Antibacterial activity of sea cucumber (*Holothuria atra*) against *Pseudomonas aeruginosa*

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**Abstract.** Sea cucumber (*Holothuria atra*) is one of the bioresources that has not been utilized optimally. This marine animal contains potentially bioactive compounds, therefore it can be used as an alternative by fishery products processing industries. This study was aimed to determine the potential of the bioactive compound in sea cucumber and to determine the antibacterial activity of sea cucumber against *Pseudomonas aeruginosa*. Experiments were carried out using diffusion method with concentration 0.50, 0.75, 1 mg/disc. The results showed that methanol extract of sea cucumber contained terpenoids, saponins, and phenolic. The diameter of methanol extract inhibition zone at 1 mg concentration of 12.25±0.05 mm and of three types of fractions used hexane fraction had a more dominant antibacterial potency with diameter inhibition zone at 1 mg concentration of 14.61±0.02 mm. Based on the results of research that the methanol extract and hexane fraction of sea cucumber has potential as an antibacterial.

**Keywords:** antibacterial, bioactive compound, sea cucumber, sea cucumber extract

1. Introduction

Sea cucumber (*Holothuria atra*) is a marine biota that has many benefits. Bioactive compounds in *H. atra* have the potential to be used as antibacterial and antifungal agents. Abraham *et al* (2002) reported that holothurian species such as *H. atra*, *H. scabra*, *Actinopyga miliaris*, and *A. echinites* had potential as antifungal and antibacterial activities of alcoholic extracts because of bioactive substances in its holothurian extracts such as steroidal sapogenins. The methanol-water extract of *H. leucospilota* exhibited antibacterial and antifungal activities at 2000 μg/mL concentration (Adibpour *et al* 2014). Antibacterial potential comes from several compounds including steroids, terpenoids, and saponins. According to Abdallah and Hassan (2012), Echinodermata has been continuing to be studied as a source of bioactive compounds. These compounds are mainly isolated from sea cucumbers and starfish as antitumor, antiviral, anticoagulant, anticancer, antimicrobial and antioxidant.

Sea cucumber *H. atra* contains compounds that act as an antibacterial that can inhibit the activity of pathogenic bacteria. Bioactive compounds can be used as an antibacterial, so they can preserve food, reduce the risk of food poisoning because it can inhibit pathogenic bacteria (Pelczar and Chan 2008).
One of important bioactive compounds in sea cucumber extract that could cause the antibacterial activity is saponins (triterpene glycosides) (Mulyndin and Kovalev 2001). Furthermore, chondroitin sulfate, sulfated polysaccharides, glycoprotein, essential fatty acids, glycosphingolipids, sterols peptides, and glycosaminoglycan (GAGs) as the bioactives could contribute as medical benefits and health functions of extracts obtained from sea cucumber (Bordhar et al 2011).

Pathogenic bacteria such as *Pseudomonas aeruginosa* that can cause disease and spread in various ways. According to Mayasari (2006), *P. aeruginosa* is resistant to several existing antibiotics due to improper used. This study was aimed to determine antibacterial activity of bioactive compounds from sea cucumber against *P. aeruginosa*.

2. Materials and methods

2.1. Materials

The materials used in this research were sea cucumber (*Holothuria atra*) from Cerocok Beach West Sumatera, pure culture of *Pseudomonas aeruginosa* (IPB), nutrient agar (Merck, Germany), nutrient broth (Merck Germany), chloramphenicol (Pharos, Indonesia), methanol (Merck, Germany), n-hexane (Merck, Germany), butanol (Merck, Germany), ethyl acetate (Merck, Germany), and Whatman 1 (Sigma-Aldrich, USA).

The apparatus used were vacuum rotary evaporator (Heidolph WB 2000), laminar airflow (Kleanzone air systems, India), incubator (Isuzu Incubator SSJ-115, Japan), petri dish (Pyrex), inoculation loop, oven (Yamato DV 41).

