Coastal and Ocean Journal

Article

The Effects of Extraction Methods on the Bioactive Compounds and Antioxidant Activity of Sea Cucumber from Labuan Bajo, Indonesia

Risya Putri Noer Azka [1], Iriani Setyaningsih [1], Ina Winarni [2], Kustiariyah Tarman [1,3*]

[1] Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University, Bogor 16680, Indonesia
[2] National Research and Innovation Agency (BRIN), Indonesia
[3] Center for Coastal and Marine Resources Studies, LRI i-MAR, IPB University, Bogor 16127, Indonesia
* Correspondence: kustiaz@apps.ipb.ac.id

Abstract: Sea cucumbers are invertebrate marine organisms with potential as a source of bioactive compounds for pharmaceutical applications. One of their benefits is serving as a source of antioxidants. This study aimed to determine the best extraction method for yield value and antioxidant activity of sea cucumbers. The methods employed included maceration, Soxhlet extraction, and microwave-assisted extraction, along with antioxidant analysis and identification of active compounds. The results showed that the maceration method produced a higher percentage yield compared to Soxhlet and microwave methods, with 3.26% for *Bohadschia marmorata* and 2.91% for *Holothuria fuscopunctata*. Identification of active compounds in both types of samples revealed the presence of phenolic compounds and steroids/triterpenoids. However, the antioxidant activity of the sea cucumber extracts was found to be weak for both species. The best antioxidant activity was obtained from maceration extraction, with IC50 values of 625 μg/mL for *Bohadschia marmorata* and 897 μg/mL for *Holothuria fuscopunctata*.

Keywords: *Bohadschia marmorata*; extraction; *Holothuria fuscopunctata*; maceration; phenol

Citation: Azka, R. P. N.; Setyaningsih, I.; Winarni, I.; Tarman, K., 2026. The Effects of Extraction Methods on the Bioactive Compounds and Antioxidant Activity of Sea Cucumber from Labuan Bajo, Indonesia. *Coastal and Ocean Journal*, (10)1: 52-61. https://doi.org/10.29244/coj.v10i1.66534

Received: 03-09-2025
Revised: 31-05-2026
Accepted: 14-06-2026
Published: 15-06-2026

Publisher's Note: Coastal and Ocean Journal (COJ) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Sea cucumbers, or *teripang* in Indonesian, are invertebrates from the phylum *Echinodermata*, class *Holothuroidea*. They have soft, elastic, elongated bodies resembling cucumbers, which is why they are called "sea cucumbers" (Husain *et al.,* 2017). These organisms are commonly found in coral reefs and deep waters and have high economic value in domestic and international markets due to their rich nutritional content. Fresh sea cucumbers contain 4% protein, 93.55% water, 1.22% ash, 0.36% fat, and 0.87% carbohydrates (One *et al.*, 2021).

Sea cucumbers are mainly used as raw materials for processed products and have potential as sources of marine-derived pharmaceuticals. Their bioactive compounds exhibit various health benefits, including antihypertensive, anti-inflammatory, antitumor, anticoagulant, wound-healing, and antioxidant properties (Rahman, 2014). *Holothuria atra* has been reported to contain phenolic and flavonoid compounds, which act as antioxidants by inhibiting free radical activity (Dwicahyani *et al.*, 2018; Laila *et al.*, 2022).

Natural antioxidants in sea cucumbers are considered safer than synthetic ones, which can be toxic. These compounds can be extracted using conventional methods such as maceration, Soxhlet extraction, and reflux, or modern techniques like Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE). The choice of extraction method affects the antioxidant activity of sea cucumbers. Studies have explored various techniques, such as maceration with ethanol or methanol (Nurhamzah *et al.,* 2022; Avigail *et al.,* 2019) and Soxhlet extraction with ethanol (Wulandari *et al.,* 2022; Misgiati *et al.,* 2024). However, research on MAE for extracting antioxidants from sea cucumbers remains limited.

