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Antioxidant Activity of Gamat (*Stichopus variegatus*) and Milk Sea Cucumbers (*Holothuria fuscocinerea*) from the Thousand Islands National Park Waters

Muhammad Septian Azhar Siregar[1*], Eri Bachtiar[1], Atikah Nurhayati[2] and Muhammad Wahyudi Lewaru[1]

[1]Department of Marine Science, Faculty of Fishery and Marine Science, Padjadjaran University, Jl. Ir. Soekarno Km. 21, Sumedang, West Java 45363, Indonesia

[2]Department of Fisheries, Faculty of Fishery and Marine Science, Padjadjaran University, Jl. Ir. Soekarno Km. 21, Sumedang, West Java 45363, Indonesia

*Correspondence : muhammad17028@mail.unpad.ac.id

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Abstract

The frequent use of synthetic antioxidant compounds can cause degenerative diseases, especially among consumers so natural antioxidant compounds are needed to reduce the risk of disease. One of the biotas that may contain potential as natural antioxidants is sea cucumber. Sea cucumbers are marine invertebrates that have therapeutic properties whose bioactive content has the potential as antioxidants. Thus, this study aims to determine the potential of two species of sea cucumber (*S. variegatus* and *H. fuscocinerea*) as natural antioxidant candidates as an alternative to synthetic antioxidants by knowing the bioactive content and analyzing the antioxidant activity of the sample. The research was started from April 1 to September 29, 2021, with methods including sampling in the waters of the Thousand Islands National Park, extraction, antioxidant testing, testing for total compound content, and data analysis on sample extracts of *S. variegatus* and *H. fuscocinerea*. The results of the DDPH antioxidant test and β-carotene bleaching assay after the one-way ANOVA test showed that the antioxidant activity value was $P < 0.05$, which means that there was a significant difference in the sample concentration on antioxidant activity. In the method and -carotene bleaching assay, the sample extracts of *S. variegatus* and *H. fuscocinerea* were 46.37% and 45.75%, respectively. Based on the value of the antioxidant test results and bioactive content, it can be concluded that the sample extracts of *S. variegatus* and *H. fuscocinerea* have very weak antioxidant activity so they cannot be used as alternative natural antioxidants to replace synthetic antioxidants.

INTRODUCTION

Free radicals are highly reactive compounds because they are easy to react and interact oxidatively with other molecules so they can cause adverse effects on the body, including damage to proteins, lipids, DNA, and cell membranes. Free radicals are capable of causing various cell and tissue disorders that result in damage

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or modification of DNA structure (Darya *et al.,* 2020), causing death, abnormalities, and cancer in cells (Young and Woodside, 2001; Sarma *et al*., 2010).

Damage caused by free radicals can trigger degenerative diseases, namely cancer, atherosclerosis, diabetes, and high blood pressure (Inggrid and Santoso, 2014). Free radicals that come from outside or inside the body can cause oxidation processes in cells as the beginning of gout and other diseases. The presence of a group of Reactive Oxidative Species (ROS) which is radical in nature causes the body to need important substances that are able to inhibit radical oxidative compounds, such as antioxidant compounds. Free radical compounds that have a negative effect on the body can be inhibited by antioxidant compounds by transferring one electron to the oxidative radical compound (Werdhasari, 2014).

Antioxidant compounds work by transferring one electron to free radical compounds so that the activity of reactive radical compounds can be inhibited. Antioxidants themselves have a positive effect on human health because they are able to provide defense for the body from free radical damage (Pangestuti *et al.,* 2016). On the other hand, antioxidant compounds have also been used in food product storage activities and are also used to reduce spoilage and rancid odors in feed products. According to Hertrampf and Piedad-Pascual (2003), fish feed exposed to air easily binds to free radical compounds. This causes chemical changes and causes nutrient degradation, and produces a rancid odor that can reduce the attractiveness of the feed. Antioxidants are divided into two parts based on natural and artificial sources, but the use of some synthetic antioxidants is carcinogenic, so it can cause cancer and gene mutations. One type of synthetic antioxidant compound used, such as BHT, has negative side effects, such as potentially toxic and carcinogenic reproductive effects in humans and other organisms (Li *et al.,* 2012; Sodhi *et al*., 2008) stated that pharmaceutical drugs for cancer have a short clinical life. Therefore, natural antioxidant candidate compounds are needed to replace synthetic antioxidants which are carcinogenic. Wijesinghe *et al*. (2013) noted that natural products can be used to treat health problems, such as in the prevention and treatment of cancer. Natural antioxidants can be obtained from land and marine organisms. Based on Darya *et al.* (2020), sea cucumber is a marine product that has antioxidant activity.

Sea cucumbers are marine invertebrates from the phylum Echinoderms that have several compounds containing secondary metabolites that have the potential as candidates for substitute natural antioxidants. In Asian medicinal traditions, sea cucumbers are used by the community as antibacterial, antifungal, anticoagulant, antihypertensive, and immune system boosters (Bordbar *et al*., 2011). One of the functional properties of sea cucumbers is an alternative medicine for cancer (Bandgar *et al*., 2010). The compounds contained in sea cucumbers are scientifically proven to be able to inhibit free radicals and prevent various degenerative diseases caused by oxidative stress in the body. Some of the compounds include triterpene glycosides (saponins), chondroitin sulfate, glycosaminoglycans (GAGs), phenolics, and essential fatty acids (Soltani *et al*., 2014; Rasyid, 2012) stated that the sea cucumber *Stichopus hermanii* has potential as an antioxidant with an IC_{50} value of 65.08 ppm. In another study conducted by Darya *et al.* (2020), the antioxidant activity of *H. scabra* has an EC_{50} value of 33.77 ± 0.24 g/ml.

Based on the statement above, it is suspected that sea cucumbers contain bioactive compounds that have the potential as candidates for natural antioxidants to substitute or alternative to current synthetic antioxidants. Therefore, the authors are interested in investigating the bioactive antioxidant content of sea cucumber extracts *H. fuscocinerea* and *S. variegatus*

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with the aim of knowing the inhibitory activity value of sea cucumber extracts *H. fuscocinerea* and *S. variegatus* against free radicals and the total value of phenolic and carotenoid content that supports antioxidant activity of sea cucumbers *H. fuscocinerea* and *S. variegatus*.

