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Isolation and Identification of Jellyfish Alkaloid (*Bougainvillia* Sp.) as Immunostimulant to Profile of Protein and Phagocyte Activity of Tiger Grouper (*Epinephelus Fuscoguttatus*)

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Abstract

Sri Andayani, M. Fadjar, and M. Farid Rahman. 2017. Isolation and Identification of Jellyfish Alkaloid (*Bougainvillia* Sp.) as Immunostimulant to Profile of Protein and Phagocyte Activity of Tiger Grouper (*Epinephelus Fuscoguttatus*). *Aquacultura Indonesiana, 18 (2): 67-71*. The aim of this research was to know the profil protein and phagocyte activity of tiger grouper (*Epinephelus fuscoguttatus*). The method used in this research was experimental method with Completely Randomized Design (RAL) consisting of 5 treatment levels ie. K=control group without giving alkaloids and feeding pellets mixed with Jellyfish alkaloid *Bougainvillia* sp., which were treatment A = 0.5 g, B = 0.75 g, C= 1 g and D = 1.25 g alkaloid/kg of feed conducted in 2 replications. It was challenge tested with *Vibrio harveyi* of 10^7 cfu/mL for 5 days. Blood plasma for protein profile and phagocyte activity was performed after giving immunostimulant on day 28 and after being infected on day 34. The results of the study were: (1) alkaloid characteristic was N-1 Benzylalcohol, 4-Octyl Piperidine) and (2) protein profile after immunostimulant addition resulted in 9 bands and sample after infection showed 7 protein bands. The phagocyte activity increases 11.64% to 80.6%. It is suggested to use 1 g of alkaloid/kg of feed as immunostimulant.

***Keywords**:* Alkaloid isolation and identification; Immunostimulant; Protein profile; Phagocytic activity; *Vibrio harveyi*

Introduction

The intensification of grouper culture has led to numbers of disease outbreaks with an increasing range of pathogens causing them. Vibriosis, a common disease caused by *Vibrio carchariae, Vibrio alginolyticus, Vibrio parahaemolyticus,* and *Vibrio harveyi* is one of the most serious problem in various stages of grouper culture. In fish farming, the mortality rate of the seeds up to 99% which is mainly caused by pathogenic bacteria infection (Harikrishnan *et al.*, 2011).

The rate of *Vibrio harveyi* outbreak in tiger grouper hatchery fish can be calculated within few hours. *Vibrio* sp. attack can cause the destruction of organs in fish and the wounds on the skin (Moriarty, 1997). To control the disease, particularly bacterial diseases, various types of antibiotics have been used such as chloramphenicol, erythromycin and oxytracycline, but apparently a lot of antibiotics raise the resistance of new bacteria strains in the response to disease (Harikrishnan *et al.*, 2010; Hamed, *et al.*, 2003). Thus, it is necessary to control the disease using natural materials, which are still limited to saponins and rotenon, so it needs a new break through in the utilization of jellyfish that are environmentally friendly as immunostimulants (Harikrishnan *et al.*, 2010; Sakai,1999).

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. Various types of antibiotics which may cause resistance have been used to control the disease, especially bacterial disease. Thus immunostimulant, a substance that can improve cellular and humoral non specific immune is used as an alternative (Dugenci, *et al*., 2003). Celluler non specific immune parameter is phagocytosis activity, while non-specific humoral activity is protein profiles.

Materials and Methods

Extraction of jellyfish (*Bougainvillia* sp.) alkaloids using the modified method of Maldoni (1991). The extract was centrifuged and the supernatant was analyzed by Thin Layer Chromatography (TLC), UV Spectrophotometer, Irradiated Red (IR) and Hydrogen Nuclear Magnetic Resonance (H'-NMR).

The method used in this research was experimental method with Completely Randomized Design (RAL) consisting of 5 treatment levels ie. K = control group without giving alkaloids and feeding pellets mixed with Jellyfish alkaloid *Bougainvillia* sp. which were treatment A= 0.5 g alkaloids/kg of feed, B= 0.75 g alkaloid/kg of feed, C= 1 g alkaloids / kg of feed, and D= 1.25 g alkaloid/kg of feed conducted in 2 replications. The test animals used were tiger grouper (*Epinephelus fuscoguttatus*) 7 - 8 cm in size and at the age of day 90. The fish was kept in a water-filled tub with 15 liters volume containing 10 fishes (5 levels of treatment), with the density of 10 fishes per tub. The adjustment or adaptation of fish to pellet feed was done for 2 weeks. The challenge test was done with *Vibrio harveyi* of 10^7 cfu/mL for 5 days. Blood plasma taking for protein profile was performed after giving immunostimulant on day 28 and after being infected on day 34, samples were then analyzed using electrophoresis method (SDS-PAGE). Blood plasma taking for observation of phagocytes activity was observed on day 1 before treatment, day 28 (after immuno-stimulatory administration), on day 30 (after day 1 of infection), day 32 (after infected on day 3) and day 34 (after being infected on day 5).

