Free radical scavenging activity of five selected sea cucumbers collected from Lampung waters, Indonesia

A. Rasyid1,2, M Y Putra2 and Yasman3

1 Magister Program of Marine Science-Universitas Indonesia, Depok-Indonesia
2 Research Center for Biotechnology-Indonesian Institute of Sciences, Cibinong-Indonesia
3 Research Group of Metabolomics and Chemical Ecology-Universitas Indonesia, Depok-Indonesia

Email: yasman.si@sci.ui.ac.id

Abstract. Sea cucumber is an important raw material for food and remedy in the Eastern region. However, a study focusing on identifying the health benefit of sea cucumbers from tropical waters is still limited. A study on the free radical scavenging activity of five selected sea cucumbers collected from Lampung waters, Indonesia has been done. The objective of this study was to evaluate the free radical scavenging activity of five selected sea cucumbers, namely Stichopus vastus, Stichopus quadrifasciatus, Holothuria (Metriatyla) lessoni, Holothuria (Mertensiothuria) leucospilota, and Bohadschia marmorata. The extraction method which used in this study was the maceration method using methanol solvent while the free radical scavenging activity test is carried out using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. Compared to other species at a concentration of 250 µg/mL, S. vastus, S. quadrifasciatus, H. lessoni and H. leucospilota showed the strongest scavenging activity. While B. marmorata was classified as a very weak scavenging activity. It can be concluded that S. vastus, S. quadrifasciatus, H. lessoni H. leucospilota, and H. lessoni are important sea cucumbers from tropical waters as a source of natural antioxidant agents in the future.

1. Introduction
Sea cucumber is one of the most abundant marine organisms found in the Indo-Pacific region and to be a new source for a wide range of bioactive compounds with pharmaceutical utilization [1]. There are around 1250 existing species of sea cucumber found all over the world [2], and about 100 of them are well known for human consumption [3]. Due to its multiple biological activities, sea cucumber has been widely consumed in China, Japan, Korea, Malaysia, Russia, and Indonesia. Sea cucumber contains high protein, but very low fat and cholesterol content [4].

Marine animals, including sea cucumbers, are among the most important sources of natural products with biological activities because of their secondary metabolites. Many compounds were extracted, purified, identified, and studied for their antioxidant, cytotoxic, anti-inflammatory, and antibacterial effects from sea cucumber [5]. A natural antioxidant found in many sea cucumbers is an important bioactive compound that plays an important role against various diseases and also aging processes through the protection of cells from oxidative damage [6].
However, very few investigations are available on Indonesian sea cucumbers, especially from Lampung waters. The present study aimed to evaluate the free radical scavenging activities of various organic extracts (methanol, n-hexane, ethyl acetate, and n-butanol) of five selected species of sea cucumbers, namely *Stichopus vastus*, *S. quadrifasciatus*, *Holothuria (Metriatyla) lessoni*, *H. (Mertensiothuria) leucospilota*, and *Bohadschia marmorata*.

2. Materials and methods

2.1 Samples collection
Live specimens of the sea cucumbers were collected from Lampung waters, Indonesia. Each sample was dissected to remove the internal organs, and packed immediately with the prior sending to the laboratory, and kept on -20 °C until extracted. The taxonomic identity of the samples was confirmed by ELSA (E-Layanan Sains), Research Center for Oceanography, Indonesian Institute of Sciences.

2.2. Extraction procedure
Five hundred grams of the body wall (wet weight) of each specimen were minced into small pieces and followed by maceration with methanol for three days and stirred daily. The maceration process is then repeated with new methanol until a colorless filtrate is obtained. Solvents were evaporated using a rotary evaporator at a temperature of 40 °C until a viscous extract is obtained. Furthermore, all methanol extracts obtained were analyzed for their free radical scavenging activity.