METHODOLOGY

Ethical Approval

The research involving the use of samples of sea cucumber species (*S. variegatus* and *H. fuscocinerea*) from the waters of the Thousand Islands National Park has been approved by the Thousand Islands National Park Office, Jl. Salemba Raya No.9 Lt. III, Central Jakarta, DKI Jakarta, Indonesia.

The collection of sea cucumber samples (*S. variegatus*) and sea cucumber species (*H. fuscocinerea*) was conducted with consideration for the welfare and conservation of the species and in accordance with the regulations and guidelines in place at the Thousand Islands National Park.

The use of sea cucumber species (*S. variegatus*) and sea cucumber species (*H. fuscocinerea*) in this research will be conducted in accordance with animal ethics and welfare principles, ensuring the comfort and health of the animals. Handling of the animals will be carried out by a trained team to minimize potential pain or stress to the animals. The researchers are responsible for the safety and health of the animals used in this research, as well as paying special attention to the conservation of the natural environment and the surroundings of the Thousand Islands National Park.

Place and Time

The research was conducted in March – June 2021 at the Microbiology Laboratory Building 3 and Marine Biotechnology Laboratory Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University and Central Laboratory of Padjadjaran University.

A sampling of gamat sea cucumber (*S. variegatus*) and milk sea cucumber (*H. fuscocinerea*) was carried out on March 2021 in the waters of the Thousand Islands National Park, DKI Jakarta (Lat -6°15'17.5" S, Lon 106°37'6.3" E).

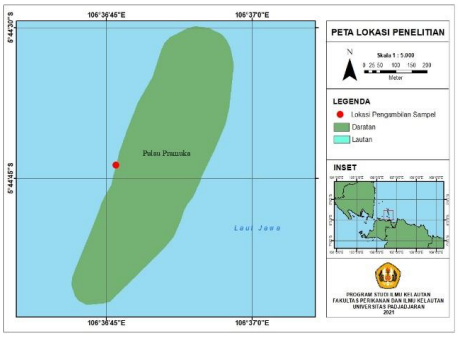

Figure 1. Map of sampling locations for sea cucumbers *S. variegatus* and *H. fuscocinerea* on Pramuka Island, Waters of Thousand Island National Park, Thousand Island, DKI Jakarta.

Research Materials

The solution of DPPH (2,2-diphenyl-1-picrylhydrazil) and β-carotene used as a support in the antioxidant test samples were obtained from the Central Laboratory of Padjadjaran University, Padjadjaran University, Jatinangor.

The sampling and extraction of sea cucumber (*S. variegatus*) and sea cucumber milk (*H. fuscocinerea*) were carried out using research instruments such as Oven (Memmert, Germany), Erlenmeyer flask (Pyrex, United States), and Rotary evaporator (N-1100, EYELA, Japan). The antioxidant assays including the DPPH method,

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total phenolic content, total carotenoid content, and B-carotene bleaching assay were conducted using research instruments such as micropipettes (Eppendorf Research, Eppendorf, Germany), volumetric pipettes (Eppendorf Reference, Eppendorf, Germany), and UV-VIS spectrophotometer (Evolution 201, Thermo Fisher Scientific, United States).

Research Design

The method used in this research is purposive sampling and experimental laboratory. In this research, several stages need to be carried out, starting from the sampling stage, cleaning by separating the meat from the stomach contents of the sea cucumber sample, cutting, drying the sea cucumber sample, simplification, antioxidant test (DPPH method and β-carotene bleaching assay), total content test. phenolic, and total carotenoid content test. The antioxidant test of the DPPH method was carried out using concentrations in the sample extracts of *H. fuscocinerea* and *S. variegatus*, namely 10, 8, 6, and 4 mg/mL (previously, preliminary tests were carried out at concentrations of 5; 2.5; 1.25; and 0.625 mg/mL). mL) (Fawzya *et al*., 2020). The antioxidant test using the β-carotene bleaching assay method used a concentration of 10 mg/mL on the sample extracts of *H. fuscocinerea* and *S. variegatus* (Althunibat *et al*., 2009). Test of total phenolic content (Darya *et al.*, 2020) and carotenoids (Gross, 1991) using a concentration of 10 mg/mL in the sample extracts of *H. fuscocinerea* and *S. variegatus*.

Work Procedure

Extraction

Prior to extraction, three sea cucumbers *H. fuscocinerea* and *S. variegatus* were sampled from the waters of the Thousand Islands National Park, cleaned using clean water, then the guts of the sea cucumbers were removed and cut into cubes. After being cut according to shape and size, samples of sea cucumbers were dried in an oven at 60 °C for 30 minutes (Syahputra *et al.,* 2021). 20 g of dry sea cucumber samples were extracted by maceration method using 96% ethanol with a volume of 1:5 (w/v) for 3 times 24 hours. After filtering, the liquid extract was concentrated using a rotary evaporator at 40 °C. The concentrated extract obtained was evaporated over a water bath until thickened (Hanjaya *et al.,* 2013).

Antioxidant Test Method DPPH

The DPPH free radical inhibition method was used to determine the antioxidant activity of sea cucumber extracts (Brand-Williams *et al*., 1995). Antioxidant testing of *H. fuscocinerea* and *S. variegatus* sample extracts was repeated three times. The extract concentrations of samples were 2, 4, 6, 8, and 10 (mg/mL) (Fawzya *et al.,* 2020). The measurement standard used an α-tocopherol compound with the same concentration as the sample. The concentration of DPPH solution used is 0.76 mM (Brand-William *et al*., 1995). After the sample and standard solutions were reacted with DPPH in a 96-well microplate (160 μL sample + 40 μL DPPH), absorbance measurements were carried out using UV-VIS spectrophotometry at a wavelength (λ) of 517 nm.

The absorbance values obtained from the sample extracts of *S. variegatus* and *H. fuscocinerea* sea cucumbers were included in the calculation of the antioxidant activity value with the following formula (Rasyid, 2012):

$$\% \text{ Inhibition} = \frac{(A - B) - (C - D)}{C - D} \times 100$$

Where:

A = absorbance sample
B = absorbance control sample
C = absorbance control negative
D = absorbance blank

Antioxidant Test Method β-carotene Bleaching Assay

The total antioxidant activity of sea cucumber extract and α-tocopherol was

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measured according to the method of Jayaprakasha *et al*. (2001) with several modifications.

