

Characterization of *Thelenota ananas* and Its Potential as a Natural Source of Antioxidants

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ABSTRACT

The sea cucumber *Thelenota ananas*, commonly found in tropical waters, possesses significant biological potential. This study aims to assess the antioxidant potential of *T. ananas* extract obtained from the Manado sea. The simplicia was analyzed for organoleptic properties, moisture content (9.8%), and ash content (23%). Bioactive compounds were identified through color and foam reactions, while antioxidant activity was measured using the DPPH method. The extract was found to contain saponins, terpenoids, and alkaloids. The antioxidant activity of the extract, with an IC50 value of 99.8 ppm, demonstrates strong antioxidant properties, though less potent than the control, vitamin C (IC50 of 45.5 ppm). These findings suggest that while *T. ananas* extract exhibits substantial antioxidant activity, it is still less effective than vitamin C. This research highlights the potential of *T. ananas* as a natural antioxidant source for the development of health products.

Keywords: *Thelenota ananas*; characterization; antioxidant; DPPH

INTRODUCTION

Sea cucumbers (Holothuroidea) are echinoderms that play a critical role in marine ecosystems, particularly as decomposers of organic matter on the seafloor. One species of interest is *Thelenota ananas*, found primarily in tropical waters, including Indonesia and Australia. This species is economically valuable for human consumption and is also known for its rich bioactive compounds with potential medicinal applications, especially in combating oxidative stress.

Oxidative stress arises when there is an imbalance between the production of free radicals and the body's ability to neutralize them with antioxidants. It is a major cause of degenerative diseases, including cancer, heart disease, and diabetes. Free radicals, generated during metabolism, can damage cells, DNA, proteins, and lipids, contributing to aging and the development of various chronic conditions. This highlights the significance of oxidative stress in the progression of cardiovascular, neurodegenerative, and metabolic diseases (Leyane et al., 2022; Liguori et al., 2018). As a result, the search for effective natural antioxidants, particularly from marine sources, has become increasingly important in disease prevention and treatment (Pangestuti & Arifin, 2018).

Previous studies have identified several sea cucumber species as rich sources of bioactive compounds, such as flavonoids, saponins, terpenoids, alkaloids, and phenolics, all known for their antioxidant properties. These compounds help mitigate oxidative stress by scavenging free radicals and reducing inflammation (Sukmiwati et al., 2024). While much research has focused on species like *Holothuria atra* and *Holothuria scabra*, the antioxidant potential of *Thelenota ananas* remains relatively underexplored, despite its promising bioactive content, including flavonoids and terpenoids (Dehwie et al., 2021; Pangestuti & Arifin, 2018).

Given the promising bioactive compounds in *Thelenota ananas*, further research is crucial to unlock its full potential as a natural antioxidant source. Understanding its flavonoid, terpenoid, and alkaloid content could lead to the development of pharmaceutical, nutraceutical, and cosmetic products. In addition to its antioxidant properties, *Thelenota ananas* has been shown to have immune-boosting, anti-inflammatory, and wound-healing effects (Ghelani et al., 2022). Thus, this study aims to explore the potential of *Thelenota ananas* as a safe and effective

source of antioxidants and a raw material for developing health products to prevent and treat diseases associated with oxidative stress

METHOD

Sample Collection

Sea cucumber samples (*Thelelenota ananas*) were collected from the sea waters of Manado, North Sulawesi Province, Indonesia, at a depth of 10-20 meters using the scuba diving method, located between 1°27'33.3" - 1°29'35" North Latitude and 124°47'00" -124°50'29" East Longitude, in August-September 2025. The obtained sample is then cleaned of dirt and other attached organisms. After cleaning, the sea cucumber is dried naturally in the sun and processed into fine simplicia that is ready for the extraction process. Before extraction, the simplicia are cut into small pieces and crushed into powder using a mechanical grinder. Extraction is carried out using an ultrasonic extractor (sonicator) with a 96% ethanol solvent, and furthermore, the extract is evaporated using a vacuum evaporator until a viscous extract is obtained, then dried on a water bath at 50°C to avoid degradation of bioactive compounds.

