

FREE RADICAL SCAVENGING ACTIVITY OF SELECTED SEA CUCUMBER SPECIES FROM MATARAM-LOMBOK, INDONESIA

Ratih Pangestuti*, Tutik Murniasih, Masteria Yunovilsa Putra, Abdullah Rasyid, Joko Tri Wibowo, Ardi Ardiansyah, Febriana Untari

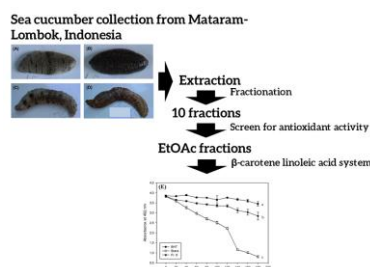
Research Center for Oceanography, Indonesian Institute of Sciences, Jl. Pasir Putih 1, Ancol Timur, Jakarta Utara, 14430, Republic of Indonesia

Article history

Received
5 November 2015
Received in revised form
25 January 2016
Accepted
27 Februari 2016

*Corresponding author
ratih.pangestuti@lipi.go.id

Graphical abstract



Abstract

Sea cucumber is an important food and remedies in Eastern hemisphere. However, less study have been performed to identify health benefit effects of sea cucumber from tropical region. In this study, radical scavenging activity of sea cucumber from Mataram - Lombok [*Holothuria leucospilota*, *Holothuria atra*, *Holothuria fuscocinerea*, and *Holothuria excellens*] were studied. Compared to other species at concentration of 0.1 mg mL⁻¹, *H. atra* extract showed strongest scavenging activity in 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (13.14 ± 2.17) % and absorbance value of β-carotene linoleic acid model at 180 min was (3.32 ± 1,12). *H. atra* extract were further fractionated, and ethyl acetate fractions (fr. 6) significantly inhibit radical activity at 180 min with the absorbance value of 2.84 ± 0.2. It can be concluded that *H. atra* is an important sea cucumber from tropical region.

Keywords: Mataram, Lombok, radical, scavenging, sea cucumber

© 2016 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Sea cucumbers are soft-bodied worm-like marine invertebrates from the class Holothuroidea. They have a leathery skin and an elongated body containing a single branched gonad. These organisms constitute 1 716 species, with the greatest biodiversity being in the Asia Pacific region. Sea cucumber is also known as "teripang or trepang" in Indonesian; "beche-de-me", a French term that means marine food product, and "balate" in Chamorro. They have economic importance in Asian countries specifically in China where several species are used in traditional medicine or eaten as delicacies [1]. Since sea cucumber are living in complex environments submitted to extreme conditions, they must adapt to the new environmental conditions to survive, and produce secondary biologically active metabolites which cannot be found

in other organisms. According to the Ming dynasty report (1368–1644 BC), the sea cucumber harbored the same medicinal properties as the herb ginseng, therefore, it also called as "haishen" which means "ocean ginseng" [2]. In recent years, scientists are heavily employed in searching for bioactive compounds from sea cucumbers to be used as potential drugs in the pharmaceutical industry and as nutraceuticals in the food industry.

Production of oxygen radicals in cellular systems is part of normal metabolism as oxygen is the ultimate electron acceptor in the electron flow system [3]. However, excess productions of the radicals, and electron flow generates uncoupled radical species such as hydroxyl radicals (OH), hydrogen peroxide (H₂O₂), and superoxide anions (O₂⁻) that lead to increase of reactive oxygen species (ROS), which are intimately contribute to the cell damage in aerobic

cells [4]. Specifically ROS is toxic in particular, as it reacts with most organic molecules with extremely high rate constants; attacks cell membranes, proteins and nucleic acids causing strand breakage, depurination/depyrimidation, and chemical modification of the bases or sugar. These oxidations are implicated in the etiology of a wide variety of diseases, including cutaneous fibrosis, chronic inflammation, atherosclerosis, cancer, hypertension, stroke, neurodegenerative, and aging [5,6]. Antioxidants may have a positive effect on human health since they can protect the human body against deterioration by ROS, including singlet oxygen, hydrogen peroxide, superoxide and hydroxyl radicals [7].