First, it is necessary to form an emulsion reagent for linoleic acid – β-carotene. One milliliter of *β*-carotene solution (0.5 mg/mL) (5 mg β-carotene added with 10 mL chloroform) was pipetted into a round bottom flask (100 ml) containing 25 mg linoleic acid and 200 μL 100% Tween 20. The mixture was then evaporated at 40 °C for 5 minutes using a rotary evaporator to remove chloroform. After evaporation, an emulsion was immediately formed by dilution and vigorous stirring of the mixture with 50 ml of distilled water.

The concentration used in the sample extract of *S. variegatus, H. fuscocinerea*, and standard (α-tocopherol) was 10 mg/mL (100 mg sample was added with 10 mL of ethanol solvent). 250 μL of aliquot emulsion was transferred to a 96-well microplate container containing 30 μL of each sample extract of sea cucumber *H. fuscocinerea* (10 mg/mL), *S. variegatus* (10 mg/mL), standard α-tocopherol (10 mg/mL) as a positive control and chloroform as a negative control. A 96-well microplate containing a mixture of sample extract solution, control, and synthetic free radicals of linoleic acid was incubated in an oven at 50 °C for 180 minutes (3 hours). The absorbance value was measured every 60 minutes starting from minute 0 to minute 180. Measurements used UV-VIS spectrophotometry at a wavelength (λ) of 492 nm.

To obtain the value of antioxidant activity, calculations were carried out using the following formula (Kumaran and Karanukaran, 2006):

$$I_{bleaching}(\%) = \left(\frac{\text{Absorbance after 2 hour test}}{\text{Absorbance intial}}\right) \times 100$$

Where:

$I_{bleaching}$ (%) = Antioxidant activity based on sample resistance to linoleic acid – β-carotene bleaching system

Absorbance initial = Initial absorbance value in the first minute (t=0) of the sample to linoleic acid – β-carotene

Total Phenolic Content Test

The method used to calculate the total phenolic content is using the Folin-Ciocalteau method (Darya *et al.,* 2020). This method uses a folin reagent to measure the total phenolic content of the sample extracts of sea cucumbers used at the extract concentration values that are in accordance with the stock solution used in the antioxidant test (Brand-Williams *et al*., 1995).

The concentration of the sample extracts of *S. variegatus* and *H. fuscocinerea* used was 10 mg/mL (100 mg of sample extract was added with 10 mL of ethanol solvent). The standard used in assisting the calculation is gallic acid with concentrations of 30, 20, 15, and 10 (mg/mL).

To obtain the total phenolic content of the ethanolic extract of the sea cucumber samples *H. fuscocinerea* and *S. variegatus*, 800 μl of Folin-Ciocalteau reagent was needed, 2.0 ml of 7.5% sodium carbonate and 4 ml of deionized water were added to 200 μl of the *H. fuscocinerea* sample (10 mg/mL), *S. variegatus* (10 mg/mL), and gallic acid were then homogenized. After incubation for 90 minutes in the dark at room temperature, the solution turned blue. The absorbance of the mixture was measured at 765 nm using a UV-VIS spectrophotometer. Calibration curves were plotted from standard gallic acid solutions in ethanol at concentrations of 30, 20, 15, and 10 ppm. The gallic acid linear equation was used to calculate the total phenolic content of the sample which was equivalent to gallic acid GAE mg/g. The following equation is used to calculate the total phenolic content.

$y = ax + b$

Where:

y = absorbance value of *H. fuscocinerea* and *S. variegatus* extract samples

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a and b = standard linear equation regression constant comparison (gallic acid)
x = total phenolic content of *H. fuscocinerea* and *S. variegatus* extract samples (mg/mL)

After obtaining the total value of phenolic content, the weight of the extract and solvent used was divided so that the total value of phenolic content was equivalent to gallic acid (GAE mg/g).

Total Carotenoid Content Test

Test of carotenoid levels refers to Gross (1991). Each sample extract of the sea cucumbers was weighed as much as 50 mg using an analytical balance and dissolved in 5 mL of acetone p.a in a test tube to obtain a concentrated solution of *H. fuscocinerea* and *S. variegatus* sample extracts (10 mg/mL). Sample extracts were measured for absorbance at 645 nm, 663 nm, and 480 nm. The absorbance results that have been obtained are calculated to obtain the total value of carotenoid content which is equivalent to the concentration of carotenoids on the weight of the sample extract (Carotenoids mol/g).

The carotenoid content of the sample of sea cucumbers can be calculated using the following formula (Gross, 1991):

$$\text{Carotenoid } \mu\text{mol/g} = \frac{\{A480 + (0{,}114 \times A663) - (0{,}638 \times A645)\} \times V \times 1000}{112{,}5 \times 0{,}1 \times 10}$$

Where:
A 663 = absorbance wavelength 663 nm
A 645 = absorbance wavelength 645 nm
A 480 = absorbance wavelength 480 nm
V = extract volume

Data Analysis

The statistically significant differences in antioxidant values of the DPPH and β-carotene bleaching assay methods were analyzed using analysis of variance (one-way ANOVA). The total phenolic and carotenoid content in the sample extract will be known for its correlation value with the value of antioxidant activity (%Inhibition) DPPH method and β-carotene bleaching assay using Pearson's correlation analysis. Analysis of ANOVA and Pearson's correlation was carried out using a data processing application in Microsoft Excel software. The difference at the 95% confidence level ($P < 0.05$) was considered statistically significant (Darya *et al*., 2020).

RESULTS AND DISCUSSION

Antioxidant Method DPPH

The results of the data obtained were a one-way ANOVA test with a significant degree of 0.05 (5%) the value of $F_{count} > F_{table}$ or 635.9 > 4.6 and the P value of 4.55 x 10-13 which means P < 0, 05. Based on the one-way ANOVA test, a statement was obtained that the concentration of the sample extract with the antioxidant resistance value (%) had a significant difference. Based on Niki (2014), α-tocopherol solution is one of the standard comparisons for a good antioxidant test and is commonly used because of its good properties easily soluble in fat. α-tocopherol solution It is also able to inhibit and limit the entry of peroxide radicals which react to form lipid peroxidation in the body. The results of the same test carried out by Althunibat *et al.* (2009) in the antioxidant test of the free radical compound DPPH, ethanol extract, and water samples of sea cucumbers *H. scabra*, *H. leucospilota*, and *S. chlorontus* have the same low antioxidant inhibition value (IC_{50}) when compared to the comparison standard of antioxidant test. α-tocopherol ranged from 2.13 to 10 mg/ml (Table 1).