Organoleptic Testing

Organoleptic tests were performed to assess the physical properties of the simplicia of the sea cucumber *Thelelenota ananas* based on color, smell, and texture (Pangestuti & Arifin, 2018). This method allows for more objective measurements of organoleptic properties and minimizes bias from individual observations.

Moisture Testing

The simplicia of the sea cucumber *Thelelenota ananas* that has been blended and sifted using mesh sieve No. 40, is weighed as much as 2 g, then put into a sealed porcelain cross whose weight is known. The sample is heated in the oven at 105°C for 2 hours. After that, the sample is cooled in a desiccant and weighed together with its cross until a constant weight is obtained. This test is performed in three iterations to ensure consistent results. The moisture content is calculated using the following formula:

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

Where:

A = empty cross weight (g)

B = sample weight (g)

C = sample weight + cross after heating at 105°C.

This method refers to the accepted standards in the analysis of the moisture content of natural materials (Harborne, 1998).

Ash Level Testing

A total of 2 g of sea cucumber simplicia that had been blended and sifted with a mesh sieve No. 40, were weighed and put into a porcelain cross whose weight was known. The sample is then heated in a kiln at 600°C until it is completely cooled. After that, the sample is cooled in a desiccant and weighed back. This procedure is repeated until a constant weight is obtained. The test is performed in two replays to ensure valid results. The ash rate is calculated using the following equation:

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

Where:

A = empty cross weight (g)

B = sample weight (g)

D = sample weight + cross after heating at 600°C.

This procedure refers to the standard methods used in the analysis of natural materials (Badan POM RI, 2005).

Bioactive Compound Screening

Extraction was carried out by ultrasonic method using a 96% ethanol solvent with a ratio of 1:10 for 2x30 minutes. The extracts obtained are evaporated using a vacuum evaporator until a thick liquid is obtained, then evaporated on top of a water bath until it becomes a thick extract.

The extract solution obtained is then prepared by mixing 2 g of thick extract with 96% ethanol until the volume reaches 20 mL. Screening of bioactive compounds is carried out by qualitative tests for alkaloids, flavonoids, terpenoids, saponins, and tannins using appropriate reagents, based on which are described as follows Harborne (1998), Kumar et al. (2020).

Alkaloid Test

1 ml of the extract solution is added 10 drops of 2N sulfuric acid, then shaken. After separating into two layers, the top layer is divided into two test tubes. The first filtrate is added 3 drops of Mayer's reagent, and the formation of white deposits indicates the presence of alkaloids. The second filtrate is added 3 drops of Dragendorff reagent, and the formation of a brick-red deposit indicates an alkaloid (Harborne, 1998).

Terpenoid Test

A total of 0.5 ml of extract is added to the test tube, then 2 ml of chloroform is mixed into the solution. 3 ml of concentrated sulfuric acid is added to form the bottom layer. A reddish-brown color appears on the interface, indicating the presence of terpenoids (Kumar et al., 2020)

Flavonoid Tests

0.5 ml of plant extract and 5 ml of distilled water are added to the test tube, then filtered. 5 ml of diluted ammonium solution is added to the filtrate, then concentrated with 0.5 ml of sulfuric acid. A yellow color appears, which indicates the presence of flavonoids (Kumar et al., 2020).

Saponin Test

A total of 0.5 ml of extract is put into the test tube, then 5 ml of distilled water is added. The solution was shaken vigorously and the presence of a ±1 cm high foam that was stable for more than 10 minutes indicated the presence of saponins (Kumar et al., 2020).

Tannin Test

A total of 0.5 ml of extract and 5 ml of distilled water are put into the test tube, then 1% ferris chloride is added. A dark green color is formed, which indicates the presence of tannins (Kumar et al., 2020).

