

Potential of Sea Cucumbers as Fuctional Foods

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Abstract

Sea cucumbers have great potential for managing water resources because they have filter feeder properties that can clean water from harmful substances. Apart from that, sea cucumbers also contain active compounds which have properties as functional foods and can help prevent cancer. Samples of sea cucumbers were collected from Karimunjawa, Central Java, and Surabaya. The research included tests for bacteria, chemicals, and heavy metals on dried sea cucumbers, as well as amino acid analysis. Two species of sea cucumbers were identified: *Holothuria atra* and *Stichopus variegatus*. *H. atra* had a higher amino acid content and the highest collagen content among all the amino acids (14,600mg.100g⁻¹). Both types of sea cucumber samples contain amino acids that have anti-inflammatory, antioxidant, and anticancer properties. This is due to their content of saponins, tannins, flavonoids, steroids, and terpenoids, which are beneficial for health. Additionally, *S. variegatus* has been found to have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. The amino acid analysis of *S. variegatus* shows the presence of sulfate and alanine compounds, which are not found in *H. atra*. Research has shown that sea cucumbers can be consumed as supplements or additional food to support health and prevent various diseases, including cancer. The use of sea cucumbers as functional food ingredients also has the potential to manage water resources sustainably. In conclusion, *H. atra* and *S. variegatus* sea cucumbers have great potential for managing water resources for functional food and cancer prevention efforts. Their high content of amino acids and other phytochemical compounds makes them a good choice for development as functional food ingredients.

Keywords: amino acids, anti-bacterial, health, saponin, sea cucumber

Introduction

Sea cucumbers are marine animals that live in warm and tropical waters. They have many benefits and excellent properties for human health. Some of these benefits include being a high source of protein, containing active compounds that have antioxidant, antitumor, and anti-inflammatory effects, as well as being able to boost the immune system (Pringgenies et al., 2018; Danga et al., 2019).

Sea cucumbers have both economic potential and can be processed into various products such as food, medicines, and cosmetics. They also have a high market value in the international market. Sea cucumbers, also known as sea cucumbers, are a type of marine animal that offers numerous health benefits. In some countries, sea cucumbers have been used as food and traditional medicine

ingredients for a long time. They are rich in nutrients and bioactive compounds like collagen, omega-3 fatty acids, minerals, and antioxidants (Pringgenies et al., 2018; Husain et al., 2023).

Currently, sea cucumber is receiving attention in functional food research and efforts to prevent cancer. Many studies have been conducted to uncover the potential of sea cucumber in managing aquatic resources and using it as a food ingredient with additional health benefits. Functional food refers to food that provides extra health advantages beyond basic nutrition. Processed sea cucumbers, such as crackers or other processed foods, are believed to offer similar benefits to raw sea cucumbers. However, there is still a lack of comprehensive research comparing the differences between processed sea cucumbers and sea cucumbers collected directly from the sea in terms of their health benefits and

their effects on cancer prevention. On the other hand, sea cucumbers also serve as marine organisms for cultivation and have antibacterial properties. (Pringgienies *et al.*, 2018; Djunaedi *et al.*, 2021, Pringgienies *et al.*, 2021).

Understanding these differences is important because it can help the community and food producers comprehend the specific health benefits, they can derive from sea cucumbers. Furthermore, this research will enhance our understanding of the potential of sea cucumbers in managing water resources, including conservation and sustainable management efforts.

By gaining a better understanding of the potential of sea cucumbers as a functional food and how they differ from processed sea cucumbers, it is hoped that people will be encouraged to include sea cucumbers in their healthy diets. Furthermore, this research can also contribute to cancer prevention efforts by highlighting sea cucumbers as a food source that is high in bioactive compounds and antioxidants.

This research aims to investigate the potential of sea cucumbers as a functional food by studying sea cucumbers collected directly from the sea. The research results will provide a comprehensive understanding of the potential of sea cucumbers in managing water resources for functional food and cancer prevention. The findings of this study are expected to provide valuable information for the community, food producers, and the scientific community in optimizing the use of sea cucumbers to promote the health and sustainability of water resources.