2.2. Methods

This research was carried out through several stages including extraction by maceration, phytochemical content analysis, and extract fractionation using hexane, ethyl acetate, and butanol, and antibacterial activity assay with concentrations of 0.5:0.75 and 1 mg/disc, and control (Chloramphenicol).

2.2.1. Extraction and fractionation. Extraction was carried out on sea cucumbers *H. atra* using the maceration method (Harborne 2006). A 600 g of sea cucumber macerated with methanol solvent for 3 days with a ratio of 1:2 (w/w). The obtained macerate was concentrated with a vacuum rotary evaporator at 40°C, until a crude extract was obtained. The methanol extract fractionated in stages was started with 200 mL n-hexane solvent. Furthermore, the filtrate of n-hexane fraction and residual fraction were obtained. The n-hexane fraction was separated, residual fraction was then fractionated with 200 mL ethyl acetate to obtain the filtrate of ethyl acetate fraction and residual fraction. The ethyl acetate fraction was separated, residual fraction was then fractionated with 200 mL n-butanol. Then, the filtrate of n-butanol fraction and residual fraction were obtained. The fractionated extract was concentrated with a vacuum rotary evaporator.

2.2.2. Analysis of bioactive compound. The method used for the bioactive compound analysis of sea cucumber extract was qualitatively based on the Harborne method (2006). The bioactive compound was tested to detect the presence of alkaloid compounds, flavonoids, phenols, steroids, and saponins.

2.2.3. Analysis of antibacterial activity. Analysis of antibacterial activity was carried out through a diffusion method based on Sukmiwati et al (2018). Test microbes used by *Pseudomonas aeruginosa*, 100 μL microbial suspension samples were pipetted and put into sterile petri dishes, 12 mL of nutrient agar were added, then the petri dishes were shaken until homogeneous and allowed to solidify. A 10 μL chloramphenicol as a positive control the test solution was dripped onto sterile disc paper, incubated for 18-24 hours at 37°C. Then the antibacterial testing was carried out with sea cucumber
extract samples, hexane fraction extract, butanol fraction extract and ethyl acetate fraction extract with a concentration of 50%, 75%, and 100%, respectively.

3. Results and discussion

3.1. Proportion of sea cucumber *H. atra*

The sea cucumber had body length of 10-20 cm, width of 3-4 cm, and weight of 200-300 grams (figure 1). The body of sea cucumbers in general consists of skin, body mass, stomach content (viscera, gonad, and impurities) (figure 2). The body proportion of sea cucumber *H. atra* can be seen in table 1.

**Table 1. The body proportion of sea cucumber *H. atra*.**

| Parts of sea cucumber                        | Proportion (%) (Whole body of the sea cucumber) |
|---------------------------------------------|-------------------------------------------------|
| Body mass                                   | 39.46                                           |
| Skin                                        | 21.68                                           |
| Stomach content (viscera, gonad, impurities, and water) | 38.86                                           |

*Karnila et al (2011)*

Table 1 shows that sea cucumber *H. atra* had a greater proportion of body mass than that of other body parts. The proportion of sea cucumber *H. atra* was greater than the proportion of sea cucumber *H. scabra*. The stomach was dominated by water. Karnila et al (2011) stated that water and impurities in sea cucumbers consisting of food debris in the digestive tract are part of the body of sea cucumbers. Sea cucumbers have the ability to eat by filtering water and eating sand or sedimentary soil particles and rotten food scraps. As a result, a lot of sand would remain in the food channel.
3.2. Bioactive compound

The bioactive compound analysis was carried out to determine the content of secondary metabolites in sea cucumber *H. atra*. The bioactive compounds of the sea cucumber methanol extract were presented in table 2.

| Bioactive compound | Reagent | Result | Information |
|--------------------|---------|--------|-------------|
| Alkaloid           | Meyer   | -      | Not formed white sediment |
|                    | Dragendorff | -    | White |
| Flavonoid          | Cyanidin test | - | Not formed blue |
| Phenol             | FeCl₃ 1% | +      | Blue |
| Terpenoid          | Liebermann-Burchard | + | Brown |
| Saponin            | H₂O     | +      | Formed foam |

