2. Characterization with infrared spectroscopy

The spectroscopy infrared used to support data of spectra H’-NMR in determined chemical structure of alkaloid extract. Result identify infrared spectroscopy indicate that analysed molecular contain some function chain which is the each the function chain give absorption band at specific area as Figure 2.

Figure 2. Spektra IR molecular of isolat *Aeginura* sp.

Band absorbance appearance at area 3441,32 cm\(^{-1}\) show the existence of vibras stretch from function bond of O-H. Vibras stretch N-H of primary amine and of secunder give absorption at wave number area 3750-3000 cm\(^{-1}\). Possibility of molecular function chain of alkena in system of aromatic supported from absorbance band appearance in wave number 3022,73 arising out cm\(^{-1}\) from existence
vibrasi stretch C-H. System of aromatic in analysed molecular is also supported of area 1714.87 cm$^{-1}$. According to [12] that hydrocarbon of aromatic at wave number area 2000-1650 cm$^{-1}$.

The ring of pirimidin is molecular supported by absorbance at area 1643.50 cm$^{-1}$. Chain of C-N at wave number area 1280.85 cm$^{-1}$ and 1360.10 cm$^{-1}$. Vibrasi stretch C=N will give absorbance at wave number 1689-1471 cm$^{-1}$.

3.1.3. Characterization with spectroscopy H’-NMR
Spectroscopy H’-NMR is technique determination of molecular structure giving environmental information of hydrogen atom chemistry, amount of hydrogen atoms in every function chain and environment which nearby every hydrogen atom [13]. Following Figure 3. H’NMR spectra.

Figure 3. Spektra H’-NMR of isolat Aeginura sp.

Absorption on area friction of chemistry $\delta$ 5.372 ppm estimate with equivalent integration relative by 1 hydrogen atom show indicate of proton which H chain atom O in hydroxide bond (OH). Absorption at $\delta$ 4.313 ppm which forming very typical multiplet for the chain of alcohol bond of metilena (CH$_2$). On 8.1 – 8.3 ppm absorption from pirimidin substitusi with atom N.

The result of analysis with a spectroscopy H’-NMR supported by spectroscopy UV and infrared (IR), the molecular structure of the alkaloid contained in the chloroform extract is shown in Figure 4. 3.1. Characterization of alkaloids Aeginura sp

The result of analysis with a spectrophotometer H’-NMR spectrum supported by ultraviolet (UV) and infra-red spectrum (IR), the molecular structure of the alkaloid contained in the chloroform extract is shown in Figure 4.

Figure 4. Chemical structure of alkaloid substance from Aeginura sp. -(1-metil-1-fenil nonil)-3-metanoil pirimidin
3.2 Kidney histopathology

Based on the results of the study, the condition of the tiger grouper's kidney after being given an alkaloid immunostimulant showed a normal histological form with the appearance consisting of [10], [14], [15] were (1) bowman capsule (2) glomerulus (3) Tubulus proximal. (B) After tested challenge with Vibrio kidney damage such as (1) cloudy swelling of epithelial cells (2) Nekrosis. Samples were taken from all three parts of Figure 5.

![Figure 5](image)

**Figure 5.** (A) Normal cross section of the kidney after an alkaloid immunostimulant consisting of (1) bowman capsule (2) glomerulus (3) Tubulus proximal, (B) After tested challenge with Vibrio kidney damage such as (1) cloudy swelling of epithelial cells (2) Nekrosis, (3) Vacuolation, 400 x HE, (bar = 100 μm)

| Treatment                  | Average scoring type of damage (%) | Total damage | Level of Damage |
|----------------------------|-----------------------------------|--------------|-----------------|
| Infected fish              | Cloudy swelling 11.67 Vacuolatio 3.67 Necrosis 42.27 | 42.27 | Medium |
| A(0.5 gr Alkaloid/kg feed) | 9.06 5.39 8.05 22.50             | Low          |
| B(0.75 gr Alkaloid/kg feed)| 8.17 5.37 7.17 20.71             | Low          |
| C(1 gr Alkaloid/kg feed)   | 5.33 4.67 3.47 13.47             | Low          |
| D(1.25 gr Alkaloid/kg feed)| 8.33 4.67 3.67 16.67             | Low          |

Figure 5 shows the controlled treatment kidney-attached bacteria. After tested challenge with *V. harveyi* kidney damage such as (1) cloudy swelling of epithelial cells (2) Necrosis, (3) Vacuolation, Treated A, B, C and D after given alkaloid, low damage in fish kidney. As a result of *V. harveyi* bacterial invasion of the kidney a little cause anatomical changes. Because Alkaloid compounds can damage nucleic acids (DNA and RNA) of bacteria as the basic structure of these alkaloids are
alkylating agents and other substances that react covalently with purine and pyrimidine bases so that they can join to DNA / RNA as well as cut its hydrogen bonding [15],[16].