Table 1. The results of the Two-Way ANOVA test on the abundance of erythrocytes.

Sample	Antioxidant activity DPPH		Antioxidant activity β-carotene bleaching assay
	Inhibition (%)	IC_{50} (mg/mL)	Inhibition (%)
S. variegatus	51	6.31 ± 0.56	46.37 ± 1.04
H. fuscocinerea	48	10.29 ± 0.67	45.75 ± 0.89

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The table of observations on the antioxidant test of the ethanol extract of sea cucumber samples *S. variegatus* and *H. fuscocinerea* (Table 1), shows that the average value of inhibition against the synthetic radical DPPH is 51% and 46%, respectively. The value of the effective concentration that was able to inhibit 50% of the synthetic radical compound DPPH (IC_{50}) at the concentration of the extract of the gamat sea cucumber and milk samples used (4, 6, 8, and 10 mg/mL) was 6.31 ± 0.56 and 10 respectively 10.29 ± 0.67 mg/mL. Based on the IC_{50} quality standard, the antioxidant ability of the sample used is that the IC_{50} value shown in the antioxidant test results of the ethanol extract samples of *S. variegatus* and *H. fuscocinerea* (Table 1) has an IC_{50} value of more than 0.2 mg/mL so that the value of antioxidant activity includes into the classification of very weak antioxidant abilities. Similar results were shown in research by Fawzya *et al.* (2020), which presented the results of antioxidant tests on hydrolyzed collagen obtained from samples of *S. variegatus* sea cucumber. The antioxidant test results (IC_{50}) obtained are 5.25 ± 0.15 mg/mL. The results obtained indicate that the antioxidants produced are equally weak.

The results of the test of the antioxidant activity value of sea cucumbers are closely related to the content of bioactive compounds contained in their body. The bioactive abilities contained in the body of sea cucumbers can be influenced by several factors. The bioactive content found in sea cucumbers is influenced by ecological factors that are not in accordance with the habitat. According to Esmat *et al.* (2013), the phenolic compounds contained in sea cucumbers come from phytoplankton and macroalgae. Likewise, Mfilinge and Tsuchiya (2016) stated that sea cucumbers consume algae and detritus as a source of phenolic compounds. Sea cucumbers in unfavorable environmental conditions tend to adapt by increasing the secretion of structural and functional bioactive compounds (Rizzo and Giudice, 2018). According to Mondol *et al*. (2017), marine organisms produce various bioactive compounds due to the diversity of biological resources found in the harsh environment including the decline in habitat quality.

The antioxidant activity of the extracts of *S. variegatus* and *H. fuscocinerea* samples of sea cucumbers was very weak due to the low content of phenolic compounds in the extracts of sea cucumbers that support antioxidant activity. According to Alper and Gunes (2020), the phenolic compounds identified were dihydroxybenzoic acid (153,890 g/g extract), gallic acid (133.169 g/g extract), and ellagic acid (109.258 g/g extract).

Antioxidant Method β-carotene Bleaching Assay

The results of the antioxidant test using the *β*-carotene bleaching assay method which was carried out for 3 hours of testing after the ANOVA test was carried out with a significant degree of 0.05 (5%) known for the value of $F_{count} > F_{table}$ or 12.37 > 4.6 and the value of $F_{count} > F_{table}$ or 12.37 > 4.6 and P is 0.003 which means P < 0.05. Based on the one-way ANOVA test, it was found that the extracts of the sea cucumbers sample *Stichopus variegatus* and *Holothuria fuscocinerea* had significantly different inhibitory abilities towards the bleaching system of linoleic acid – *β*-carotene radicals, respectively, the values were 46.37 ± 1.04% and 45.75 ± 0.89%. Antioxidant test results (Althunibat *et al.,* 2009) on *Holothuria sp*. and *Stichopus sp*. using the *β*-carotene bleaching assay method showed that there was antioxidant activity based on the inhibition value of the sample extract against the bleaching system of linoleic acid – *β*-carotene lipid peroxide at the number of 35.92% to 80.58%.

Likewise, research results (Pangestuti *et al.,* 2016) showed a significant difference in the sample extract of *H.*

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atra with a concentration of 0 – 1 mg/mL. Based on the researcher's statement regarding the results of antioxidant test research using the *β*-carotene bleaching assay method on sea cucumber samples, it shows that the extracts of *S. variegatus* and *H. fuscocinerea* sea cucumber samples contain carotenoid compounds that support antioxidant activity, but the value of antioxidant activity is based on the inhibition value (% Inhibition). The results obtained are classified into the category of weak antioxidants (Althunibat *et al.,* 2009; Pangestuti *et al.,* 2016).

The weak value of the antioxidant activity of the sea cucumber sample extract is thought to be due to ecological factors that do not support the habitat in general. Apart from food, there are several factors affecting the phenolic compounds in sea cucumbers. According to Min *et al*. (2015), an increase in CO_2, nitrogen deposition temperature, and drought can increase the production of phenolic compounds, leading to changes in sediment organic matter dynamics. Phenolic compounds are generally used to control the rate of decomposition of sedimentary organic matter to stabilize organic carbon in the ecosystem. Increased ocean acidity can affect the structure and function of phenol chemical compounds. According to Jin *et al*. (2015), ocean acidity increases phytoplankton phenolic production by 46 – 212%, as a source of phenolic which grows under the concentration of CO_2 in the surrounding environment.

Natural and Synthetic Antioxidant

To determine the potential of natural antioxidants in sea cucumbers *S. variegatus* and *H. fuscocinerea*, a comparison of sea cucumbers' natural antioxidant activity values with synthetic compounds (BHT and BHA) was carried out.

Based on the comparison table of natural antioxidants in sea cucumber extracts with synthetic antioxidants, it was found that the antioxidant activity of sea cucumber extracts was lower than that of synthetic antioxidant compounds (Table 2).

Table 2. Comparison of natural antioxidant activity of sea cucumber extract and synthetic antioxidant DPPH method and β-carotene bleaching assay.