Information: + Detected, - Not detected

*H. atra* sea cucumbers contained terpenoids, saponins, and phenols. Sukmiwati *et al* (2018) reported sea cucumber *Stichopus vastus* extract contained saponins, terpenoids, and steroid compounds. These compounds have been reported to have antibacterial activity. Phenol compounds can damage cell membranes, deactivate enzymes and denaturation proteins decreasing membrane permeability. Changes in permeability of the cytoplasmic membrane disrupts the transportation of important organic ions into the cell, resulting in inhibition of growth and even cell death (Damayanti and Suparjana 2007). The terpenoid compound can inhibit transport across the thicker cell membranes of bacteria because of large polysaccharide groups and some sulfates from most of the triterpene antibacterial agents (Farouk *et al* 2007). Saponin is a class of compounds that can inhibit or kill microbes by reacting with membrane sterols, the main effect of saponins on bacteria is the release of proteins and enzymes from the cells. Therefore, saponins are a class of compounds that are active in inhibiting cell growth (Hardiningtyas 2009).

3.3. Antibacterial activity

The test results of inhibition of antibacterial activity based on inhibition zone diameter of sea cucumber *H. atra* extract, hexane fraction, butanol fraction and ethyl acetate fraction against *Pseudomas aureginosa* bacteria were shown in table 3.

| Extract and Fraction | Inhibitory zone (mm) | 50% | 75% | 100% |
|----------------------|----------------------|-----|-----|------|
| Methanol extract     | 8.58±0.03            | 11.78±0.02 | 12.25±0.05 |
| Hexane fraction      | 12.56±0.03           | 13.46±0.02 | 14.61±0.02 |
| Butanol fraction     | 5.33±0.02            | 6.39±0.02  | 7.85±0.01  |
| Ethyl acetate fraction | 5.26±0.01           | 6.68±0.02  | 7.44±0.01  |
| Control (Chloramphenicol) | 27.06±0.53          | 27.74±0.52 | 32.25±0.43 |

Table 3 shows that the hexane fraction had higher antibacterial activity compared to other fraction with the diameter of the inhibitory zone produced was 14.61±0.02 mm at a concentration of 100%. Sukmiwati *et al* (2018) stated that the maximum inhibitory concentration of *S. vastus* extract against *P. aureginosa* was 10.97±0.00 mm at a concentration of 100% in n-hexane fraction. Aries *et al* (2015) stated that the content of the antibacterial component found in the n-hexane fraction was more dominant than the other fractions, with an average inhibition zone of nearly 13 mm, indicating that the test material in the fraction had the potential to be antibacterial. Roihanah *et al* (2012) reported that n-hexane solvent was the most effective solvent in inhibiting bacterial growth, this is because bioactive
compounds contained in sea cucumbers are easily soluble in non-polar solvents which can act as antibacterial ingredients.

Butanol fraction and ethyl acetate fraction had weak inhibitory antibacterial activity of 7.44±0.01 mm and 7.85±0.01 mm at a concentration of 100% compared to methanol extract of sea cucumber *H. atra* and hexane fraction. Manoppo *et al* (2017) reported that the compounds contained in sea cucumber *H. edulis* extract, chloroform fraction, n-hexane and methanol had antibacterial activity that was less effective or weakly inhibited at around 2.00 to 3.60 mm, but the fraction broad-spectrum, indicating that the content of the compound has antibacterial activity against gram-positive bacteria and gram-negative bacteria.

The difference in antibacterial activity can be influenced by the amount of secondary metabolic content contained in the sample. Pleczar and Chan (2008) stated that secondary metabolites able to lyse erythrocytes to inhibit microorganisms.

4. Conclusions

Sea cucumber *H. atra* extract contained bioactive compounds such as phenols, terpenoids, and saponins. Sea cucumber *H. atra* extract and n-hexane fraction had high antibacterial activity against *P. aeruginosa*.

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