Characterization, antioxidant and antibacterial activity of cultivated sea cucumbers from Bali, Indonesia

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Abstract. Sea cucumber is a marine commodity widely distributed throughout Indonesian waters. Indonesia exported sea cucumber to Hongkong, Japan, Korea, Singapore, Taiwan, Malaysia and Australia. This study aims to characterize sea cucumber, determine the antioxidant, and antibacterial activity of cultivated sea cucumbers. Sea cucumbers are cultivated in the pond for 12 months, with the weight and size average 160.9 grams, and 15.3 cm at harvest. The sea cucumbers were characterized by measuring their proximate and heavy metals content. The samples were extracted using water at 60°C, 70°C, 80°C, and soxhletation with 70% ethanol. Furthermore, the antibacterial analysis was tested on three bacteria, namely \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, and \textit{Escherichia coli} and antioxidants were analyzed using the ABTS method. The results showed that the sea cucumber protein content was 78.77%, fat content was 1.66%, and carbohydrate was 3.80%. Heavy metal content of cultivated sea cucumbers, including As 1.12 mg/kg, Pb 0.15 mg/kg, Ni, Hg, and Cd were not detected. Antibacterial test results showed all the extract of sea cucumber could not inhibit the growth of bacteria. The best antioxidant activity was found in dried sea cucumbers extracted using the soxhletation method with IC\textsubscript{50} value 62.72 μg/mL.

Keywords: antibacterial activity; antioxidant; extraction; sea cucumber; soxhletation

1. Introduction

Sea cucumbers are one of the aquatic commodities that have high economic value and potential for pharmaceutical and nutraceuticals field, especially antioxidants. Some types potential of commercial sea cucumbers are \textit{Holothuria scabra} (sand sea cucumbers), \textit{Holothuria edulis} (black sea cucumbers), \textit{Holothuria vacabunda}, \textit{Holothuria vatiensis} (red sea cucumbers) \textit{Holothuria marmorata} (brown sea cucumbers), and \textit{Stichopus horrens} [1]. Sea cucumbers bioactive compound have been scientifically proven to reduce free radicals and prevent various degenerative diseases caused by excess free radicals. The compound are triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycans (GAGs), phenolics, and essential fatty acids [2].

Research on antioxidants activity of sea cucumber has been carried out, especially sea cucumbers taken directly from nature, Rasyid [3] reported that \textit{Stichopus hermanii} sea cucumbers have potential as antioxidants with an IC\textsubscript{50} value of 65.08 ppm. \textit{Holothuria scabra} has an IC\textsubscript{50} value of 33.77 ± 0.24 mg/mL [4], \textit{Holothuria atra} has antioxidant activity with IC\textsubscript{50} value 126.19 ppm, sea cucumber \textit{Bohadschia vitiensis}, \textit{Stichopus cf. quadrifasciatus}, \textit{Pearsonothuria graeffei}, and \textit{Holothuria atra} were
classified low active as antioxidant with IC$_{50}$ values of 454.28 ± 14.4; 713.51 ± 9.1; 801.57 ± 8.4 and 1535.47 ppm respectively [5]. The high antioxidant activity in sea cucumbers is influenced by the bioactive compounds that found in sea cucumbers, including lectins [6], sterols, triterpene glycosides [7], chondroitin, chondroitin sulfate E [8], sterols [9] vitamins B1, B2, B6, A, C, D, and E, amino acids, fatty acids myristic, palmitic, stearic, docosahexaenoic acid (DHA), eikosapentaenoic acid (EPA), carotenoids, flavonoids and polyphenols [10]. [11] state that the total phenol content is 21.08 ± 0.49; 14.325 ± 0.21; 14.033 ± 0.33 and 10.67 ± 0.12 mg GAE/g sample, and the carotenoid levels were 23.28 ± 0.07; 25.78 ± 0.56; 11.85 ± 0.04; and 41.44 ± 0.008 µmol/g sample.

Beside an antioxidant activity, sea cucumbers also potential as antibacterial properties. Previous research on sea cucumbers as antibacterial showed that the ethyl acetate fraction in sea cucumbers can inhibit the growth of Propionibacterium acnes and Staphylococcus epidermidis bacteria with a minimum inhibitory concentration (MIC) of 62.5 mg/mL, and 125 mg/mL [12]. The study of cultivated sea cucumbers is still limited a lot of study just focus on wild sea cucumber from the sea. currently the Centre for Aquaculture Research and Fisheries (BBRBKPP) - KKP, Gondol, Bali has been able to cultivate sea cucumbers (Holothuria scabra), but the potential cultivated sea cucumber as antioxidants and antibacterial have not investigated yet, thus this study aims to determine the antioxidant and antibacterial activity from cultivated sea cucumber (Holothuria scabra) using several extraction methods.