Antioxidant	Antioxidant DPPH		Antioxidant *β*-carotene bleaching assay	Reference
	Inhibition (%)	IC_{50} (mg/mL)	Inhibition (%)	
Natural antioxidant				
S. variegatus	51	6.31 ± 0.56		
		5.25		(Fawzya *et al.,* 2020)
H. fuscocinerea	48	10.29 ± 0.67		
H. scabra		> 10	35.92 ± 2.87	(Althunibat *et al.,* 2009)
H. leucospilota		5.44 ± 0.15	55.85 ± 3.38	
S. chlorontus		> 10	73.87 ± 3.04	
H. atra			13.14 ± 2.17	(Pangestuti *et al.,* 2016)
Synthetic antioxidant				
BHT	89.16 ± 1.83	0.02 ± 0.001	98 ± 2	(Ceylan *et al*., 2015)
		0.019	78.78 ± 1.59	(Guangrong *et al.,* 2008)
		0.021		(Ricci *et al*., 2005)
		0.042		(Ricci *et al*., 2005)
		0.086		(Ricci *et al*., 2005)
BHA	80.01 ± 1.78	0.035 ± 0.007	79.15 ± 0.24	(Ceylan *et al*., 2015)

The low antioxidant value in the sea cucumber sample extract is thought to be

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due to the extraction factor. According to Chen *et al.* (2015), the endogenous (enzymatic) antioxidant system can be damaged during processing (particle size reduction and heating), certain materials (salts and organic acids), and storage conditions (presence of oxygen). Sample extracts that still contain salt can reduce antioxidant activity. According to Lee *et al.* (1999), NaCl salt can reduce the activity of antioxidant enzymes catalase, glutathione peroxidase, and superoxide dismutase in carrying out its role as an antioxidant function.

Another factor that is thought to affect the natural sample extract of sea cucumbers has low antioxidant activity compared to synthetic antioxidant compounds is the ability to stabilize heat. The weak value of antioxidant activity in the sea cucumber sample extract is thought to be due to low thermal stability. The antioxidant compound α-tocopherol used as a standard for comparison has antioxidant stability against heat. α-tocopherol compounds have the ability to maintain a constant antioxidant quality at temperatures between 80 °C to 110 °C (Brewer, 2011).

Total Phenolic Content

Based on the observation table (Table 3) on the total value of phenolic content in the samples of *Stichopus variegatus* and *Holothuria fuscocinerea* obtained from the calculation of the equation gallic acid ($y = 0.0395x - 0.11$; $R2 = 0.9923$), it was found that the highest total phenolic content was in the sample of *S. variegatus* followed by *H. fuscocinerea*. The highest total phenolic content was found in the sample extract of *S. variegatus* with a value of 41.43 ± 0.67 gallic acid equivalent (GAE) mg/g followed by the phenolic content of the sample extract of *H. fuscocinerea* with a value of 27.39 ± 0.8 GAE. mg/g. The results of the total phenolic content shown in Table 3 are the phenolic content values obtained from the stock solution extracts of *S. variegatus* and *H. fuscocinerea* samples used for antioxidant assay (10 mg/mL) using DPPH and *β*-carotene bleaching assay methods.

The total phenolic content in aquadest extract and organic solvent (ethanol) in samples of *H. leucospilota, H. scabra*, and *S. chlorontus* sea cucumbers based on research results (Althunibat *et al.*, 2009) has a sequential value of 4.85 – 9.70 GAE mg/g and 1.53 – 2.90 GAE mg/g. According to Mamelona *et al.* (2007), the total phenolic content in samples of sea cucumber body parts (gonads, muscles, and respiratory system) *Cucumaria frondose* varied from 22.5 – 236.0 GAE mg/g dry weight (dw). Based on the statement of the researchers regarding the evidence of the total phenolic content of sea cucumbers, the total phenolic content of the sample extracts of *S. variegatus* and *H. fuscocinerea* obtained was thought to be classified as high phenolic content.

The results of the measurement of the total phenolic content in the sample extracts of *S. variegatus* and *H. fuscocinerea* were tested by Pearson correlation with a significant degree of 0.05 (5%) (Table 4). Based on the analysis, the probability value (P-value) is $P < 0.05$, which means that there is a significant difference between the two variables, namely antioxidants with total phenolic content. For the correlation value (r) obtained in the Pearson correlation test, the antioxidant (barrier) values obtained sequentially by the DPPH method with the total phenolic content of the sample extracts of *S. variegatus* and *H. fuscocinerea*, respectively, are -0.986 and -0.850. Likewise, the results of the correlation between antioxidants (barriers) using the *β*-carotene bleaching assay method with the total phenolic content respectively (-0.896 and -0.999). Based on the correlation value, it was obtained that the total value of phenolic content had a strong relationship with the antioxidant activity of the sample extracts of *S. variegatus* and *H. fuscocinerea*. The good relationship between total phenolic content and the value of antioxidant activity (% Inhibition) is supported by the opinion of

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(Althunibat *et al.*, 2009) regarding research on anticancer and antiproliferative tests of *H. leucospilota*, *H. scabra*, and *S. chloronotus* sample extracts from sea cucumbers. Althunibat *et al.* (2009) show that the presence of total phenol and flavonoid content has an important role in effective antioxidant defense against oxidative stress and degenerative disorders.

The antioxidant activity of the extracts of *S. variegatus* and *H. fuscocinerea* samples of sea cucumbers was very weak due to the low content of phenolic compounds in the extracts of sea cucumbers that support antioxidant activity. According to Alper and Günes (2020), the phenolic compounds identified were dihydroxybenzoic acid (153,890 g/g extract), gallic acid (133.169 g/g extract), and ellagic acid (109.258 g/g extract).

Table 3. Total phenolic and carotenoid content in sample extracts of sea cucumber *Stichopus variegatus* and *Holothuria fuscocinerea*.

Samples	Total Phenolic Content (mg GAE/g)	Total Carotenoid Content (μmol/g)
Stichopus variegatus	41.43 ± 0.67	38.37 ± 0.22
Holothuria fuscocinerea	27.39 ± 0.8	33.64 ± 0.07

Table 4. Correlation of phenolic and carotenoid content to antioxidant activity (DPPH and β-carotene bleaching assay) based on Pearson correlation significant difference 0.05 (5%).