Results

Characterization of Bougainvillia sp alkaloids

Characterization of spectroscopy

Based on thin layer chomatography (TLC) preparative, the chloroform and metanol ratio used were 2:8. This dilution on TLC was used for the analyses of *spectroscopy* UV, Infrared and H'NMR. The characterization molecule by UV gives spectral results, absorbance data and maximum wavelength from molecular jellyfish (Creswell *et al*., 1985).

The qualitative result of chloroform extract absorption showed a strong wavelength was about 238.8 nm and wavelength at 285 nm which indicate of chromophore with electronic transition n $\rightarrow\pi^*$. Transition can relate to carbonyls bond (C=O) or C=N and N-C=C alkaloid characteristic. Based on analysis of molecular in chloroform extract, not saturated molecule was containing aromatic nucleus and heteroatom and carbonyl of C=N was character of alkaloid.

Characterization with infrared spectroscopy

The spectra copy infrared used to support data of spectra H'-NMR in determined chemical structure of alkaloid extract. Identify result of infrared spectroscopy indicate that analyzed molecular contain some function chain which is the each of the function chain give absorption band at specific area.

Band absorbance appearance at area 3441,32 cm^{-1} show the existence of vibration stretch from function bond of O-H. Vibration stretch N-H of primary amine and secondary amine 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 per cm from existence of vibration stretch C-H. System of aromatic in analyzed molecular is also supported of area 1714.87/cm. According to Silverstein *et al*. (1981) stated that hydrocarbon of aromatic at wave number area was 2000-1650/cm.

The ring of pyrimidin is molecular which supported by absorbance at area 1643.50/cm. Chain of C-N at wave number area 1280.85/cm and 1360.10/cm. Vibration stretch C=N will give absorbance at wave number 1689-1471/cm.

Characterization with spectroscopy H'-NMR

Spectroscopy H'-NMR is technique determination of molecular structure that giving environmental information of hydrogen atom chemistry and amount of hydrogen atoms in every function chain and environment which nearby with every hydrogen atom (Creswell *et al.,* 1982).

Absorption on area friction of chemistry δ 5.372 ppm was estimate with equivalent integration relative by 1 hydrogen atom that indicated of proton which H chain atom O in hydroxide bond (- OH). Absorption at δ 4.313 ppm which forming very typical multiple for the chain of alcohol bond of methylene ($-CH2^-$), were on 8.1–8.3 ppm absorption from pyrimidin substitution with atom N.

The result of analysis with a spectroscopy H'-NMR supported by spectroscopy UV and infrared (IR). As the result of analysis with spectrophotometer H'-NMR spectrum supported by ultraviolet (UV) and infra-red spectrum (IR), the molecular structure of the alkaloid of *Bougainvillia* sp. contained in the chloroform extract is shown in Figure 1.

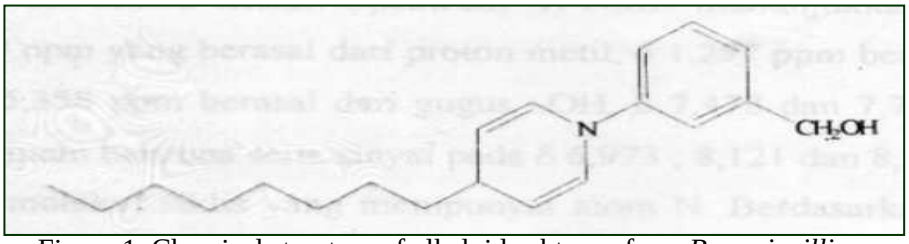


Figure 1. Chemical structure of alkaloid subtance from *Bougainvillia* sp. (N-1 – Benzilalkohol, 4 – Octyl Piperidin)

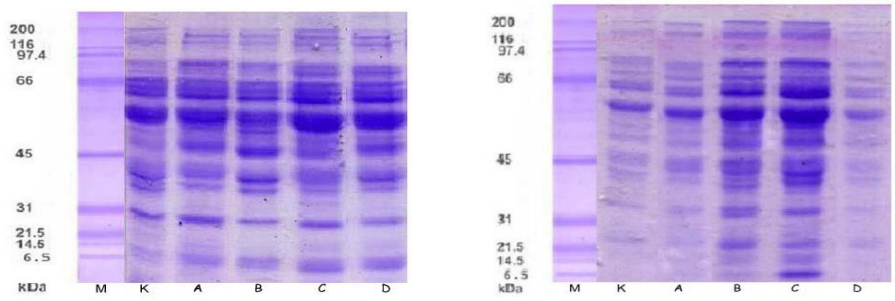
Protein profile

The results of alkaloid influence jellyfish (*Bougainvillia* sp.) were non specific humoral response on tiger grouper before and after being infected by the bacterium *Vibrio.* One of humoral defense mechanism indicator is the number of protein found in fish blood plasma that increased by administering extracts of plants/animals (Dugenci *et al*., 2003). The result protein profil of tiger grouper with electrophoresis technique is described in Figure 2.