The selection of an extraction method depends on the properties of the target compounds, as different techniques influence yield and antioxidant activity (Yulianti *et al*., 2020). Identifying the most effective and efficient method is essential for maximizing antioxidant compound extraction. This study aims to determine the best extraction method for optimizing yield and antioxidant activity from sea cucumbers.

2. Materials and Methods

2.1. Materials and Equipments

The study utilized *Bohadschia marmorata* and *Holothuria fuscopunctata* collected from Labuan Bajo, East Nusa Tenggara, Indonesia. The chemical reagents included ethanol (Supelco), methanol (Supelco), distilled water, DPPH (HiMedia India), and anisaldehyde reagent for phytochemical analysis. The laboratory equipment used comprised beakers (Iwaki), a Soxhlet extractor, a microwave (SIGMATIC SMO-25SSG), a rotary vacuum evaporator (BUCHI), a chromatography chamber, silica gel TLC plates (Merck), micropipettes, UV lamps (254 nm and 366 nm), and a microplate reader (SPECTROstar Omega).

2.2. Sample Preparation

Fresh sea cucumbers were washed thoroughly and identified based on morphology, coloration, and internal skeletal structures (spicules) using taxonomic references (Massin, 1996, 1999; Samyn & Massin, 2003; FAO, 2012). The specimens were cleaned to remove mucus and dissected to extract the body tissue while discarding internal organs. The cleaned samples were then cut into small pieces, weighed, and prepared for extraction.

2.3. Extraction Methods

2.3.1 Maceration (Modified from Bawole *et al*., 2021)

Sea cucumber tissue was macerated in 70% ethanol at a ratio of 1:3 (w/v) for 3 × 24 hours, with solvent replacement every 24 hours. The extracts were filtered, evaporated at 40°C using a rotary evaporator, weighed, and stored at 4°C for further analysis.

2.3.2 Soxhlet Extraction (Modified from Wulandari *et al.*, 2022)

Fifty grams of sea cucumber tissue were placed in filter paper inside the Soxhlet extractor and extracted with 250 mL of 70% ethanol for 14 hours (7 cycles) at 60–70°C without solvent replacement. The filtrate was evaporated at 40°C, weighed, and stored at 4°C.

2.3.3 Microwave-Assisted Extraction (Modified from Kamaluddin *et al.*, 2014)

Fifty grams of sea cucumber tissue were mixed with 250 mL of 70% ethanol and subjected to MAE for two cycles of 5 minutes at 2 W and 56°C. The extract was filtered, evaporated at 40°C, and stored at 4°C.

2.3.4 Antioxidant Assay

A stock solution of the sea cucumber extract was prepared at a concentration of 1,000 µg/mL by dissolving 2 mg of the extract in 2 mL of methanol. The extract solution was then diluted to create a series of concentrations: 1,000, 500, 250, 125, and 62.5 µg/mL. A 0.1 µM DPPH solution was prepared by dissolving DPPH crystals in methanol, ensuring the solution was protected from sunlight. The blank solution was prepared by mixing 100 µL of DPPH solution with 100 µL of methanol. The DPPH stock solution was mixed with the diluted extract solutions at a 1:3 ratio, with 60 µL of DPPH solution and 180 µL of extract solution. The mixture was homogenized and incubated at 37 °C for 30 minutes in a dark environment. Absorbance measurements were conducted using a microplate reader at a wavelength of 517 nm. The percent inhibition and IC50 values were subsequently calculated.

3. Results

3.1. Extraction Yield

The extraction yield of sea cucumbers varied across different methods. The highest yield was obtained through maceration, with values of 3.26% for *Bohadschia marmorata* and 2.91% for *Holothuria fuscopunctata*, while Soxhlet extraction resulted in the lowest yield (1.89% and 1.95%, respectively). MAE yielded 2.39% and 2.79%. Results are shown in Table 1.