Antioxidant Activity Test

The antioxidant activity test was carried out using the DPPH method (1,1-diphenyl-2-picrylhydrazil) following the study (Dolongtelide et al., 2023; Stuttgart et al., 2021). Extracts that had been prepared with different concentrations (20 ppm, 40 ppm, 80 ppm, and 100 ppm) were incubated with DPPH solution. Absorbance was measured at a wavelength of 517 nm

using a UV-Vis spectrophotometer to calculate % inhibition. This measurement was carried out three times with ascorbic acid as a comparison. The following equation is used to calculate the % inhibition:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\%$$

Where:

A_{control} is the control absorbance (DPPH with ethanol).

A_{sample} is the absorbance of the sample.

Preparation of DPPH Reagents

The DPPH solution is made by dissolving 4 mg of DPPH powder in 100 mL of 96% ethanol.

Sample Solution Preparation

A 1000 ppm stock solution is made by dissolving 10 mg of sea cucumber extract in 10 mL of 96% ethanol. Sample solutions with concentrations of 20 ppm, 40 ppm, 80 ppm, and 100 ppm were prepared by mixing 0.2 mL, 0.4 mL, 0.8 mL, and 1 mL of stock solution with 96% ethanol to a volume of 10 mL.

The entire antioxidant activity test was performed with three iterations to ensure consistent results, and the results were analyzed to determine the IC₅₀ value, which was calculated using a linear regression equation of the % inhibition curve to the sample concentration.

RESULTS AND DISCUSSION

Organoleptic Test

Organoleptic testing of pineapple sea cucumber simplicia (*Thelenota ananas*) was carried out using the five senses to assess color, smell, taste, and texture (Mehra et al., 2024). The test results are shown in **Table 1** and **Figure 1**.

Table 1. Results of Organoleptic Test of Simplicia of Pineapple Sea Cucumber

No	Observation	Result
1	Color	White-orange
2	Smell	Typical sea cucumbel
3	Taste	Salty
4	Shape	Dense, prickly



Figure 1. Simplicia Pineapple Cucumber

The results of organoleptic tests showed that sea cucumber simplicia is powdery, white-orange in color, smells typical of sea cucumbers, and has a salty taste. This color and smell are in accordance with the natural characteristics of sea cucumbers, while the salty taste may come from the internal mineral salt content of its marine habitat (Pangestuti & Arifin, 2018). The

dense and prickly texture is consistent with the sea cucumber's body wall structure which contains calcium carbonate spicule, which acts as a mechanical protector. The test was repeated three times to ensure consistency of results, and the average scores between panelists showed minimal variation (<5%), confirming the reliability of the observations.

Organoleptic analysis is important to ensure the quality of simplicia as a pharmaceutical and food raw material. Color, smell, taste, and texture are the initial parameters to assess the freshness and authenticity of natural ingredients before further extraction and testing (Harborne, 1998).

Water Rate and Ash Rate

Testing of moisture content and ash content was carried out at the Integrated Laboratory of Sam Ratulangi University Manado using oven and kiln methods (Badan POM RI, 2005).

Table 2. Results of Pineapple Sea Cucumber Ash and Water Content Test

No	Test	%
1	Water rate	9.80
2	Ash rate	20.1

The moisture content of 9.80% (**Table 2**) met the requirements of simplicia (<10%), indicating that simplicia drying has been effective and the risk of microbial growth is low. This result is supported by research conducted by Finarti et al. (2020) that the water content of sea cucumbers *H. vacabunda* is significantly with a moisture content of 12.09%. The ash content of 22.1% indicates a fairly high mineral content, in contrast to the ash content of sand sea cucumber (*Holothuria scabra*) and black sea cucumber (*Holothuria atra*) with low ash content of 5.19% and 1.93% (Elfath et al., 2020). The test was conducted in three tests to ensure consistency of results, which showed a variation of <0.5% between tests. These results are consistent with the research of Pangestuti et al. (2020) which reported that sea cucumber ash content is 18–22%. The high ash content supports the nutritional potential of sea cucumbers as a mineral source, while the low moisture content extends the shelf life of simplicia.