In the recent past, bioactive metabolites and extract from sub-tropical sea cucumber have shown significant radical scavenging activities [8,9]. However, less study have been performed to identify the potential uses of tropical sea cucumber. The current study was designed to identify the most active sea cucumber species as radical scavengers. Furthermore, effects of solvent fractions derived from sea cucumber on the in scavenging free radical were also investigated.

2.0 MATERIALS AND METHODS

2.1 Materials

Sea cucumber were collected from Mataram Lombok, Indonesia. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), β -carotene, ascorbic acid, linoleic acid, were purchased from Sigma Chemical Co. Other chemicals and reagents used were of analytical grade commercially available.

2.2 Field Sample

Sea cucumber sample were collected from Mataram-Lombok, Indonesia (08°48.212'S, 116°30.031'E, at 1 m to 2 m depth) on March 2015. The samples were washed with tap water and wrapped with cotton soaked in ethanol. Sea cucumbers were kept at -20 °C prior further analysis.

2.3 Preparation of the Sea Cucumber Extract

Sea cucumbers were chopped into small pieces and analytical grade ethanol was used as solvent for the extraction. The extraction was performed for 3 d at room temperature and repeated for three times. After the extraction, solvent was filtered out and vacuum evaporated to obtain the concentrated sea cucumber ethanolic extract. The yield of each extract was calculated and kept at -20 °C prior further analysis.

2.4 Preparation of the Sea Cucumber Fraction

The most active concentrated ethanolic extract was lipophilized and dissolved in distilled water for further fractionation. The dissolved extract was then fractionated with different solvent (hexane, ethyl acetate, methanol and butanol). The yield of each fraction was calculated and kept at -20 °C prior further analysis.

2.5 DPPH Radical Scavenging Assay

The sea cucumber ethanolic extract or fraction were prepared in different concentrations, ranging from 0.1 mg mL⁻¹ to 1 mg mL⁻¹ for each sample and analyzed in triplicate. 160 μ L of an ethanolic solution or fraction of the tested sample were added to 40 μ L of 50 μ M DPPH solution in 96 well plates. DPPH solution was added and incubated in the dark for 30 min. Absorbance values were read at 517 nm using microplate reader Infinite® 200 PRO (Tecan Austria GmbH). Ascorbic acid are used as are used as reference compounds under the same experimental conditions. Radical scavenging activity was calculated compared with the absorbance of the untreated control group.

2.6 The β -carotene Bleaching Assay

Approximately 5 mg of β -carotene (type I synthetic, Sigma-Aldrich) was dissolved in chloroform (10 mL). The carotene-chloroform solution was pipetted into a boiling flask containing linoleic acid (25 mg, Sigma-Aldrich) and 200 μ L Tween® 40 (Sigma-Aldrich). Chloroform was removed using a rotary evaporator (IKA) at 40 °C for 5 min, and distilled water (50 mL) was added to the residue slowly with vigorous agitation, to form an emulsion. An aliquot (250 μ L) of the β -carotene-linoleic acid emulsion was distributed in each of the 96-wells plates. Methanolic solutions (30 μ L) of the sample extracts and BHT at 1 000 mg L⁻¹ were added. An equal amount of methanol was used for control. The 96 well plates were incubated at 50 °C, and the absorbance was measured at 492 nm using microplate reader Infinite® 200 PRO (Tecan Austria GmbH). Readings of all samples were performed immediately at zero time and every 20 min up to 180 min).

2.7 Statistical Analysis

The data were presented as the mean \pm SD, with three replications. Differences between the means of the individual groups were assessed by one-way ANOVA with Duncan's multiple-range tests. Differences were considered to be significant at $p < 0.05$. The statistical software package SPSS v.16 (SPSS Inc., Chicago, IL, USA) was used for the analysis.

3.0 RESULTS AND DISCUSSION

3.1 Sea Cucumber Identification and Production Yields of Ethanolic Extracts

Sea cucumber were identified at Research Center for Oceanography-LIPI. Sea cucumber collected from Mataram were identified as *Holothuria (Mertensiothuria) leucospilota*; *Holothuria (Halodeima) atra* Jaeger, 1833; *Holothuria (Stauropora) fuscocinerea* Jaeger, 1833; and *Holothuria (Platyperona) excellens* (Ludwig, 1875) (Figure 1). Voucher specimens of each species were deposited in Natural Product Laboratory, Research Center for Oceanography-LIPI.