Materials and Methods

Sea cucumber were collected from the waters of Karimunjawa Islands, Central Java. Sampling was done at night during low tide.

Sample extraction

The sea cucumber samples were cleaned to remove any dirt. Then, they were soaked in fresh water overnight to eliminate salt and parasites that may be attached to their bodies. The sea cucumbers then were dried in a drying cabinet at a temperature of 45 °C for two days (Pringgienies, 2014). The process of sample extraction follows the method described by Pringgienies *et al.* (2018). Each prepared sample was cut into pieces measuring 2-5 cm. The extraction process begins by dissolving 200 g of the prepared sample in 1000 ml of methanol solvent. The mixture was then covered with aluminum foil. The extraction was carried out using the maceration

method. The solvent was replaced every 24 h, with occasional stirring, and this process was repeated 3 times. The maceration results are filtered using the Whatman filter paper. The filtrate obtained was then concentrated using a rotary evaporator at 40 °C until it forms a paste. This resulting filtrate was a crude extract of the sea cucumber, which will be used for further analysis.

Antibacterial activity test

The ability of the sea cucumber extract sample to produce antibacterial activity was tested using the overlay method. The extracted sample was applied to the surface of Zobell agar media, forming small circles with an inoculating needle. Each petri dish was divided into 6-8 quadrants for the inoculation of symbiotic bacteria. The bacterial inoculation was then incubated at room temperature for 48 h. The tested pathogenic bacteria were *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The bacteria were cultured in 10 ml of liquid Zobell media and incubated on a shaker at 120 rpm for 24 h. Pathogenic bacterial culture (1 ml) was taken and transferred to 100 ml of warm liquid Zobell soft agar media. The bacterial culture and media were poured onto agar media previously inoculated with symbiotic bacterial isolates and incubated for 48 h at room temperature. The formation of inhibition zones around the isolates indicates antibacterial activities (Pringgienies and Dananjoyo, 2011).

Phytochemical test

A phytochemical test was conducted on samples of sea cucumber methanol extract, following the method described by Pringgienies *et al.* (2021). The purpose of the test was to determine the presence of saponins, tannins, flavonoids, quinones, alkaloids, steroids, and terpenoids in the samples. The research was conducted exclusively on wet sea cucumbers, specifically the *H. atra* variety. Processed sea cucumbers are not tested due to their exposure to additional chemical processing, such as heating, which can potentially impact the phytochemical results of the sea cucumbers.

Heavy metal content

The dried and cleaned sea cucumber samples were ground to make sea cucumber flour. The sea cucumber flour samples were then put in a round plastic container and brought to Diponegoro University Integrated Laboratory. They were tested for the presence of heavy metals like Arsenic (As), Lead (Pb), Cadmium (Cd), and Mercury (Hg) using the Atomic Absorption Spectrophotometer (AAS) method and Mercury Analyzer in the Testing and Calibration Laboratory at Diponegoro University in Semarang.

Absorption Spectrophotometer (AAS) method following standard procedures from Mutiah *et al.* (2022), Absorption Spectrophotometer analyzed with different wavelength based on targeted heavy metal that want to analyzed.

Amino acid analysis

The sample used for amino acid analysis was sea cucumber powder collected from Karimunjawa waters and processed sea cucumber crackers collected from the market in Surabaya. Before the analysis, the sample was prepared by measuring the protein content and undergoing acid or base hydrolysis. The hydrolyzed sample was then analysed using HPLC (High Performance Liquid Chromatography) following standard procedures (Pringgenies *et al.*, 2016). This analysis converts proteins into amino acids, allowing them to be detected by chromatography. The results of the analysis were detected by a detector and recorded by a recorder. The computer then displays the data as retention time and peak area graphs, which correspond to the characteristics of each amino acid.

Result and Discussion

The sea cucumber sample found in the Karimunjawa waters has been identified as the *Stichopus variegatus* species, which is characterized by its golden or yellowish color. This is why it is also referred to as the golden sea cucumber.