Bioactive Compound Screening

Phytochemical screening of pineapple sea cucumber simplicia was carried out qualitatively with a color reaction test and a foam test (Farak et al., 2020; Harborne J. B., 1998). The results are shown in **Table 3**.

Table 3. Screening Results of Bioactive Compounds of Pineapple Sea Cucumbers

No	Senyawa	Test Results	Information
1	Alkaloid	+	White sediment (Mayer)
2	Steroid/Terpenoid	+	Red discoloration (Terpenoids)
3	Flavonoid	-	Unchanged
4	Saponin	+	Foam formed
5	Tanin	-	Does not change blue/green

Sea cucumber simplicia contains alkaloids, terpenoids, and saponins that have the potential to be antioxidant, antibacterial, and anti-inflammatory (Pangestuti & Arifin, 2018). The consistency of the test triplicate results showed high reproducibility and confirmed the presence

of these bioactive compounds. Flavonoids and tannins were not detected, likely because qualitative methods were less sensitive or the concentration of compounds was too low.

Alkaloids are known to be able to stabilize free radicals through electron donation, as well as act as chelators of transition metal ions such as Fe^{2+} and Cu^{2+} that are involved in the Fenton reaction that produces hydroxyl radicals (Zhang et al., 2020). Terpenoids, especially the type of triterpenoids that are widely found in marine animals, work by inhibiting the formation of ROS through regulation of prooxidant enzymes such as NADPH oxidase and increasing the activity of endogenous antioxidant pathways (Mantiniotou et al., 2025). Meanwhile, saponins have the ability to inhibit lipid peroxidation and prevent cell membrane damage by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), as well as helping to restore redox homeostasis at the cellular level (Wijesekara et al., 2024). To strengthen these findings, it is important to conduct quantitative comparisons with other sea cucumber species. Based on the data available from (Pangestuti & Arifin, 2018c), it is known that the total content of antioxidant compounds in *Thelenota ananas* extract resulted in an IC_{50} value of 72.5 $\mu\text{g}/\text{mL}$ in the DPPH test. This value was lower (meaning stronger activity) compared to *Stichopus hermanni* which showed an IC_{50} of 110.3 $\mu\text{g}/\text{mL}$, and *Holothuria atra* with an IC_{50} of 89.7 $\mu\text{g}/\text{mL}$ under equivalent test conditions. This difference indicates that *Thelenota ananas* has a higher antioxidant potential than other species, which is most likely due to the unique composition of its bioactive compounds, in particular its high content of triterpenoids and saponins.

DPPH Antioxidant Activity Test

Sea cucumber extract was tested using the DPPH method, with 96% ethanol as the solvent and vitamin C as a positive control (Table 4). Each concentration was tested in triplicates to ensure reproducibility (Brand-Williams, 1995; Sukmiwati et al., 2024).

Table 4. Results of % Inhibition and IC_{50} of Pineapple Sea Cucumber Extract

Concentration (ppm)	Deterrence (Absorbance)	Average	% Inhibisi	IC_{50} (ppm)
20	0,689 / 0,685 / 0,691	0,688	17,40	
40	0,643 / 0,649 / 0,650	0,647	22,32	99,62
60	0,571 / 0,569 / 0,572	0,571	31,52	
80	0,503 / 0,499 / 0,513	0,505	39,40	
100	0,395 / 0,391 / 0,398	0,395	52,64	
DPPH Control	0,841 / 0,815 / 0,844	0,833	-	