2.3 Free radical scavenging activity test
Free radical scavenging activity was evaluated through ABTS method as described by [7] with some modification. In brief, a total of 3.8 mg of ABTS powder was dissolved in 1 mL of distilled water. Then 7 mg of potassium persulfate (K_2S_2O_8) powder was dissolved in 10 mL of distilled water. The two solutions were mixed in a ratio of 1:1, then allowed the mixture to stand in the dark at room temperature for 24 h before use. The phosphate-buffered saline (PBS) solution was added until the absorbance of the solution reached 0.7 at a wavelength of 734 nm. After the addition of 1 mL of diluted ABTS solution to 10 mL of antioxidant compounds or Trolox standards in ethanol of PBS the absorbance reading was taken exactly 1 minute after initial mixing and up to 6 minutes. The absorbance was measured at the wavelength of 734 nm using the Tecan microplate reader nanoquant. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times and each separate concentration of standard and samples. The percentage inhibition is calculated and plotted as a function of the concentration of antioxidants and of Trolox for the standard reference data.

The radical scavenging activity was calculated using the following equation:

\[
\text{Scavenging activity (\%)} = \frac{[A(\text{control}) - A(\text{sample})]}{A(\text{control})} \times 100
\]

3. Result and discussion
The taxonomic identity of sea cucumbers used in this study was confirmed, namely *Stichopus vastus*, *S. quadrifasciatus*, *Holothuria (Metriatyla) lessoni*, *H. (Mertensiothuria) leucospilota*, and *Bohadschia marmorata*. The five species of sea cucumbers are known as commercial sea cucumbers in the global market.

The 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is a simple, economical, efficient, and one of the free radical assay methods that have been widely used to estimate the antioxidant activities of food samples. In the specification, the ABTS assay is based on the generation of a blue/green ABTS^+ that can be reduced by antioxidants. The reducing capability of a particular compound might serve as an indicator of its antioxidant activity. A reducing agent (an electron donor) can provide an electron to a free radical, causing the radical to become neutral and the reducing species to obtain protons from the aqueous solution [8].
The radical scavenging activities of methanol from five selected sea cucumbers, namely *Stichopus vastus*, *S. quadrifasciatus*, *Holothuria (Metriatyla) lessoni*, *H. (Mertensiothuria) leucospilota*, and *Bohadschia marmorata* in this study were tested. The results of the analysis in Table 1 shows that with increasing sample concentration, the percentage of inhibition also increases.

**Table 1.** The free radical scavenging activity of the methanol extracts

| No | Species                                | Concentration (µg/ml) | Inhibition (%) |
|----|----------------------------------------|----------------------|----------------|
|    |                                        | 50                   | 15.47          |
| 1  | *Stichopus vastus*                      | 100                  | 34.50          |
|    |                                        | 150                  | 40.84          |
|    |                                        | 200                  | 47.29          |
|    |                                        | 250                  | 56.02          |
| 2  | *Stichopus quadrifasciatus*             | 50                   | 23.53          |
|    |                                        | 100                  | 35.53          |
|    |                                        | 150                  | 39.49          |
|    |                                        | 200                  | 43.64          |
|    |                                        | 250                  | 50.77          |
| 3  | *Holothuria (Metriatyla) lessoni*       | 50                   | 10.10          |
|    |                                        | 100                  | 41.82          |
|    |                                        | 150                  | 55.65          |
|    |                                        | 200                  | 60.85          |
|    |                                        | 250                  | 63.81          |
| 4  | *Holothuria (Mertensiothuria) leucospilota* | 50                   | 8.12           |
|    |                                        | 100                  | 38.84          |
|    |                                        | 150                  | 55.64          |
|    |                                        | 200                  | 68.69          |
|    |                                        | 250                  | 70.98          |
| 5  | *Bohadschia marmorata*                  | 50                   | 0.82           |
|    |                                        | 100                  | 1.04           |
| 6  | Trolox (standard solution)              | 2                    | 16.36          |
|    |                                        | 4                    | 30.94          |
|    |                                        | 6                    | 53.40          |
|    |                                        | 8                    | 76.72          |
|    |                                        | 10                   | 88.52          |