The sea cucumber known as *Stichopus variegatus* has a soft and flexible body, with small

spines covering its surface. It has a long and cylindrical shape. The processed sea cucumber is assigned as *Holothuria atra*, which has a black body color, although there are other color variations such as dark brown or dark gray. The body surface of the *Holothuria atra* sea cucumber is generally rough and covered in many fine spines that resemble hairs, as shown in Figure 1. The extraction results of *S. variegatus* sea cucumbers and the processed results of *H. atra* sea cucumbers are shown in Figure 2.

Phytochemical screening and antibacterial activity

The sea cucumber sample was analyzed for phytochemicals, and it was found that the *Stichopus variegatus* species contained saponins, tannins, flavonoids, steroids, and terpenoids. However, quinones and alkaloids were not detected. It is important to note that no phytochemical analysis or antibacterial tests were conducted on processed sea cucumbers.

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The test results on the gram-positive pathogenic bacterium *Staphylococcus aureus* and

Table 1. Phytochemical Test Results of *Stichopus variegatus* sea cucumber extraction

Compound	Saponins	Tannins	Flavonoids	Quinones	Alkaloids	Steroids	terpenoids
Activity	+	+	+	-	-	+	+



a



b

Figure 1. Identified samples of *Stichopus variegatus* (a) and *Holothuria atra* (b).

Table 2. Results of pathogenic bacteria testing on extracted *Stichopus variegatus* sea cucumber

Test Bacteria	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>
Activity	+			+
Heavy metal content (ppm)	Arsenic (As)	Plumbum (Pb)	Cadmium (Cd)	Mercury (Hg)
<i>H. atra</i>	0,00001	0,05	0,02	0,0005
<i>S. variegatus</i>	0,00001	0,05	0,02	0,0005



Figure 2. The extraction results of *Stichopus variegatus* sea cucumber (a) and the processed product of *Holothuria atra* sea cucumber made into sea cucumber floss (b).

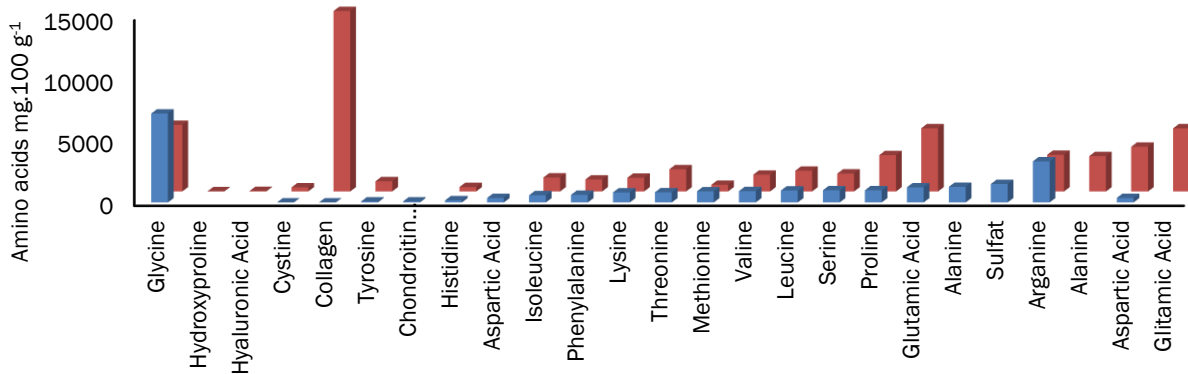


Figure 3. Amino acid content: *Stichopus variegatus* and *Holothuria atra*

the gram-negative pathogenic bacterium. *E. coli* in the sample extract of sea cucumber showed that the sea cucumber sample extraction has positive activity against both test bacteria, *Staphylococcus aureus* and *E. coli* bacteria, as shown in Table 2.