Table 1. Yield of 70% ethanol extracts from two sea cucumber species obtained using different extraction methods

Sea cucumber species	Extraction methods	Yield (%)
Bohadschia marmorata	Maceration	3.26 ± 0.4
	Soxhletation	1.89 ± 1.34
	MAE	2.39 ± 0.32
Holothuria fuscopunctata	Maceration	2.91 ± 1.18
	Soxhletation	1.95 ± 0.81
	MAE	2.79 ± 0.34

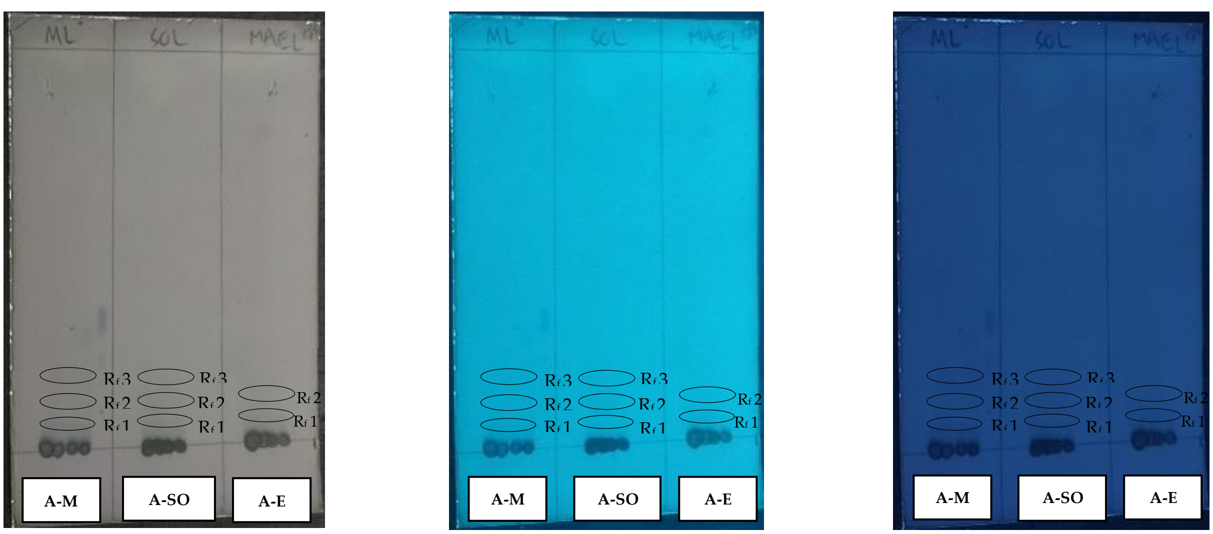
These results were lower than previous studies, which reported 4.43% for maceration (Dwicahyani *et al*., 2018) and 5.5% for Soxhlet extraction using fresh samples (Wulandari *et al*., 2021). Maceration yielded higher extraction efficiency than Soxhlet and MAE due to its lower extraction temperature, preventing solvent evaporation. Ethanol (70%) was used as a solvent due to its effectiveness in extracting both polar and semi-polar compounds (Yuliana *et al*., 2017). Solvent volume also played a role, as an insufficient amount could limit compound extraction, while excessive use could lead to solvent loss through evaporation (Wahyuningsih *et al*., 2024).

The sample condition also influenced yield. Fresh samples contained high moisture, which could hinder compound extraction, while dried samples produced higher yields (Ardiansyah *et al*., 2019). Smaller sample sizes increased surface area for solvent interaction, enhancing extraction efficiency (Yulianti *et al*., 2020).

Extraction duration and cycles also impacted yield. Maceration (3 × 24 hours) allowed prolonged solvent contact, facilitating better compound extraction. Soxhlet extraction (14 hours, 7 cycles) had lower yields due to continuous solvent recirculation, leading to compound degradation (Kasiramar, 2018). MAE (5 minutes, 2 cycles) produced yields comparable to maceration despite high temperatures, as microwave radiation rapidly heated the sample, breaking cell walls and releasing compounds (Gupta *et al*., 2012). However, the lack of solvent replacement and fewer extraction cycles may have limited MAE yields compared to maceration.