The scoring on the histopathological state of the fish' kidneys is classified as follows; less than 30% damage is categorized low, damage between 30%-70% is categorized medium, and more than 70% damage is categorized high [17]. The scoring is presented in Table 2.

The result of the scoring shows that the 4 treatment with given alkaloid 0,5 gr, 0,75gr, 1 gr and 1,25 gr dosage are able to decrease the damage level on the tissues of the fish’ kidneys. The average total damage of treatment C =1gr Alkaloid dosage is found to have the lowest value compared to another treatment with the percentage damage of 13,47% which is categorized low. Those results show better value than the infected fish without any medication with total damage of 42.27% within the medium category.

The highest damage is found in the cloudy swelling in which 18.50% found in the infected fish, 5,33-9,06% found in the fish treated A,B,C, and D alkaloid dosage. Necrosis damage follows with the average percentages of the infected fish at 12.10%, fish treated with Alkaloid dosage 0,5 gr , 0,75gr, 1 gr and 1,25 at 3,47-8,05 %.

3.3. Relatif percent survival

The result showed that after immunostimulant all treatments the best RPS tiger grouper treatment test C = 1gr alkaloids / kg of feed was 100%, followed by treatment B of 70.59%, D of 35.29% and the smallest RPS value was treatment A of 23.53%. The average RPS obtained at the end of the study of each treatment with different replicates can be seen in Table 3 below.

| Treatment    | Replicates | Total | SR(%) ±sd | Death (%) ±sd | RPS(%) ±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 | 100±0 |
| 1,25 gr alkaloid | 50 50 100 | 55±0,7071 | 45±0,7071 | 35,29±8,3184 |
| Kontrol       | 10 20 30  | 15±0,7071 | 85±0,7071 |             |

The results of the lowest dose RPS were 23.53%, according to [5] that the main factor determining the influence of a compound depends on the dose and the concentration of the compound itself. because the alkaloids concentration of Aeginura sp jellyfish as immunostimulant ingredients through feeding on tiger grouper fish has not provided an immune response, nor is the dosage too high the immunostimulant effect can not increase immunity, because the fish body is unable to respond to the mechanisms of cellular and humoral response, so that antibodies are not formed and can also cause toxic effects.

Aeginura sp jellyfish sp is a cytotoxic bioactive compound. These toxins can disrupt or destroy cell membranes of bacteria, which can disrupt transport of compounds in and out of cells. However, the results of this study were better than the other treatment.[18] which used 2% + β-glucan 2% immunostimulant nucleolide for 21 days maintenance of 37% RPS in Rainbow Trout after Vibrio anguillarum infection.
4. Conclusion
The result of the scoring shows that the 4 treatment with given alkaloid 0.5 gr, 0.75gr, 1 gr and 1.25 gr dosage are able to decrease the damage level on the tissues of the fish’ kidneys. The average total damage of treatment C =1gr Alkaloid dosage is found to have the lowest value compared to another treatment with the percentage damage of 13.47% which is categorized low. Those results show better value than the infected fish without any medication with total damage of 42.47% within the medium category. Relative Percent The best Survival on treatment C = 1 gr alkaloid / kg of feed was 100% followed by treatment B of 70.59%, D of 35.29% and the smallest RPS value was treatment A of 23.53%.

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Acknowledgments
This research was supported by the project of DP2M PHB. The author would like to thank Ninis, Indri, Nafis, Dina, Anggi, Merry, Firdi, at the Faculty of Fisheries and Marine sciences UB and Esti kurniati and afriandini at Laboratory Organic Chemistry, Faculty of mathematic and Life Sciences, University of Brawijaya Malang East Java, Indonesia