Correlation (r)	Antioxidant DPPH (%)	Antioxidant β-carotene bleaching assay (%)
Total Phenolic Content		
S. variegatus	0.986	0.896
H. fuscocinerea	0.850	0.999
Total Carotenoid Content		
S. variegatus	0,959	0.862
H. fusocinerea	0.793	0.996

Total Carotenoid Content

Based on the test results of total carotenoid content, samples of sea cucumber *S. variegatus* and *H. fuscocinerea* had different carotenoid content values. The highest total carotenoid content was found in *S. variegatus* sea cucumber samples with a value of 38.37 ± 0.22 carotenoids mol/g sample extract, followed by *H. fuscocinerea* samples with a value of 33.64 ± 0.07 carotenoids mol/g sample extract (Table 3).

In the research by Avigail *et al.* (2019), the calculated value and comparison of the highest to lowest carotenoid content was obtained, starting from the sample extract of the sea cucumber *H. atra* 42.84 mol/g sample, *Stichopus* sp. 25.87 mol/g sample, *P. graeffei* 23.33 mol/g sample, and *B. vitiensis* 11.816 mol/g sample. Likewise, Chasanah *et al.* (2016) researched the comparison value of the carotenoids of sea cucumbers *Stichopus* sp. and *Actinopyga* sp. obtained from Karimunjawa waters with the same sea cucumbers obtained from Lampung waters. The carotenoid content in 6 species of sea cucumber is at 8.72 – 42.25 mol/g sample. Based on several studies related to the total value of the carotenoid content of sea cucumbers, the results obtained on the total value of the carotenoid content in the sample extracts of *S. variegatus* and *H. fuscocinerea* were appropriate and thought to have good numbers.

The results of the measurement of the total carotenoid content in the sample extracts of *S. variegatus* and *H. fuscocinerea* were tested for Pearson correlation with a significant degree of 0.05 (5%) (Table 4). Based on the analysis, the probability value (P-value) is P <0.05, which means that there is a significant difference

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between the two variables, namely antioxidants and the total value of carotenoid content. For the correlation value (r) obtained in the Pearson correlation test, the antioxidant (barrier) values obtained sequentially by the DPPH method with the total carotenoid content of the sample extracts of *S. variegatus* and *H. fuscocinerea*, respectively, are -0.959 and 0.793. Likewise, the results of the correlation between antioxidants (barriers) using the *β*-carotene bleaching assay method with the total carotenoid content respectively (-0.862 and -0.996). Based on the correlation value, it was obtained that the total value of carotenoid content had a strong relationship with antioxidant activity in the sample extracts of *S. variegatus* and *H. fuscocinerea*.

According to Supriyono (2008), carotenoid compounds are thought to have a participating role in antioxidant activity. Carotenoids are tetraterpenoid compounds as well as pigments that give living things an orange or red color. Carotene has a beneficial role for health such as antioxidant, enhancing intercellular relationships, and anticarcinogenic. (Miller *et al*., 1996) stated that each carotenoid compound showed different free radical scavenging activity due to the number of functional groups and conjugated double bonds.

The high correlation between the total carotenoid content and the antioxidant activity of the sample extracts of *S. variegatus* and *H. fuscocinerea* sea cucumbers was due to the interaction between lipophilic carotenoid compounds and linoleic acid-free radicals of the same nature. According to Pangestuti *et al.* (2016), the oxidation reaction model of the bleaching system of linoleic acid – *β*-carotene. Radical linoleic acid (LOO·) is formed and reacts with high unsaturated *β*-carotene molecules and in the absence of antioxidants it will accelerate the bleaching of linoleic acid – *β*-carotene. The linoleic acid bleaching system can be slowed down by the presence of antioxidant compounds or compounds in sea cucumber extract that can donate hydrogen atoms to bind to free radical compounds to form antioxidant radicals (A·) and lipid peroxide compounds (ROOH).

CONCLUSION

The results showed that the antioxidant activity of sea cucumber extracts *S. variegatus* and *H. fuscocinerea* based on the values obtained from both methods (DPPH and *β*-carotene bleaching assay) (6.31 mg/mL and 46.37%) and (10.29 mg/mL and 45,75%). For the supporting test, the values obtained from the measurement of the total phenolic and carotenoid content in the sample extracts of *S. variegatus* and *H. fuscocinerea* respectively were (41.43 ± 0.67 GAE mg/g and 38.37 ± 0.22 mol/g) and (27.39 ± 0.8 GAE mg/g and 33.64 ± 0.07 mol/g). Based on the antioxidant activity values obtained from the extracts of samples of natural marine ingredients from sea cucumbers *S. variegatus* and *H. fuscocinerea* are included in the category of weak antioxidants, and natural antioxidants in sea cucumbers cannot replace synthetic antioxidants.

CONFLICT OF INTEREST

The author conducted this research based on personal self-interest, as the author is a member of the research team and hopes that the findings will enhance their reputation as a part of experts in the field of marine science.

AUTHOR CONTRIBUTION

Muhammad Septian Azhar Siregar: Designed and planned the research, conducted data collection, analyzed data, and wrote the manuscript. Eri Bachtiar: Contributed to data collection and data analysis, and provided critical revisions to the manuscript. Atikah Nurhayati: Provided critical input, and composed, and revised specific sections of the manuscript. Muhammad Wahyudin Lewaru: Involved in research design, and data collection, and

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provided critical input in manuscript writing.

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REFERENCES

Alper, M. and Günes, M., 2020. Evaluation of cytotoxic, apoptotic effects and phenolic compounds of sea cucumber *Holothuria tubulosa* (Gmelin, 1791) extracts. *Turkish Journal of Veterinary & Animal Sciences*, *44*(3), pp.641-655. https://doi.org/10.3906/vet-1909-80

Althunibat, O.Y., Hashim, R.B., Taher, M., Daud, J.M., Ikeda, M.A. and Zali, B.I., 2009. In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. *European Journal of Scientific Research*, *37*(3), pp.376-387. https://www.researchgate.net/profile/Muhammad-Taher/publication/279554996_In_Vitro_Antioxidant_and_Antiproliferative_Activities_of_Three_Malaysian_Sea_Cucumber_Species/links/00b4952c7abdac6120000000/In-Vitro-Antioxidant-and-Antipro liferative-Activities-of

Avigail, Y., Yudiati, E. and Pringgenies, D., 2019. Aktivitas antioksidan dan kandungan total fenolik pada teripang di Perairan Karimunjawa, Jepara. *Journal of Marine Research*, *8*(4), pp.346-354. https://doi.org/10.14710/jmr.v8i4.24600