3.2. Identification of Active Compounds

Sea cucumber extract compounds were identified using thin-layer chromatography (TLC) with silica gel 60 RP-18 as the stationary phase and methanol:water (6:4) as the mobile phase. Phenolic compounds were the primary target due to their significant antioxidant activity. Anisaldehyde reagent was used to detect phenols, terpenoids, and steroids (Stahl, 1969). Compound presence was determined by color spots and Rf values under visible and UV light (254 nm and 366 nm). Results are shown in Figure 1 and Table 2.

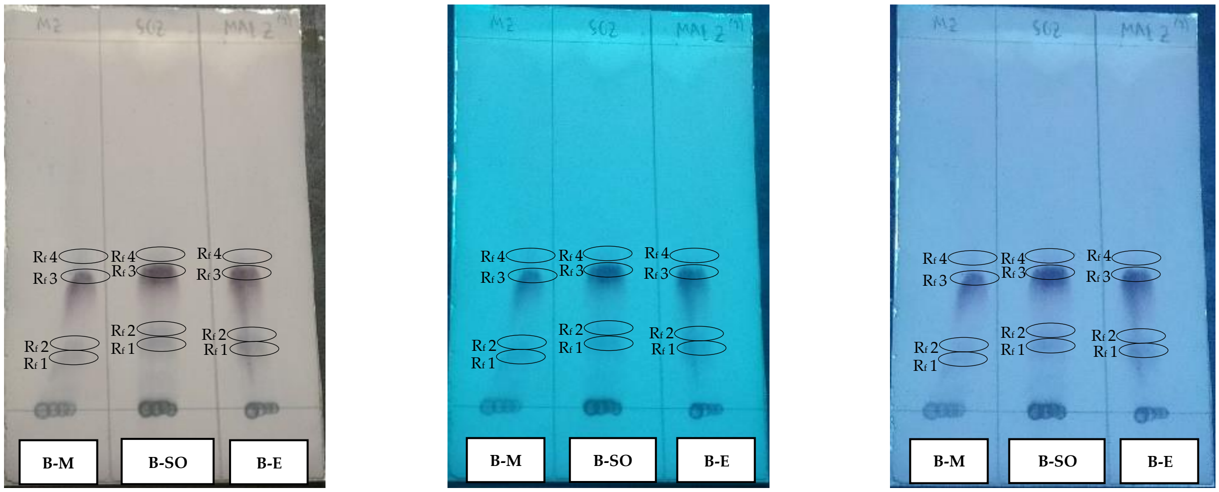


Sample A, Anisaldehyde, Visible Light

Sample A, Anisaldehyde, UV 254 nm

Sample A, Anisaldehyde, UV 366 nm

Figure 1. TLC profile of sea cucumber extracts *Bohadschia marmorata* (A) obtained using maceration (M), Soxhlet extraction (SO), and MAE (E) methods



Sample B, Anisaldehyde, Visible Light — Sample B, Anisaldehyde, UV 254 nm — Sample B, Anisaldehyde, UV 366 nm

Figure 2. TLC profile of sea cucumber extracts *Holothuria fuscopunctata* (B) obtained using maceration (M), Soxhlet extraction (SO), and MAE (E) methods

Table 2. Detection results of compounds in sea cucumber extracts using different extraction methods

Sample	Reagent	Rf value	Spot color	Suspected Compound
A-M	Anisaldehyde	0,1	Green	Terpenoid
		0,18	Yellow	Phenol
		0,29	Purple	Steroid/triterpenoid
A-SO	Anisaldehyde	0,1	Green	Terpenoid
		0,12	Yellow	Phenol
		0,16	Purple	Steroid/triterpenoid
A-E	Anisaldehyde	0,1	Blue-green	Terpenoid
		0,13	Purple	Steroid/triterpenoid
B-M	Anisaldehyde	0,13	Purple	Steroid/triterpenoid
		0,2	Blue	Steroid/triterpenoid
		0,34	Dark purple-black	Steroid/triterpenoid
		0,4	Blue	Steroid/triterpenoid
B-SO	Anisaldehyde	0,16	Purple	Steroid/triterpenoid
		0,2	Blue	Steroid/triterpenoid
		0,36	Dark purple-black	Steroid/triterpenoid
		0,4	Blue	Steroid/triterpenoid
B-E	Anisaldehyde	0,16	Purple	Steroid/triterpenoid
		0,2	Greenish-blue	Steroid/triterpenoid
		0,34	Dark purple-black	Steroid/triterpenoid
		0,4	Blue	Steroid/triterpenoid