The analysis results for heavy metals Arsenic (As), Lead (Pb), Cadmium (Cd), and Mercury (Hg) in the sea cucumbers *Holothuria atra* and *Stichopus variegatus* indicated that they were rarely detected

or not present. The detection limits for Arsenic (As) were 0.01 ppm, Lead (Pb) was 0.05 ppm, Cadmium (Cd) was 0.03 ppm, and Mercury (Hg) was 0.0005 ppm. However, the analysis results showed that none of the samples contained detectable levels of these metals.

The results of the amino acid analysis showed the content of various amino acids in unprocessed *Stichopus variegatus* sea cucumber. The amino acids

were listed in descending order of their content in milligrams per 100 g: Glycine (7190), Arginine (3320), Sulfate (1488), Alanine (1260), Glutamic Acid (1217), Proline (980), Serine (980), Leucine (960), Valine (915), Methionine (898), Threonine (819), Lysine (810), Phenylalanine (610), Isoleucine (580), Aspartic Acid (360), Histidine (168), Chondroitin Sulfate (79), Tyrosine (75). The contents of Collagen and Cystine were not detected, as shown in Figure 3.

The processed sea cucumbers were directly obtained from the market in Surabaya. The price of fried sea cucumbers of the *H. atra* is Rp. 30,000.100 g⁻¹. On the other hand, the price of unprocessed sea cucumbers of the *S. variegatus* species varies greatly depending on the type of sea cucumber. Specifically, the price of the *Stichopus variegatus* species is Rp. 325,000.100 g⁻¹. The reason for using different samples from the same class, which is the Holothuridae sea cucumber, is to inform the public that the more popular sea cucumbers for consumption have different nutritional values and health benefits. It has been proven that raw sea cucumbers have antibacterial activity (Pringgienies et al., 2014), antifungal activity (Pranoto et al., 2012), and potential as an anticancer agent (Pringgienies et al., 2018). However, the general public is only familiar with the name sea cucumber.

Research results indicate that the sea cucumber species *Stichopus variegatus* contains various compounds, including saponins, tannins, flavonoids, steroids, and terpenoids. It has been found to have antibacterial activity against pathogenic bacteria such as *S. aureus* and *E. coli*. Additionally, sea cucumbers contain compounds like saponins, phenolics, and polysaccharides, which have the potential to act as antioxidant and anti-inflammatory agents. These effects are significant in preventing the development of cancer and reducing the risk of inflammation that contributes to cancer.

Both processed and unprocessed sea cucumber samples contain saturated and unsaturated amino acids. The saturated amino acids found in these samples are Collagen, Arginine, Threonine, Leucine, Valine, Lysine, Hyaluronic Acid, Hydroxyproline, and Glutamine Hydrochloride. On the other hand, the unsaturated amino acids present are Glycine, Alanine, Glutamic Acid, Proline, Serine, and Aspartic Acid. Overall, the amino acid content in both types of sea cucumbers indicates that *H. atra* sea cucumbers consistently have higher amino acid content compared to *Stichopus variegatus* sea cucumbers.

The collagen content is highest in *H. atra* sea cucumber (14600 µg.100g⁻¹) and undetectable in *S.*

variegatus sea cucumber. The high content in processed sea cucumbers is likely due to frying them in cooking oil. The frying process increases the concentration of collagen in sea cucumbers. Frying alters the protein structure in sea cucumbers and is believed to enhance collagen bioavailability. Collagen is generally known to be beneficial for skin health. Furthermore, research has shown that the highest content of glycine is found in *S. variegatus* sea cucumber (7190 mg.100g⁻¹) and is higher than in *H. atra* sea cucumber (5390 mg.100g⁻¹). There is abundant information on the benefits of glycine in preventing various diseases and disorders, including cancer. Supplementation with the appropriate dose of glycine is effective in treating metabolic disorders in patients with cardiovascular disease, certain inflammatory diseases, obesity, cancer, and diabetes (Razak et al., 2022).

Research on *S. variegatus* sea cucumber showed that it contains sulfate compounds and Alanine, which are not found in *H. atra* sea cucumber. Sulfate compounds have long been known to have wound healing properties. Meanwhile, Alanine is commonly found in commercial supplements or health drinks. Consuming Alanine can enhance muscle endurance and improve athletic performance.