Bandgar, B.P., Gawande, S.S., Bodade, R.G., Totre, J.V. and Khobragade, C.N., 2010. Synthesis and biological evaluation of simple methoxylated chalcone as anticancer, anti-inflammatory, and antioxidant agents. *Bioorganic & Medical Chemistry*, *18*(3), pp.1364-1370. https://doi.org/10.1016/j.bmc.2009.11.066

Bordbar, S., Anwar, F. and Saari, N., 2011. High-value components and bioactives from sea cucumbers for functional foods-A review. *Marine Drugs*, *9*(10), pp.1761-1805. https://doi.org/10.3390%2Fmd9101761

Brand-Williams, W., Cuvelier, M.E. and Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), pp.25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brewer, M.S., 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety, 10*(4), pp.221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x

Ceylan, Y., Usta, K., Usta, A., Maltas, E. and Yildiz, S., 2015. Evaluation of antioxidant activity, phytochemicals and ESR analysis of Lavandula stoechas. *Acta Physica Polonica A*, *128*(2B), pp.B-483-B-488. http://dx.doi.org/10.12693%2FAPhysPolA.128.B-483

Chasanah, E., Fawzya, Y.N., Tarman, K., Januar, H.I. and Nursid, M., 2016. Fatty acid profile, carotenoid content, and in vitro anticancer activity of Karimunjawa and Lampung sea cucumber. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 11*(3), pp.117-124. http://www.bbp4b.litbang.kkp.go.id/squalen-bulletin/index.php/squalen/article/view/269

Chen, L.Y., Cheng, C.W. and Liang, J.Y., 2015. Effect of esterification condensation on the Folin–Ciocalteu method for the quantitative measurement of total phenols. *Food Chemistry*, *170*, pp.10-15. https://doi.org/10.1016/j.foodchem.2014.08.038

Cite this document as Siregar, M.S.A., Bachtiar, E., Nurhayati, A. and Lewaru, M.W., 2023. Antioxidant Activity of Gamat (*Stichopus variegatus*) and Milk Sea Cucumbers (*Holothuria fuscocinerea*) from the Thousand Islands National Park Waters. *Journal of Aquaculture and Fish Health*, *12*(3), pp.390-404.

Darya, M., Sajjadi, M.M., Yousefhadi, M., Sourinejad, I. and Zarei, M., 2020. Antifouling and antibacterial activities of bioactive extracts from different organs of the sea cucumber *Holothuria leucospilota*. *Helgoland Marine Research*, *74*, 4. https://doi.org/10.1186/s10152-020-0536-8

Esmat, A.Y., Said, M.M., Soliman, A.A., El-Masry, K.S.H. and Badiea, E.A., 2013. Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (*Holothuria atra*) against thioacetamide intoxication in rats. *Nutrition*, *29*(1), pp.258-267. https://doi.10.1016/j.nut.2012.06.004

Fawzya, Y.N., Putra, N.A., Witarto, A.B. and Patantis, G., 2020. Golden sea cucumber: Identification and the antioxidant activity of its collagen hydrolysates *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *15*(3), pp.119-129. http://www.bbp4b.litbang.kkp.go.id/squalen-bulletin/index.php/squalen/article/view/511

Gross, J., 1991. *Pigments in Vegetables. Chlorophylls and carotenoid*. An avi Book. Van Nostrdan Reinhold. New York, p.351.

Guangrong, H., Jiaxin, J. and Dehui, D., 2008. Antioxidative and antibacterial activity of the methanol extract of *Artemisia anomala* S. Moore. *African Journal of Biotechnology*, *7*(9), pp.1335-1338. https://www.ajol.info/index.php/ajb/article/view/58671

Hanjaya, S., Darjito and Purwonugroho, D., 2013. Pengaruh pH dan waktu kontak pada adsorpsi Cd (II) menggunakan adsorben kitin terfosforilasi dari limbah cangkang bekicot (*Achatina fulica*). *Jurnal Ilmu Kimia Universitas Brawijaya*, *2*(2), pp.503-509. http://kimia.studentjournal.ub.ac.id/index.php/jikub/article/view/348

Hertrampf, J.W. and Piedad-Pascual, F., 2003. *Handbook on ingredients for aquaculture feeds*. Kluwer Academic Publisher. London. p.569.

Inggrid, H.M. and Santoso, H., 2014. Ekstraksi antioksidan dan senyawa aktif dari buah kiwi (Actinidia deliciosa). Lembaga Penelitian dan Pengabdian kepada Masyarakat, Universitas Katolik Parahyangan, Bandung, *2*, p.43. https://journal.unpar.ac.id/index.php/rekayasa/article/view/1253

Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K., 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chemistry*, *73*(3), pp.285-290. https://doi.org/10.1016/S0308-8146(00)00298-3

Jin, L., Li, D., Alesi, G.N., Fan, J., Kang, H.B., Lu, Z., Boggon, T.J., Jin, P., Yi, H., Wright, E.R., Duong, D., Seyfried, N.T., Egnatchik, R., DeBerardinis, R.J., Magliocca, K.R., He, C., Arellano, M.L., Khoury, H.J., Shin, D.M., Khuri, F.R. and Kang, S., 2015. Glutamate dehydrogenase 1 signals through antioxidant glutathione peroxidase 1 to regulate redox homeostasis and tumor growth. *Cancer Cell*, *27*(2), pp.257-270. http://dx.doi.org/10.1016/j.ccell.2014.12.006

Kumaran, A. and Karunakaran, R.J., 2006. Antioxidant activities of the methanol extract of *Cardiospermum halicacabum*. *Pharmaceutical Biology*, *44*(2), pp.146-151. https://doi.org/10.1080/13880200600596302

Lee, C.M., Boileau, A.C., Boileau, T.W., Williams, A.W., Swanson, K.S., Heintz, K.A. and Erdman Jr, J.W., 1999. Review of animal models in carotenoid research. *The Journal of Nutrition*, *129*(12), pp.2271-2277. https://doi.org/10.1093/jn/129.12.2271

Li, H., Tsao, R. and Deng, Z., 2012. Factors affecting the antioxidant potential

Cite this document as Siregar, M.S.A., Bachtiar, E., Nurhayati, A. and Lewaru, M.W., 2023. Antioxidant Activity of Gamat (*Stichopus variegatus*) and Milk Sea Cucumbers (*Holothuria fuscocinerea*) from the Thousand Islands National Park Waters. *Journal of Aquaculture and Fish Health*, *12*(3), pp.390-404.