After spraying with anisaldehyde reagent, the TLC plates showed different spot colors, including green, yellow, blue, and purple. The detected compounds included phenols and steroids/triterpenoids. According to Wagner & Bland (1996), phenols appear as yellow to brown spots, while steroids/triterpenoids range from blue to purple. This aligns with Putram *et al*. (2017), who found that blue and purple spots indicate steroids/triterpenoids, while yellow spots confirm phenols in sea cucumber extracts. However, phenols were not fully detected, possibly due to their low concentration or degradation from high extraction temperatures and anisaldehyde drying. Lubis *et al.* (2023) noted that active compound levels in sea cucumbers are influenced by habitat, diet, age, and predator interactions.

In addition, this assay is a widely recognized limitation of qualitative phytochemistry screening, which relies on simple color-change, precipitation, or foaming reactions to detect major compounds abundantly contained in the extracts, while low-concentration compounds will not be detected. This phytochemical assay is a preliminary test, which is designed for speed and cost-effectiveness method.

Silica gel 60 RP-18 TLC plates function differently from standard silica gel 60 F_{254} plates. The latter allows non-polar compounds to move faster, reaching higher Rf values, while RP-18 plates retain non-polar compounds and allow polar compounds to migrate further. Despite differences in spot positioning, compound identification remains consistent (Sjursnes *et al*., 2014). Thus, the detected steroids/triterpenoids were confirmed as non-polar due to their low Rf values.

3.3. Antioxidant Activity

Antioxidant activity measures a compound's ability to neutralize free radicals. IC_{50} (Inhibitory Concentration 50) is used to assess the strength of active compounds in combating free radicals. The IC_{50} values of *Bohadschia marmorata* and *Holothuria fuscopunctata* extracts using different extraction methods are shown in Figure 2

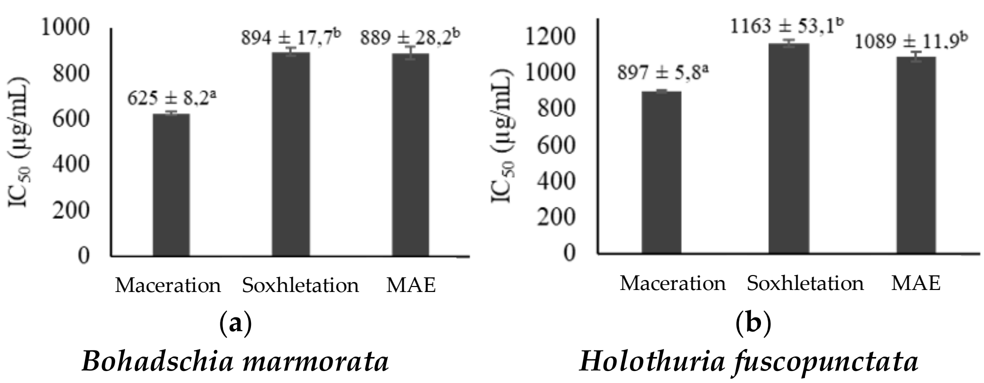


Figure 3. Antioxidant activity values of sea cucumber extracts obtained using different extraction methods

The analysis showed that different extraction methods significantly affected the antioxidant activity of sea cucumber extracts ($p < 0.05$). The lowest IC_{50} values, indicating the strongest antioxidant activity, were obtained from maceration (625 µg/mL for *Bohadschia marmorata* and 897 µg/mL for *Holothuria fuscopunctata*). In contrast, Soxhlet extraction yielded the highest IC_{50} values (894 µg/mL and 1163 µg/mL), followed by MAE (889 µg/mL and 1089 µg/mL). Since lower IC_{50}

values indicate stronger antioxidant activity, all extracts were classified as very weak antioxidants (>200 ppm) (Molyneux, 2004).