The production yields of ethanolic extracts from all sea cucumbers are given in Figure 2. The extraction yields of the four sea cucumber extracts ranged from 2 to 6 %. *H. atra* yielded the highest amount of ethanolic extract (5.67 %) compared to other sea cucumber species. The lowest extraction yield was obtained from *H. excellens* (2.69 %). The extraction yields of sea

cucumber ethanolic extracts widely varied depending on the sea cucumber species and extraction solvent. The type of solvent used in this study was ethanol, the extraction yield from *H. atra* was about two fold higher than *H. excellens*. This indicates that ethanol was found to be more effective in extracting *H. atra*, suggesting that *H. atra* body wall was more soluble in ethanol compare to other sea cucumber species used in this study. As compared to the results of the present study, [10] showed considerable variations in extraction yield among different sea cucumber species. They observed significant differences in total phenolic content among different sea cucumber species which also correlate to the total phenols contents. The main sources of sea cucumbers food are phenolic rich materials such as phytoplankton and particles derived from degrading marine macroalgae. It might be assumed that *H. atra* contain more phenolic contents and hydrophobic compounds compare to other species.

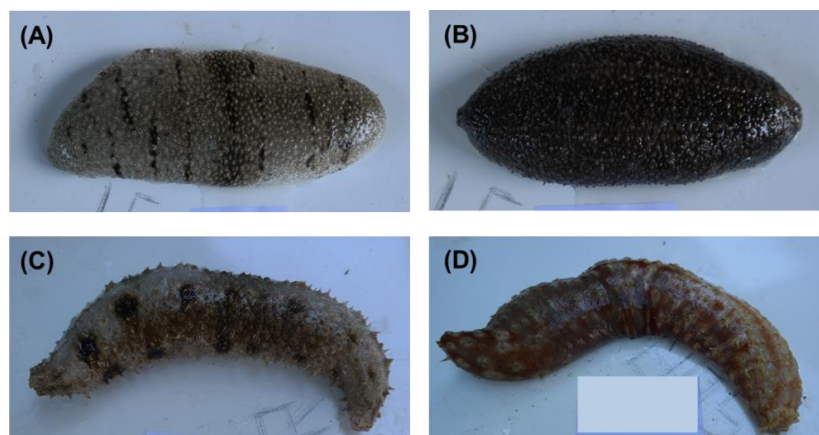


Figure 1. Sea cucumber from Mataram- Lombok. (a) *Holothuria (Mertensiothuria) leucospilota*; (b) *Holothuria (Halodeima) atra* Jaeger, 1833; (c) *Holothuria (Stauropora) fuscocinerea* Jaeger, 1833; and (d) *Holothuria (Platyperona) excellens*

3.2 Radical Scavenging Activity of Sea Cucumber Extracts in DPPH system

DPPH assay is a simple, economical and one of the most widely used methods is to estimate the antioxidant activities of food samples. The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of DPPH as the concentration of the sea cucumber extracts increased, the free radical quenching ability also increased. The DPPH scavenging results (Figure 3) show that *H. atra* and *H. excellens* have significantly ($p < 0.05$) higher free radical scavenging ability than other extracts. However, compared to ascorbic acid at same concentration (0.1 mg mL^{-1}); *H. atra* and *H. excellens* showed lower scavenging activity (3.2 to 1.8

fold lower). Because of different extraction, measurement methods and units used in various antioxidant activity studies on sea cucumber reported in the literature, direct comparison of our results on radical scavenging activity of sea cucumber extracts with other studies is not feasible.