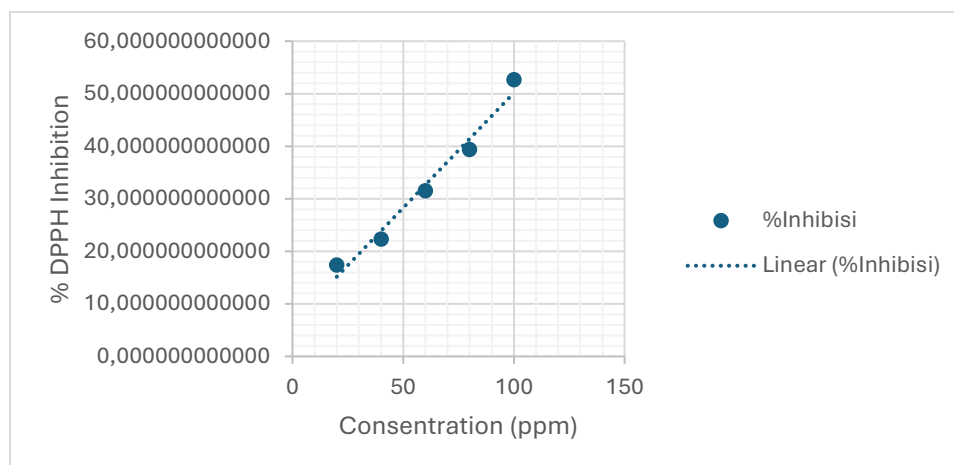


Figure 3. Graph of the Relationship of Extract Concentration with % DPPH Inhibition

The IC₅₀ value was calculated using a linear regression equation of the % inhibition to concentration curve. In this study, the value of IC₅₀ = 99.62 ppm for *Thelenotia ananas* extract showed that the extract had strong antioxidant activity (Figure 3). Based on the classification generally accepted in the literature, the value of IC₅₀ can be categorized as follows:

- IC₅₀ < 50 ppm : Antioxidant activity is very strong.
- 50 ppm < IC₅₀ < 100 ppm : Strong antioxidant activity.
- 100 ppm < IC₅₀ < 200 ppm : Moderate antioxidant activity.
- IC₅₀ > 200 ppm : Weak antioxidant activity.

This classification has been widely accepted in various studies that evaluate the antioxidant activity of various natural compounds, both from plants and marine animals (Gao et al., 2017) (Choi et al., 2024). Based on this classification, IC₅₀ = 99.62 ppm indicates that *Thelenotia ananas* extract has strong antioxidant activity, in accordance with the results of previous studies that showed the potential of sea cucumber extract in inhibiting free radical activity (Ayubi et al., 2024). These results are in line with findings that certain sea cucumber species, including *Thelenotia ananas*, can be a bioactive source with significant antioxidant potential, which can be harnessed in the development of pharmaceutical products or nutraceuticals based on natural ingredients. This antioxidant activity is thought to come from a combination of bioactive compounds such as saponins, terpenoids, and polysaccharides that are able to neutralize free radicals, stop lipid peroxidation, and stabilize cell membranes (Dong et al., 2018). Its activity was lower than that of the vitamin C control (IC₅₀ = 45.5 µg/mL), but still showed significant potential as a natural antioxidant. The use of positive controls ensured that the change in absorbance was indeed due to the antioxidant activity of the extract, not other experimental factors.

CONCLUSION

This study shows that *Thelenotia ananas* has significant potential as a natural source of antioxidants that can be developed for health products. The results of the antioxidant activity test using the DPPH method showed that *Thelenotia ananas* extract had strong antioxidant activity with an IC₅₀ value of 99.62 ppm, which indicated its ability to ward off free radicals and the potential to prevent oxidative damage that can cause various degenerative diseases. In addition, bioactive compounds such as alkaloids, terpenoids, and saponins found in this sea cucumber extract contribute to the antioxidant activity detected. Therefore, *Thelenotia ananas* can be used as a raw material in the development of functional products for health, especially in the prevention and therapy of degenerative diseases related to oxidative stress.

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