These findings differ from previous studies, where fresh *B. marmorata* and *H. scabra* extracts had IC_{50} values of 100.66 μg/mL and 43.58 μg/mL (Nugroho *et al*., 2021), and dried *H. scabra* showed an IC_{50} of 33.77 μg/mL (Hossain, 2022). The weak antioxidant activity observed may be due to the crude nature of the extracts, which still contained interfering compounds such as salts and minerals. Environmental factors also influence antioxidant content, as sea cucumbers produce higher levels of secondary metabolites in stressful habitats (Avigail *et al*., 2019). Additionally, the extracted body part affects activity, with internal organs showing higher antioxidant potential than body walls due to their nutrient content (Oktaviani *et al*., 2015).

Among the extraction methods, maceration produced the strongest antioxidant activity, likely because it does not involve heat, preserving thermolabile compounds such as flavonoids. In contrast, Soxhlet extraction and MAE involve high temperatures (60–70°C and 56°C, respectively), which may degrade flavonoids and reduce antioxidant activity. The results show that the bioactive compounds in the sea cucumber extracts were sensitive to the heat. This finding is in line with previous studies (Wulandari *et al*., 2021; Yuliantari *et al.,* 2017). Antioxidant activity is influenced by compound concentration, temperature, oxygen exposure, lipid content, and chemical composition (Sayuti & Yenrina, 2015).

The antioxidant activity of the samples corresponded with the phenolic compounds detected qualitatively in the samples. Both samples contained steroid and triterpenoid, however, only sample A (*Bohadschia marmorata)* was detected to have phenol and terpenoid as previously reported. Therefore, we suggest that this antioxidant activity is in corresponding with the phenol and triterpenoid contents. This finding is consistent with previous studies (Misgiati *et al.,* 2024; Debi *et al.,* 2025).

5. Conclusions

The maceration method was the most effective extraction technique in terms of yield and antioxidant activity compared to other methods. Maceration resulted in a higher extraction yield and lower IC_{50} values, indicating stronger antioxidant activity than Soxhlet and microwave-assisted extraction. All sea cucumber samples contained steroid/triterpenoid compounds. However, the antioxidant activity of the extracts was classified as very weak. The best antioxidant activity was observed in maceration extracts, with IC_{50} values of 625 μg/mL for *Bohadschia marmorata* and 897 μg/mL for *Holothuria fuscopunctata*.

Author Contributions: Conceptualization, K.T., I.W. and I.S.; supervision, K.T., I.S. and I.W.; designed and performed the experiments and analyzed the data, R.P.N.A. and I.W., writing—original draft preparation, R.P.N.A., K.T. and I.W., writing—review and editing, R.P.N.A., K.T., and I.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in 2024 by the State Budget (APBN) through the Rumah Program scheme of the Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Indonesia.

Data Availability Statement: The data presented in the study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank Dr. Misgiati, Mr. Bima and Ms. Dela for the technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

Ardiansyah, A., Rasyid, A., Nugroho, A., 2019. Antioxidant test and BSLT test on crude extract *Holothuria scabra* with various solvents. Jurnal Ilmu dan Teknologi Kelautan Tropis. 11(3), 627-636. https://doi.org/10.29244/jitkt.v11i3.24169

Avigail, Y., Yudiati, E., Pringgenies, D., 2019. Antioxidant activity and total phenolic content in sea cucumber extracts from Karimunjawa waters, Jepara. Journal of Marine Research. 8(4), 346-354. https://doi.org/10.14710/jmr.v8i4.24600

Bawole, A.S.W., Wewengkang, D.S., Antasionasti, I., 2021. Antioxidant activity of sea cucumber (*H. atra*) extract using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. PHARMACON. 10(2), 863-867. https://doi.org/10.35799/pha.10.2021.34036

Debi, P.R., Barua, H., Ahmmed, M.K., Bhowmik, S., 2025. Therapeutic potential of sea cucumber-derived bioactives in the prevention and management of brain-related disorders: a comprehensive review. Marine Drugs. 23(8), 310. https://doi.org/10.3390/md23080310

Dwicahyani, T., Sumardianto, Rianingsih, L., 2018. Bioactivity testing of *Holothuria atra* extract against *Staphylococcus aureus* and *Escherichia coli*. Journal of Fisheries Processing and Biotechnology. 7(1), 15-24.