3.3 Radical Scavenging Activity of Sea Cucumber Extracts in β -carotene Linoleic Acid System

Real food generally consists of multiple phases in which lipids and water co-exists with some emulsifier; therefore, antioxidant assay using heterogeneous system such as an emulsion is also required. Heat-induced oxidation of an aqueous emulsion system of linoleic acid was used to estimate the antioxidant activity of sea cucumber extracts. In addition, β -carotene has been used as a target molecule. In the

β -carotene-linoleic acid coupled oxidation model system, the linoleic acid free radical (LOO^\bullet) formed attacks the highly unsaturated β -carotene molecules and in the absence of an antioxidant rapidly bleaches the typically orange color of β -carotene which is monitored spectrophotometrically at 492 nm. With the aim of minimizing side reactions that could give misleading results in this assay, temperature at 50 °C

was chosen. The extent of β -carotene bleaching can be slowed down by the presence of antioxidant or sea cucumber extracts (AH) that donates a hydrogen atom to quench the free radical what results in antioxidant radical (A^\bullet) and lipid derivative (ROOH) formation.

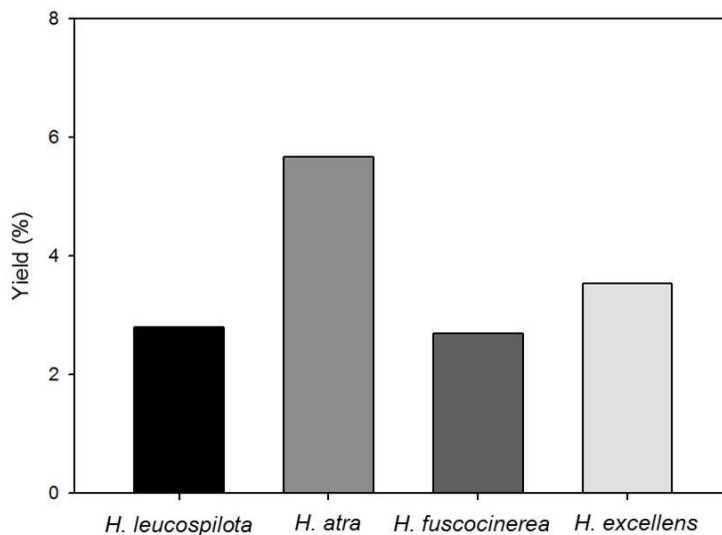


Figure 2 Production yields of ethanolic extracts from sea cucumber

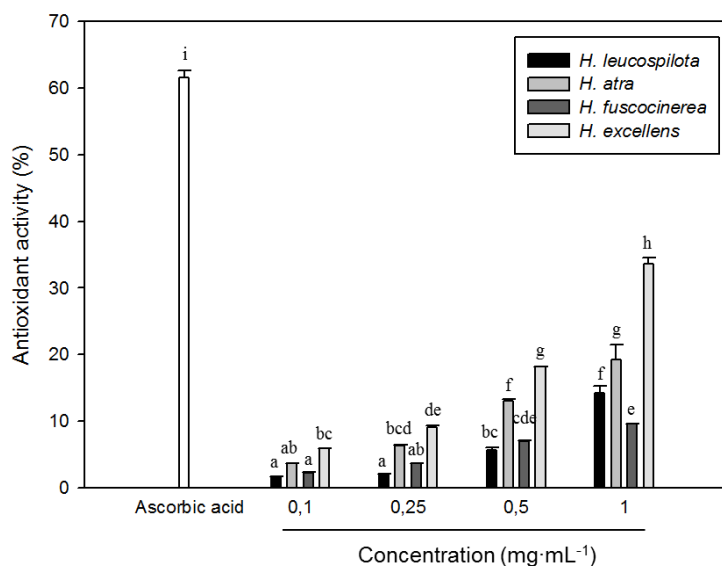


Figure 3 Antioxidant activity of sea cucumber extract defined as inhibition percentage through DPPH assay

The lowest discoloration of β -carotene refers to highest antioxidant activity. Figure 4 and Figure 5 shows the results of this investigation. The total antioxidant activities of 0.1 mg mL⁻¹ crude extracts of *H. atra* and *H. excellens* after 180 min reaction time were (3.32 ± 1.12) and (2.75 ± 0.07), respectively. The *H. atra* had higher antioxidant activity than *H. excellens*. In the

β -carotene-linoleic acid model system, we could conclude that results were consistent with the data obtained from the DPPH test indicating that the mechanisms of action of the extracts for the antioxidant activity may be identical and related to some bioactive compounds. Therefore, in the present study *H. atra* was selected for further purifications.