2. Method
This research was conducted in July - October 2020, at the Laboratory of drug discovery and development, Research centre for biotechnology - LIPI. The raw material was cultivated sea cucumbers from Centre for Aquaculture Research and Fisheries (BBRBKPP) - KKP, Gondol, Bali. Samples were extracted using several methods, namely infusion, water extraction with temperature 60, 70, and 80 ºC, and soxletation using 70% ethanol solvent in wet and dry sea cucumber samples. Then the extracted sea cucumber was concentrated with a rotary evaporator at a temperature of 40 ºC in order to obtain a crude extract. The extract was analyzed for yield, chemical composition [13], antioxidant analysis [14], and antibacterial activity [15].

3. Result and discussion

3.1. Characterization of sea cucumber
The raw material used in this study was cultivated sea cucumber from Centre for Aquaculture Research and Fisheries (BBRBKPP) - KKP, Gondol, Bali. The raw material for sea cucumbers have an average weight of 160.9 grams, average size of 15.3 cm with a harvest age of 12 months. Morphology and characteristics of sea cucumber as raw material can be seen in figure 1 and table 1.
Table 1. Characteristics of cultivated sea cucumber (Holothuria scabra).

| No | Parameter       | Mount               |
|----|----------------|---------------------|
| 1  | Harvest age    | 12 month            |
| 2  | Average size   | 15.3 ± 2.34 cm     |
| 3  | Average weight | 160.9 ± 48.39 gram |

Holothuria scabra was extracted using various methods that can be applied in industry, specifically using infusion method, water extraction using different temperature (60, 70, 80 °C), and soxhlet extraction using ethanol 70%. The extracted filtrate was evaporated using a rotary evaporator. The principle of the rotary evaporator is to evaporate the solvent by rotating the flask as a filtrate container to obtain the extract precipitate. The temperature used in this evaporation is 40 °C so that the bioactive compounds are not damaged. According to Harborne [16] the use of a vacuum rotary evaporator to concentrate the extracted solution with a small volume, preferably using a temperature of 30-40 °C. The yield of the extracted sea cucumber can be seen in table 2.

Table 2. Yield extracted from sea cucumber (Holothuria scabra).

| No | Extraction method                  | Yield   |
|----|-----------------------------------|---------|
| 1  | Infusion                          | 4.11%   |
| 2  | Water extraction on 60 °C         | 3.9%    |
| 3  | Water extraction on 70 °C         | 3.78%   |
| 4  | Water extraction on 80 °C         | 3.72%   |
| 5  | Soxhlet EtOH 70% (dry sea cucumber)| 7.7%    |
| 6  | Soxhlet EtOH 70% (wet sea cucumber)| 5.5%    |

The results of sea cucumber extraction using several methods showed that the highest yield was obtained from soxhlet extraction using ethanol from dry sea cucumber sample of 7.7%, while the wet sea cucumber soxhlet extract was 5.5%, the water extraction using different temperatures 60, 70 and 80°C had no significant effect on the yield of sea cucumbers, which ranged from 2.7-3.9%, while sea cucumbers extracted using the infusion method had yields of 4.11%. This is influenced by the difference in the solvent used. Ethanol solvent is more attractive secondary metabolite components in sea cucumbers than water solvents. Ernawati [17] states that polar solvents such as methanol and ethanol can attract quaternary alkaloid compounds, phenolic components, carotenoids, and tannins, saponins, alkaloids, polyhydroxy steroid.

3.2. Chemical composition of sea cucumber (H. scabra)
The chemical composition of cultivated sea cucumber H. scabra can be seen in table 3. The results showed that cultivated sea cucumber (H. scabra) had 8.22% of water content, 78.77% of protein, 7.55% of ash, 1.66% of lipid, 5.84% of fiber and 3.80% of carbohydrates. The same results showed by Herliyani et al. [18] that the dried wild sea cucumber extract had a high protein content of 79.59%, 9.8% of ash, 0.5% of lipid, and 7.3% of water content. These results corresponding with the SNI standard for dry sea cucumbers (No: SPI-kan/02/29/1987) [19-21]. Standard of dry sea cucumbers have a minimum ash content of 7% and a maximum water content of 20%. The high levels of protein in H. scabra are caused by the sea cucumber's body is composed of collagen. Sea cucumber contains high protein, like other fishery products. Protein is a food substance that is very important for the body because it functions as a source of energy, building blocks and regulators [22].
Table 3. Chemical composition of cultivated sea cucumber (*H. scabra*).  

| No | Parameter       | *H. Scabra (cultured)* | *H. Scabra*| SNI 19,20,21 |
|----|-----------------|------------------------|------------|--------------|
| 1  | Protein (%)     | 78.77                  | 79.59      |              |
| 2  | Ash (%)         | 7.55                   | 9.8        | Min. 7       |
| 3  | Lipid (%)       | 1.66                   | 0.5        |              |
| 4  | Water (%)       | 8.22                   | 7.3        | Max. 20      |
| 5  | Fiber (%)       | 5.84                   |            |              |
| 6  | Carbohydrate (%)| 3.80                   |            |              |

3.3. Antioxidant activity
The bioassay of antioxidant activity of sea cucumbers using the ABTS (Decolorization Assay of Antioxidant Capacity Reaction Pathways) method. The results of antioxidant analysis on several types of sea cucumber extract using the ABTS method can be seen in table 4.