Food and Agriculture Organization (FAO), 2012. Commercially important sea cucumbers of the world. Food and Agriculture Organization, Rome, Italy.

Gupta, A., Naraniwal, M., Kothari, V., 2012. Modern extraction methods for bioactive plant extracts. International Journal of Applied and Natural Sciences. 1(1), 8-26.

Hossain, A., Dave, D., Shahidi, F., 2022. Antioxidant potential of sea cucumbers and their beneficial effects on human health. Marine Drugs. 20(8), 521. https://doi.org/10.3390/md20080521

Husain, G., Tamanampo, F.W.S., Manu, G.D., 2017. Structure of sea cucumber (Holothuroidea) communities in the Nyaregilaguramangofa Island coastal area, South Jailolo, West Halmahera, North Maluku. Platax Scientific Journal. 5(2), 177-188.

Kamaluddin, M.H., Lutfi, M., Hendrawan, Y., 2014. The impact of microwave-assisted extraction (MAE) on the extraction of antioxidant catechins from green tea leaves (*Camellia sinensis*): study on extraction time and material-to-solvent ratio. Journal of Tropical Agricultural Engineering and Biosystems. 2(2), 147-155.

Kasiramar, G., 2018. The significant role of the Soxhlet extraction process in phytochemical research. Mintage Journal of Pharmaceutical and Medical Sciences. 7(1), 43-47.

Laila, F., Resmeiliana, I., Yulianti, W., Supardan, A.D., 2022. Evaluation of phenolic compound content, total flavonoids, and in vitro antioxidant activity in methanolic extract of Dayak onion (*Eleutherine palmifolia*). KOVALEN: Journal of Chemistry Research. 8(3), 298-307. https://doi.org/10.22487/kovalen.2022.v8.i3.16175

Lubis, F., Najmi, N., Lisdayanti, E., Nasution, M.A., 2023. Food preferences of sea cucumber (*Holothuria atra*) in Lhok Bubon Beach waters, West Aceh. Journal of Fisheries. 13(1), 89-97.

Massin, C., 1999. Reef-dwelling Holothuroidea (Echinodermata) of the Spermonde Archipelago (Southwest Sulawesi, Indonesia). Zoologische Verhandelingen. 329, 1-144.

Misgiati, Winarni, I., Murniasih, T., Novriyanti, E., Tarman, K., Safithri, M., Setyaningsih, I., Cahyati, D., Pratama, B.P., Wirawati, I., 2024. The anticancer and antioxidant potential of local sea cucumber *Holothuria edulis*, an ecology balancer of Labuan Bajo marine ecosystem. Case Studies in Chemical and Environmental Engineering. 9, 100625. https://doi.org/10.1016/j.cscee.2024.100625

Molyneux, P., 2004. The use of the stable free radical DPPH (diphenylpicrylhydrazyl) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology. 26(2), 211-219.

Nugroho, A., Harahap, I.A., Ardiansyah, A., Bayu, A., Rasyid, A., Murniasih, T., Setyastuti, A., Putra, M.Y., 2021. Antioxidant and antibacterial activities in 21 species of Indonesian sea cucumbers. Journal of Food Science and Technology. 59(1), 239-248. https://doi.org/10.1007/s13197-021-05007-6

Nurhamzah, L.Y., Oktini, T.W., Fahmi, A.S., 2022. Stability of antioxidants in black sea cucumber (*Holothuria atra*) extract under different temperatures and heating durations. Nutrition Scientific Journal. 1(1), 8-20.