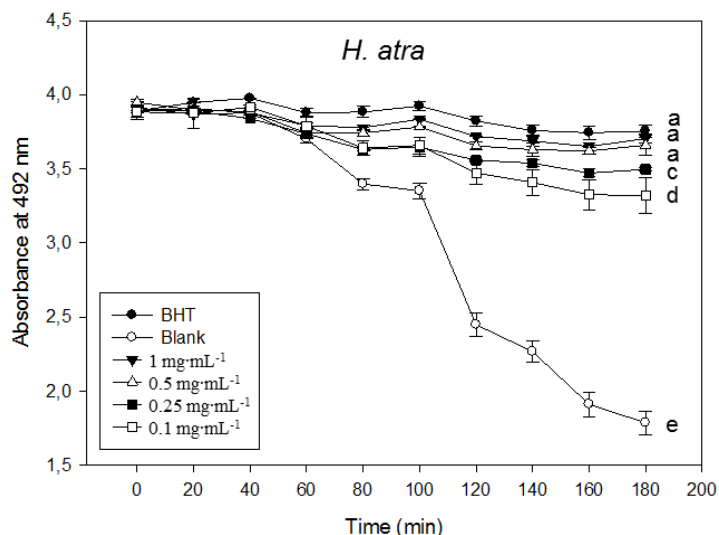


Figure 4 Scavenging activity of *H. atra* extracts in β -carotene linoleic acid systems

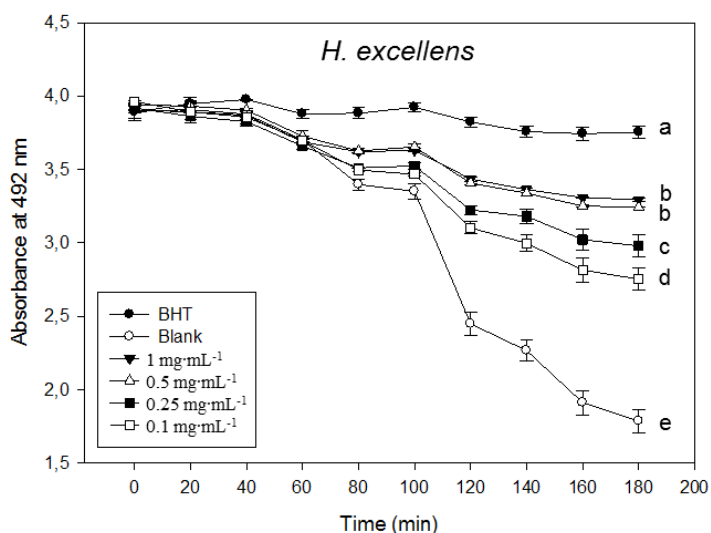


Figure 5 Scavenging activity of *H. excellens* extracts in β -carotene linoleic acid systems

3.4 Radical Scavenging Activity of *H. atra* Fractions in β -carotene Linoleic Acid System

H. atra extracts were fractionated into 10 fractions. Further, antioxidant activities of these fractions were tested by using β -carotene linoleic acid assay. The inhibition ratio of ethyl acetate fraction (fraction 6) at 180 min was found to be the greatest with the absorbance value of 2.84 ± 0.2 , and almost equal to the inhibition capacity of the positive control BHT (Figure 6). The inhibition pattern of other fractions (fraction 2-5; fraction 7-10) were significantly low. Antioxidant properties of natural extracts are generally ascribed to the presence of some bioproducts including phenolic compounds, collagen, polyphenols in different body parts of fresh and processed sea cucumber [9]. Phenolic compounds retain redox properties which allow them to act as

carbohydrates, salt, sugars, carotenoids, ascorbic acid, glutathione, peptides and pigments. The presence of antioxidant activities in sea cucumber species came in support from sea cucumber extracts from Malaysian sea cucumber species. In 2011, [11] reported the higher phenolic content of the ethyl acetate fraction of sea cucumber extracts are responsible for their bioactivities. It is expected that phenolic contents are the bioactive substances in fractions 6. Phenolic compounds, the main class of natural antioxidants, have been shown to be correlated with the antioxidant activities of many extracts. Reports on the polyphenolic composition of sea cucumbers are very rare. However, recent publications demonstrated some studies on quantification and characterization of reducing agents, hydrogen donors, single oxygen quenchers and metal chelators. Sea cucumbers food is mainly composed of phytoplankton and particles of

marine seaweed which are rich in phenolic compounds.