Protein profile after immunostimulant addition produced 9 protein bands with molecular weight 168.85 kDa, 140.68 kDa, 90.78 kDa, 58.47 kDa, 37.75 kDa, 29.97 kDa, 15.73 kDa, 12.64 kDa, and 10.15 kDa. In the sample after infection, only 7 protein bands appeared, that was by molecular weight of 170.5 kDa, 86.5 kDa, 65 kDa, 43.88 kDa, 30.87 kDa, 19.3 kDa and 12.13 kDa. Presumably the missing protein is a protein after infection of 12.13 kDa. Apparently these changes are caused by bacterial activity in the fish body post-infection, which affects the damage of certain proteins, causing the protein lost, while the presence of new proteins are synthesized which may function in helping the immune system of fish during bacteria attacks. This finding is supported by Bullock *et al.* (1985) who stated that the thinner bands of plasma protein appeared in infected fish.

Phagocytes activity

The results of alkaloid influence jellyfish (*Bougainvillia* sp.) against non-specific cellular immune response on tiger grouper before and after being infected by the bacterium *Vibrio harveyi* is shown in Figure 3. Data analysis using one-way analysis of variance (ANOVA) found significant in treatment.



I II

Figure 2. Profil of plasma protein in Tiger Grouper with elektroforesis methode 9: (I) before infection (II) after infection, (K) control, (A,B,C and D) treatment by different immunostimulant dosage, and (M) marker with weight molecule kDA

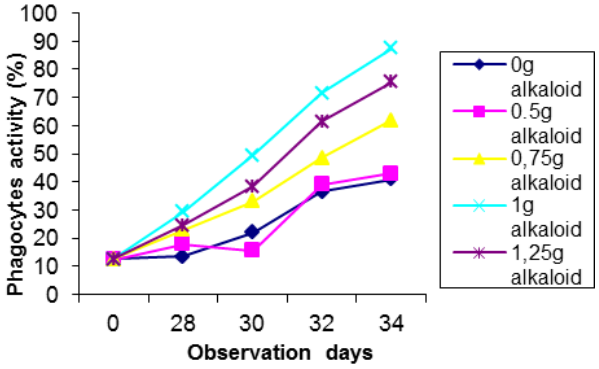


Figure 5. The relationship between phagocytes activity with observation days

Based on this trial, the best dose of alkaloid was 1g/kg of feed, which could increase the percentage of phagocytes activity by 11,6 % on day 28, 49.5% on day 30, 71.5% on day 32, and 80.6% on day 34.

Discussion

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 (Bullock *et al.*, 1985; Jawetz *et al*., 1987). These toxins can damage cell membranes by interfering the transport of compounds in and out of cells, capable of penetrating cell walls and also destroy the existing system in cells. Cytoplasmic membrane that acts on the integrity of cell permeability can also be disturbed by some anti-microbial compounds found in jellyfish that can cause leakage of cell contents. One part of cells most susceptible to the antimicrobial agent is a structural protein as found in the cell walls. After infection, monocytes will move from blood to tissue cells, monocyte cells will mature and become macrophages (Abbas and Lichtman, 2005).

Observations on macrophages showed that the treatment dose of 1 g of alkaloid/kg feed was very effective compared to other doses. The dose increased the number of phagocytes activity of macrophages after the fish was challenged with *Vibrio harveyi*. The significant increase in macrophages can kill pathogens after treatment with immunostimulants (Sakai, 1999; Andayani, 2007). Macrophages play a role in both innate and adaptive immune systems. In innate immunity, macrophages serve as phagocytic cells that eat the pathogens, produce proinflammatory cytokines and ROS production by the NADPH oxidase enzyme as well as RNS by nitric oxide (NO-) synthase. ROS and RNS are also called as oxidative burst that was formed through oxidation process. ROS formed are superoxide anion (O_2-), H_2O_2, HOCl, singlet oxygen (O^-) and hydroxyl radical (OH^-). HOCl is a powerful oxidant of H_2O_2 formed by the MPO enzyme. In adaptive immunity, macrophages act as APCs (antigen presenting cells) that present the antigen that is recognized by lymphocytes (Sahan and Duman, 2010). According to Nakanishi (2004) the ability of macrophages to kill microbial pathogens is a very important process in the mechanism of disease prevention. Providing immunostimulantin the form of alkaloid may increase the production of hydroxyl radicals which are supposed to kill *Vibrio harveyi*.

Conclussions

The chemical structure of *Bougainvillia* sp. is 1-Benzilalkohol, 4 – Piperidinoctyl. The results showed increasing phagocytic activity (from 11.64% to 80.6%). Plasma protein electrophoresis results of SDS PAGE after being given the alkaloid of 9 band and after infected by *Vibrio harveyi* of 7 bands. Bioactive alkaloids of jellyfish increased non-specific immune activity of tiger grouper and can be used as immunostimulants mainly concerned with the role of disease prevention

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