Oktaviani, D., Mulyani, Y., Rochima, E., 2015. Antioxidant and antibacterial activities of *Holothuria atra* viscera extract from Biawak Island waters, Indramayu. Journal of Fisheries and Marine Sciences. 6(2), 1-6.

One, N.S., Idiawati, N., Prayitno, D.I., Minsas, S., Fajri, M., 2021. Identification and proximate analysis of *Thelenota ananas* sea cucumber from Kabung Island. Journal of Natural Resource and Environmental Management Research. 10(1), 46-52.

Putram, N.M., Setyaningsih, I., Tarman, K., Nursid, M., 2017. Anticancer activity of active sea cucumber fractions. Journal of Indonesian Fisheries Processing. 20(1), 53-62.

Rahman, M.A., 2014. Global sea cucumber fisheries: culture potential, bioactive compounds, and sustainable utilization. International Journal of Advances in Chemical Engineering and Biological Sciences. 2(1), 53-62.

Samyn, Y., Massin, C., 2003. The holothurian subgenus Mertensiothuria (Aspidochirotida: Holothuriidae) revisited. Journal of Natural History. 37(20), 2487-2519. https://doi.org/10.1080/00222930210144334

Sayuti, K., Yenrina, R., 2015. Antioxidants: natural and synthetic. Andalas University Press.

Sjursnes, B.J., Kvittingen, L., Schmid, R., 2015. Normal and reversed-phase thin-layer chromatography of green leaf extracts. Journal of Chemical Education. 92(1), 193-196. https://doi.org/10.1021/ed400519v

Stahl, E., 1969. Thin-layer chromatography: a laboratory handbook, second ed. Springer, Berlin Heidelberg.

Wagner, H., Bland, S., 1996. Plant drug analysis: a thin layer chromatography atlas, second ed. Springer, Berlin Heidelberg.

Wahyuningsih, S., Yunita, I., Sundari, U.Y., Pagalla, D.B., Kalalinggi, S.Y., Alpian, Nurmalasari, E., Suryandani, H., Ramlah, Nasrullah, M., 2024. Natural product extraction. Gita Lentera Redaksi, Padang, Indonesia.

Wulandari, D.A., Gustini, N., Murniasih, T., Bayu, A., Sari, M., Syahputra, G., Harahap, I.A., Rasyid, A., Moria, S.B., Rahmawati, S.I., Izzati, F.N., Septiana, E., Rachman, F., Putra, M.Y., 2022. Nutritional value and biological activities of sea cucumber *Holothuria scabra* cultured in the open pond system. Journal of Aquatic Food Product Technology. 31(6), 599-614. https://doi.org/10.1080/10498850.2022.2082902

Wulandari, D.A., Murniasih, T., Sari, M., Syahputra, G., Rasyid, A., Septiana, E., Untari, F., Harahap, I.A., Ardiansyah, A., Gustini, N., Putra, M.Y., 2021. Characterization, antioxidant and antibacterial activity of cultivated sea cucumbers from Bali, Indonesia. IOP Conference Series: Earth and Environmental Science. 744, 012102. https://doi.org/10.1088/1755-1315/744/1/012102

Yuliana, Ilyas, A., Suriani, 2017. Isolation of antibacterial bioactive compounds from the ethanolic extract of sand sea cucumber (*Holothuria scabra*) from the Selayar Islands. AL-Kimia. 5(1), 71-80.

Yuliantari, N.W.A., Widarta, I.W.R., Permana, I.D.G.M., 2017. Effect of extraction temperature and time on flavonoid content and antioxidant activity of soursop leaves (*Annona muricata* L.) using ultrasonic extraction. Scientific Media and Food Technology. 4(1), 35-42.

Yulianti, W., Ayuningtiyas, G., Martini, R., Resmeiliana, I., 2020. Effect of extraction method and solvent polarity on total phenolic content of cherry leaves (*Muntingia calabura* L.). Jurnal Sains Terapan. 10(2), 41-49.