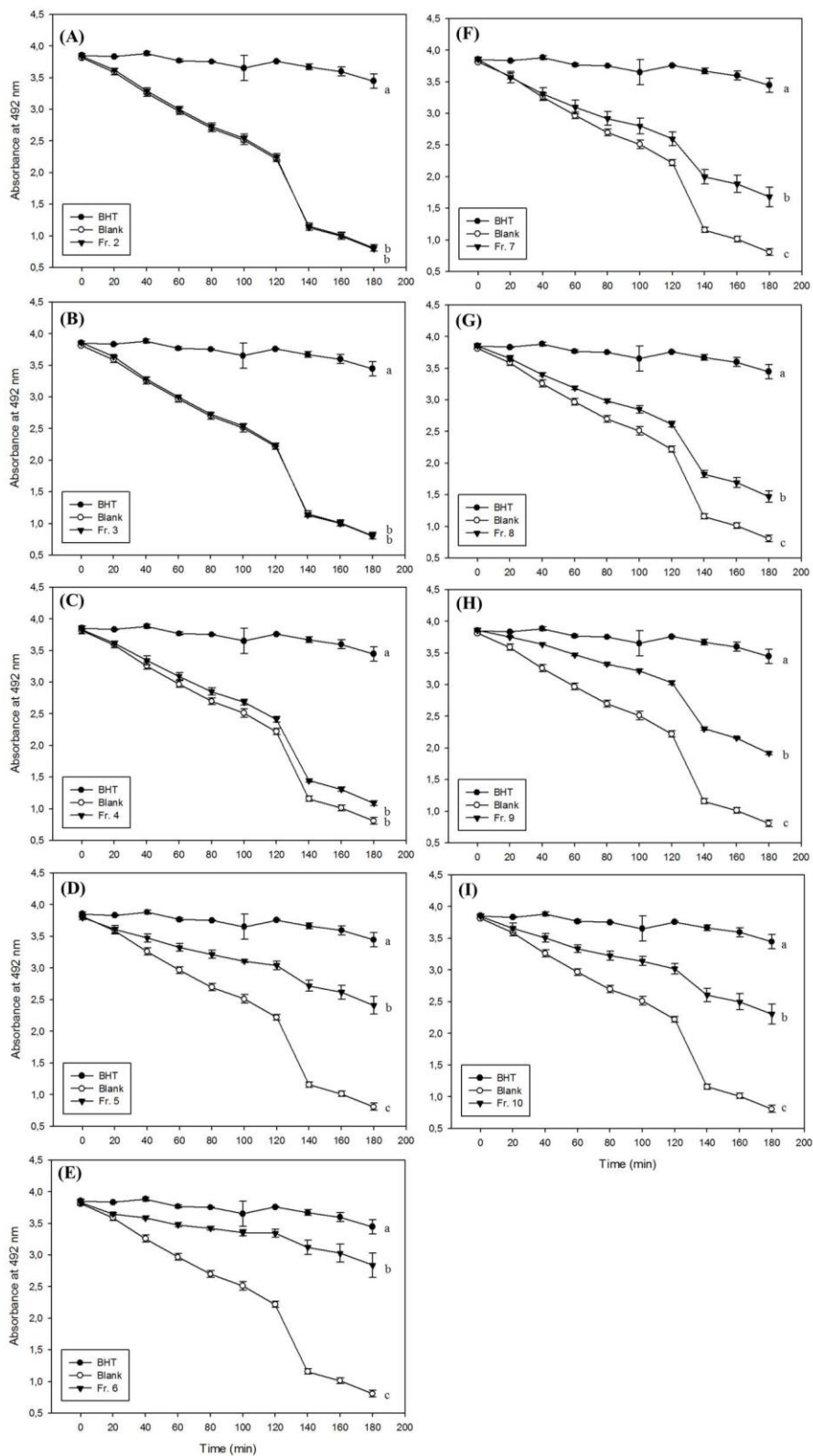


Figure 6 Scavenging activity of *H. atra* fractions in β -carotene linoleic acid systems