and health benefits of plant foods. *Canadian Journal of Plant Science*, *92*(6), pp.1101–1111. https://doi.org/10.4141/cjps2011-239

Mamelona, J., Pelletier, E., Girard-Lalancette, K., Legault, J., Karboune, S. and Kermasha, S., 2007. Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*. *Food Chemistry*, *104*(3), pp.1040-1047. https://doi.org/10.1016/j.foodchem.2007.01.016

Mfilinge, P.L. and Tsuchiya, M., 2016. Changes in sediment fatty acid composition during passage through the gut of deposit feeding Holothurians: *Holothuria atra* (Jaeger, 1883) and *Holothuria leucospilota* (Brandt, 1835). *Journal of lipids*, *2016*, 4579794. https://doi.org/10.1155/2016/4579794

Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M. and Rice-Evans, C.A., 1996. Antioxidant activities of carotenes and xanthophylls. *FEBS Letters*, *384*(3), pp.240-244. https://doi.org/10.1016/0014-5793(96)00323-7

Min, S.Y., Yan, M., Kim, S.B., Ravikumar, S., Kwon, S.R., Vanarsa, K., Kim, H.Y., Davis, L.S. and Mohan, C., 2015. Green tea epigallocatechin-3-gallate suppresses autoimmune arthritis through indoleamine-2, 3-dioxygenase expressing dendritic cells and the nuclear factor, erythroid 2-like 2 antioxidant pathways. *Journal of Inflammation*, *12*(1), 53. https://doi.org/10.1186/s12950-015-0097-9

Mondol, M.A.M., Shin, H.J., Rahman, M.A. and Islam, M.T., 2017. Sea cucumber glycosides: Chemical structures, producing species and important biological properties. *Marine Drugs*, *15*(10), 317. https://doi.org/10.3390/md15100317

Niki, E., 2014. Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. *Free Radical Biology and Medicine*, *66*, pp.3-12. https://doi.org/10.1016/j.freeradbiomed.2013.03.022

Pangestuti, R., Murniasih, T., Putra, M.Y., Rasyid, A., Wibowo, J.T., Ardiansyah, A. and Untari, F., 2016. Free radical scavenging activity of selected sea cucumber species from Mataram-Lombok, Indonesia. *Jurnal Teknologi (Sciences & Engineering)*, *78*(4–2), pp.179-185. https://doi.org/10.11113/jt.v78.8202

Rasyid, A., 2012. Identification of secondary metabolites compounds, antibacterial and antioxidant activities on the methanol extract of sea cucumber *Stichopus hermanii*. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *4*(2), pp.360-368. https://doi.org/10.29244/jitkt.v4i2.7799

Ricci, D., Fraternale, D., Giamperi, L., Bucchini, A., Epifano, F., Burini, G. and Curini, M., 2005. Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (Lamiaceae). *Journal of Ethnopharmacology*, *98*(1-2), pp.195-200. https://doi.org/10.1016/j.jep.2005.01.022

Rizzo, C. and Giudice, A.L., 2018. Marine invertebrates: Underexplored sources of bacteria producing biologically active molecules. *Diversity*, *10*(3), 52. https://doi.org/10.3390/d10030052

Sarma, A.D., Mallick, A.R. and Ghosh, A.K., 2010. Free radicals and their role in different clinical conditions: An overview. *International Journal of Pharma Sciences and Research*, *1*(3), pp.185-192. https://www.ijpsr.info/docs/IJPSR10-01-03-04.pdf

Sodhi, S., Sharma, A., Brar, A.P.S. and Brar, R.S., 2008. Effect of α tocopherol and selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. *Pesticide Biochemistry and Physiology*, *90*(2), pp.82-86.

Cite this document as Siregar, M.S.A., Bachtiar, E., Nurhayati, A. and Lewaru, M.W., 2023. Antioxidant Activity of Gamat (*Stichopus variegatus*) and Milk Sea Cucumbers (*Holothuria fuscocinerea*) from the Thousand Islands National Park Waters. *Journal of Aquaculture and Fish Health*, *12*(3), pp.390-404.

https://doi.org/10.1016/j.pestbp.2007.08.002

Soltani, M., Parivar, K., Baharara, J., Kerachian, A.A. and Asili, J., 2014. Hemolytic and cytotoxic properties of saponin purified from *Holothuria leucospilota* sea cucumber. *Reports of Biochemistry and Molecular Biology*, *3*(1), pp.43-50. http://rbmb.net/article-1-57-en.html

Supriyono, T., 2008. *Kandungan beta karoten, polifenol total dan aktivitas "merantas" radikal bebas kefir susu kacang hijau (Vigna radiata) oleh pengaruh jumlah starter (Lactobacillus bulgaricus dan Candida kefir) dan konsentrasi glukosa*. *Doctoral Dissertation*, Thesis, Universitas Diponegoro, Semarang.

Syahputra, G., Hariyatun, Firdaus, M. and Santoso, P., 2021. Extraction and characterization of collagen from sand sea cucumber (*Holothuria scabra*). *Jurnal Ilmu Pertanian Indonesia*, *26*(3), pp.319-327. https://doi.org/10.18343/jipi.26.3.319

Werdhasari, A., 2014. Peran antioksidan bagi kesehatan. *Jurnal Biotek Medisiana Indonesia*, *3*(2), pp.59-68. http://ejournal2.bkpk.kemkes.go.id/index.php/jbmi/article/view/1659

Wijesinghe, W.A.J.P., Jeon, Y.J., Ramasamy, P., Wahid, M.E.A. and Vairappan, C.S., 2013. Anticancer activity and mediation of apoptosis in human HL-60 leukemia cells by edible sea cucumber (*Holothuria edulis*) extract. *Food Chemistry*, *139*(1-4), pp.326–331. https://doi.org/10.1016/j.foodchem.2013.01.058

Young, I.S. and Woodside, J.V., 2001. Antioxidants in health and disease. *Journal of Clinical Pathology*, *54*(3), pp.176-186. http://dx.doi.org/10.1136/jcp.54.3.176

Cite this document as Siregar, M.S.A., Bachtiar, E., Nurhayati, A. and Lewaru, M.W., 2023. Antioxidant Activity of Gamat (*Stichopus variegatus*) and Milk Sea Cucumbers (*Holothuria fuscocinerea*) from the Thousand Islands National Park Waters. *Journal of Aquaculture and Fish Health*, *12*(3), pp.390-404.