Unsaturated amino acids in sea cucumbers, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have anti-inflammatory properties and can help reduce the risk of heart disease (Innes and Calder, 2020). These amino acids help reduce levels of bad cholesterol (LDL) and triglycerides in the blood while increasing levels of good cholesterol (HDL).

Unsaturated amino acids, particularly DHA, found in sea cucumbers, play a crucial role in the development of brain tissue. Consuming DHA can enhance cognition, memory, and learning ability. Additionally, this amino acid has neuroprotective properties that safeguard the brain against oxidative damage (Pringgienies et al., 2016). The presence of unsaturated amino acids in sea cucumbers has been shown to facilitate the synthesis of anti-inflammatory and antioxidant compounds, among others (Carletti et al., 2022; Bordbar et al. 2011; Khotimchenko, 2018). Sea cucumbers belonging to the Holothuroidea class are highly valued as food and traditional medicine in Asia due to their rich content of essential nutrients, including vitamins, minerals, and amino acids. Various compounds derived from sea cucumbers exhibit unique biological and pharmacological activities, such as anticancer, anticoagulant/antithrombotic, antimicrobial, antioxidant, antihyperlipidemic, antihyperglycemic, anti-inflammatory, antihypertensive, and radioprotective properties (Shi et al, 2016).

The research on untreated sea cucumbers reveals that they contain saponin, while processed sea cucumbers have lower saponin levels. This is because processed sea cucumbers are washed multiple times before being processed, which causes the saponin to dissolve in water and lose its bitter taste which is disliked. Saponin is known to have numerous potential health benefits. Studies have demonstrated that saponin in sea cucumbers has the potential for various activities, including anti-cancer, anti-obesity, anti-hyperuricemia, anticoagulant, antioxidant, antimicrobial, antiangiogenic, antithrombotic, anti-inflammatory, antitumor, and immunomodulatory activities (Fagbohun *et al.*, 2023). Sea cucumbers were found to have nutritional value and health benefits after extensive research. They have the potential to be functional marine foods that promote good health.

Additionally, sea cucumbers have antioxidant properties due to the presence of sulfate compounds. These compounds can protect cells in the body from damage caused by free radicals. Free radicals are known to cause oxidative stress and can contribute to the development of chronic diseases, including cancer. Gupta *et al.*, 2019)

Sea cucumber also has the potential to be an antitumor agent. Some initial studies have indicated that the sulfated compounds found in sea cucumbers have effects that can inhibit the growth of cancer cells, induce cell death in cancer cells, and prevent the spread of cancer cells. While research is still limited, sea cucumber has shown promise as a potential antitumor agent (Pringgenies *et al.*, 2018).

In addition, various compounds found in sea cucumbers (including saponins, glycosaminoglycans, chondroitin sulfate, sulfated polysaccharides, phenolates, peptides, lectins, cerebroside, and sterols) have been discovered to possess numerous bioactive properties. These properties include anticoagulant, antimicrobial, antioxidant, anti-inflammatory, anticancer, antiangiogenic, and antihypertensive effects, making them suitable for the development of functional foods. Furthermore, research indicates that sea cucumbers do not contain heavy metals.

Conclusion

Holothuria atra has higher levels of amino acids compared to *Stichopus variegatus*. *Holothuria atra* has the highest collagen content among its amino acid content, measuring 14.600mg.100g⁻¹. Both *Holothuria atra* and *Stichopus variegatus* sea cucumbers have amino acid contents that have the potential to act as anti-inflammatory, antioxidant, and anticancer agents. *Stichopus variegatus* sea

cucumber also has antibacterial properties against *S. aureus* and *E. coli* bacteria. Additionally, it contains saponins, tannins, flavonoids, steroids, and terpenoids. Both *Holothuria atra* and *Stichopus variegatus* sea cucumber species are functional foods that can be used as preventatives against cancer.

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