4.0 CONCLUSIONS

The ethanolic extracts and fractions of *H. atra* showed potent primary antioxidants as shown by their high radical scavenging capacity (13.14 ± 2.17 %). Finally the authors like to declare that this is the first report of the antioxidant effects of sea cucumber *H. atra* ethyll acetate fraction on β -carotene linoleic acid systems, up to the best of our knowledge. In addition, *H. atra* can be considered as a natural antioxidant and also be purified and used for food and pharmaceutical industries.

Acknowledgement

The authors acknowledge Ismiliana Wirawati for sea cucumber identification. This research was supported by a grant from Excellent Competitive (Riset Unggulan) funded by the Indonesian Institute of Sciences, Republic of Indonesia.

References

- [1] Pangestuti, R., B. Ryu, S. Himaya, and S. K. Kim. 2013. Optimization of Hydrolysis Conditions, Isolation, and Identification of Neuroprotective Peptides Derived from Seahorse *Hippocampus trimaculatus*. *Amino Acids*. 45(2): 369–381.
- [2] Bahrami, Y., W. Zhang, and C. Franco. 2014. Discovery of Novel Saponins from the Viscera of the Sea Cucumber *Holothuria lessona*. *Marine Drugs*. 12(5): 2633–2667.
- [3] Masella, R., R. Di Benedetto, R. Vari, C. Filesi, and C. Giovannini. 2005. Novel Mechanisms of Natural Antioxidant Compounds in Biological Systems: Involvement of Glutathione and Glutathione-related Enzymes. *The Journal of Nutritional Biochemistry*. 16(10): 577–586.
- [4] Lovell, M. A., and W. R. Markesbery. 2007. Oxidative DNA Damage in Mild Cognitive Impairment and Late-stage Alzheimer's Disease. *Nucleic Acids Research*. 35(22): 7497–7504.
- [5] Mendis, E., N. Rajapakse, and S. K. Kim. 2005. Antioxidant Properties of a Radical-scavenging Peptide Purified from Enzymatically Prepared Fish Skin Gelatin Hydrolysate. *Journal of Agricultural and Food Chemistry*. 53(3): 581–587.
- [6] Pangestuti, R., and S. K. Kim. 2013. Marine-derived Bioactive Materials for Neuroprotection. *Food Science and Biotechnology*. 22(5): 1175–1186.
- [7] Himaya, S., B. Ryu, D. H. Ngo, and S. K. Kim. 2012. A Peptide Isolated From Japanese Flounder Skin Gelatin Protects Against Cellular Oxidative Damage. *Journal of Agricultural and Food Chemistry*. 60(36): 9112–9119.
- [8] Zhao, Y., B. Li, Z. Liu, S. Dong, X. Zhao, and M. Zeng. 2007. Antihypertensive Effect and Purification of an ACE Inhibitory Peptide from Sea Cucumber Gelatin Hydrolysate. *Process Biochemistry*. 42(12): 1586–1591.
- [9] Mamelona, J., É. Pelletier, K. Girard-Lalancette, J. Legault, S. Karboune, and S. Kermasha. 2007. Quantification of Phenolic Contents and Antioxidant Capacity of Atlantic Sea Cucumber, *Cucumaria frondosa*. *Food Chemistry*. 104(3): 1040–1047.
- [10] Althunibat, O. Y., R. B. Hashim, M. Taher, J. M. Daud; M. A. Ikeda, and B. Zali. 2009. *In Vitro* Antioxidant and Antiproliferative Activities of Three Malaysian Sea Cucumber Species. *European Journal of Scientific Research*. 37(3): 376–387.
- [11] Himaya, S., B. Ryu, Z. J. Qian, Y. Li, and S. K. Kim. 2011. 1-(5-bromo-2-hydroxy-4-methoxyphenyl) Ethanone [SE1] Suppresses Pro-inflammatory Responses by Blocking NF- κ B and MAPK Signaling Pathways in Activated Microglia. *European Journal of Pharmacology*. 670(2): 608–616.