Table 4. Antioxidant activity of sea cucumber (*H. scabra*) extract using ABTS method.

| Extracted                          | Antioxidant activity IC$_{50}$ (ppm) |
|------------------------------------|---------------------------------------|
| Trolux (positive control)          | 5.73                                  |
| Haisom (commercial product)        | 6343.26                               |
| Infusion                           | 665.71                                |
| Water extraction on 60°C           | 991.92                                |
| Water extraction on 70°C           | 1005.80                               |
| Water extraction on 80°C           | 1593.27                               |
| Soxhlet EtOH 70% (wet sea cucumber)| 267.92                                |
| Soxhlet EtOH 70% (dry sea cucumber)| 62.72                                 |

The result of antioxidant activity showed that sea cucumber (*H. scabra*) soxhlet extract on dry sea cucumber had an IC$_{50}$ value of 62.72 ppm and 267.92 ppm on wet sea cucumber. According to Purnomo and Adiono [23] a compound that have strong antioxidant activity if the IC$_{50}$ value is less than 100 ppm, and has moderate activity if the IC50 value is 100-200 ppm. The soxhlet extract in dry and wet samples active as antioxidant with IC$_{50}$ <100 ppm. Therefore, these both of them have potential antioxidants compared to water and infusion extract, water extract with different temperatures 60, 70 and 80°C had an IC$_{50}$ values of 665.71 ppm, 991.92 ppm, 1005.80 ppm respectively, and 1593.27 ppm on infusion extract. The high antioxidant activity of *H. scabra* is influenced by the content of bioactive compounds in the sea cucumber. Pranoto *et al.* [24] stated that sea cucumber *H. scabra* contains flavonoids, alkaloids, saponins, steroids, and titerpenoids. Mierziak *et al.* [25] stated that several flavonoid compounds have antioxidant properties and able to inhibit the activity of the xanthine oxidase enzyme and the superoxide reaction. The results of research on other types of sea cucumbers also show their potential activity as antioxidants, including *H. atra* has antioxidant activity with an IC$_{50}$ value of 562.59 ppm, *S. horrens* has an IC$_{50}$ value of 968.78 ppm [26] *Holothuria atra* has an IC$_{50}$ value of 126, 19 ppm.
[27], *Bohadschia vitiensis*, *Stichopus cf. quadri fasciatus*, *Pearsonothuria graeffei*, and *Holothuria atra* had an IC$_{50}$ values of 454.28 ± 14.4; 713.51 ± 9.1; 801.57 ± 8.4 and 1535.47 ppm respectively [28]. Water extract with different temperatures showed that temperature difference had an effect on antioxidant activity. The increase in temperature cause the IC$_{50}$ value to increase, this condition indicate that the antioxidant activity is getting weak. It happens because high temperature can cause secondary metabolite damage.

3.4. **Antibacterial activity**

The antibacterial activity showed that the sea cucumbers extract using water extraction on different temperature, infusion extract, and soxhlet extract did not have antibacterial activity against three types of bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. This happens because the concentration extract used in the antibacterial activity test was 1000 ppm, it is possible that the inhibition zone is formed at a concentration of more than 1000 ppm so that its activity has not been detected. This argument is in accordance with Oktaviani et al. [29] research which states that *H. atra* are weak in inhibiting the growth of *Vibrio harveyi* bacteria at a concentration of 1000 ppm with the formation of an inhibition zone of 0.41, while with greater concentrations of 10,000 ppm and 100,000 ppm formed a zone of inhibition with a diameter of 7.79 mm and 5.31 mm. this activity is classified moderate active as antibacterial. The antibacterial activity of sea cucumber extract (*H. scabra*) against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* can be seen in table 5.

| Sample                              | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* |
|-------------------------------------|-------------------------|---------------------|-------------------|
| Water extraction on 60 ºC           | -                       | -                   | -                 |
| Water extraction on 70 ºC           | -                       | -                   | -                 |
| Water extraction on 80 ºC           | -                       | -                   | -                 |
| K(control)                          | 45.90                   | 42.75               | 19.20             |
| Soxhlet EtOH 70% (wet sea cucumber) | -                       | -                   | -                 |
| Soxhlet EtOH 70% (dry sea cucumber) | -                       | -                   | -                 |
| Haisom (commercial product)         | -                       | -                   | -                 |
| Infusion                            | -                       | -                   | -                 |

There is no inhibition zone in the sample.

4. **Conclusions**

Cultivated sea cucumber have a high protein content (78.77%). The antioxidant activity of cultured sea cucumbers soxhlet extracted using 70% ethanol has the potential antioxidant activity with IC$_{50}$ values of 62.72 and 267.92 ppm. Cultivated sand sea cucumbers do not have an inhibition zone against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* at a concentration of 1000 ppm.

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