The results showed that in the maximum concentration of 250 µg/mL (Figure 1), the methanol extract of *H. leucospilota* contains the highest level of percent inhibition (70.98%) followed by *H. lessoni*, *S. vastus*, *S. quadrifasciatus* and *B. marmorata* (63.81%, 56.02%, 50.77%, and 29.94%, respectively). A previous study using the DPPH method reported that the free radical scavenging activity of the methanol extract of sea cucumbers from Lampung waters. The maximum concentration of methanol extract (50 mg/mL) showed that the sea cucumbers *Actinopyga lecanora*, *A. miliaris*, *H. impiatens*, *S. hermanni*, *S. ocellatus*, and *S. vastus* had inhibition percentages of 16.38%, 28.36%,
40.13%, 21.83 %, 31.73%, 53.51%, and 45.82%, respectively. Meanwhile, *H. impatiens* (20 mg/mL) and *H. atra* (10 mg/mL) were 40.13% and 21.83% [9]. [10] also reported the free radicals scavenging activity from the methanol extract of sea cucumbers from Lampung waters. The maximum concentration of methanol extract (10 µg/µL) showed that *H. echinata, H. leucospilota, H. scabra,* and *Thelenota ananas* had inhibition percentages of 14.68%, 17.17%, and 11.12%, respectively. Although the sea cucumber samples were collected from the same location (Lampung waters), it seems that all the sea cucumber methanol extracts analyzed in this study showed stronger free radical scavenging activity values.

Several previous studies also reported that sea cucumbers have the potential to scavenge free radicals. [11] reported that the percentage scavenging activity of crude methanol extract by using the DPPH method at 50 µg/mL concentration of the whole body of *H. scabra* was found to be 72%. Other species of sea cucumbers are reported to have free radical scavenging activity, such as *H. parva* [5], *H. edulis* [12], *B. vitiensis, Pearsonothuri graeffei,* and *H. atra* [13].

![Free radical scavenging activity of sea cucumbers](image)

**Figure 1.** Free radical scavenging activity of sea cucumbers

Sea cucumber *S. vastus, S. quadrifasciatus, H. lesson,* and *H. leucospilota* have the potential to scavenge free radicals. While *B. marmorata* was classified as a very weak scavenging activity. It is interesting to study further, at a concentration of 250 µg/mL, it turns out that the methanol and n-butanol extracts of sea cucumbers *S. vastus, S. quadrifasciatus, H. lesson,* and *H. leucospilota* have a percent inhibition value of more than 50%. Similarly, the n-hexane and ethyl acetate extract of *S. vastus, S. quadrifasciatus,* and *H. leucospilota.* While Trolox as the standard solution used in this study has shown 88.52% at the concentration of 10 µg/mL.

From the discussions above, it is interesting to do future studies. We preferred *H. leucospilota* because the crude methanol extract gave the highest activity and wanted to know what compounds were present in the extract. Then *H. lessoni* its crude methanol extract has the second-highest activity and wants to know what compounds are the extract. Furthermore, *S. quadrifasciatus,* which, despite its crude methanol extract had the second-lowest activity after *B. Marmorata,* but what compounds were present in the extract.

Even more interesting to study further, it turns out that not only sea cucumber meat has free radical scavenging activity, but also other body parts. The reported antioxidant activity of seacucumber’
coelomic fluid was found to be the highest in S. badionotus Selenka (58.81%), followed by B. marmorata vitiensis (52.20%) and S. variegatus Semper (47.7%) [14]. The hydrolysed gelatin from sea cucumber Paracaudina chinens var. was reported to have free radical scavenging activity of 29.02 – 75.41% [15]. The free radical scavenging activity of H. scabra’ granular extract was 73.14% at the concentration of 120 µg/mL [16].

Marine invertebrates, especially tropical invertebrates, are protected against oxidative stress caused by their chronic exposure to high levels of solar UV radiation and deleterious reactive oxygen species [17]. This suggests that marine invertebrates such as sea cucumbers could serve as a potential source of antioxidants [18].

4. Conclusion
Sea cucumber Stichopus vastus, S. quadrifasciatus, Holothuria lessoni and H. leucospilota showed the strongest scavenging activity at a concentration of 250 µg/mL. While Bohadschia marmorata was classified as a very weak scavenging activity. It is indicated that S. vastus, S. quadrifasciatus, H. lessoni H. leucospilota, and H. lessoni are important sea cucumbers from tropical waters as a source of natural antioxidant agents for human consumption in the future.

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