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Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Antioxidant Activity of Sea-Cucumber (*Holothurian atra* and *Holothurian edulis*) From Selayar Island

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Abstract. The present investigation was carried out to characterize the bioactive compounds present in extract of *Holothurian atra and Holothurian edulis* using Gas Chromatography-Mass Spectrum (GC-MS) and antioxidan activity. The results of the GC-MS analysis provide different peaks determining the presence of 4 phytochemical compounds with different therapeutic activities. The measurement of antioxidant activity carried out using 1,1difenil-2-pikrihidazil (DPPH) method. The major, 9-Octadecenoic acid (Z)-, methyl ester (47.29), hexadecanoic acid, methyl ester (28.41), octadecanoic acid, methyl ester (4.47), and [1,1'-bibicyclo[2.2.2]Octane]-4-carboxylic acid (92.11) and minor compounds were also present. Antioxidant activity of the ethanolic extract of *Holothurian edulis* have low antioxidant activity with the value IC50 41.6828 mg / mL and ethanolic extracts of *Holothurian atra* have strong antioxidant activity with value IC50 33.514 mg / mL. From the results, it could be concluded that *Holothurian atra and Holothurian edulis* contains various bioactive compounds have various biological activities and antioxidant activity.

Keywords : GC-MS, holothurian atra, holothurian edulis , sea cucumber, selayar island

1. Introduction

Indonesian marine waters have a very high diversity of marine life that can be used for life. Apart from functioning as marine food, this marine biota is also very potential as a source of antitumor compounds, among which are derived from phylum Echinoderms [1]. Sea cucumbers are one of the members of spiny-skinned animals (echinoderms) scattered throughout the Indonesian sea waters, ranging from west to east. This animal is found in almost all beaches, ranging from shallow to 40 meters depth [2]. According to [3], the Ministry of Maritime Affairs and Fisheries made marine biotechnology a superior program since 2002. Rapidly developing marine biotechnology aims to utilize marine biota, by extracting bioactive compounds as medicines.

Bioactive compounds are secondary metabolites produced by microorganisms to defend themselves from threats that come from the environment and the animals around them. Marine animals are not protected from bacteria that tolerate high concentrations, fungi, and viruses, which may be pathogenic to these organisms, so these secondary metabolites are produced to defend themselves [4]. Secondary metabolites from marine biota have potential as anticancer, antiviral and anti-inflammatory [5].

Research on sea cucumbers as bioactives in Indonesia especially those originating from eastern Indonesia still need to be developed. Research on the potential of sea cucumbers in Indonesia, among others, as a vitality enhancer [6], antibacterial [7], antifungal [8]. Because it is necessary to do more research on other properties of sea cucumbers, for example as an antioxidant.

2. Materials and Methods

The tools used in this study were UV spectorfometrics, Erlenmeyer flasks, measuring cups, rotary evaporators, round bottom flask, analytical scales, dropper pipettes, micropipets, incubators, ose needles, knives, vials, funnels, test tubes, test tubes, knives Refrigerator, Tweezers.

The materials used are methanol, ethyl acetate, materials for testing the bioactive content are Dragendorf reagent, Mayer reagent, Mg plate, Amyl alcohol, concentrated HCl, aquadest, and DPPH (1,1-diphenyl-2-picrihydazyl).

The research method used in this study is the experimental laboratories method. The stages of the extraction process are submerging the Holothurian Atra and Holothurian Edulis in fresh water for 1 day, wash and clean the bowels of the Holothurian Atra and Holothurian Edulis, cut the meat and then dried using a solar dryer. Holothurian Atra and Holothurian Edulis dried was macerated with ethanol in a ratio of 1: 4 for 48 hours. The filtrate is then evaporated using a rotary evaporator at 40oC.

3. *Holothurian Atra* and *Holothurian Edulis* Sample Extraction

Obtained from the waters of the Selayar Archipelago, the province of South Sulawesi, which is elliptical with a brownish-gray pattern and has black streaks on its back. The abdomen is white and all over the body surface there are rough spots, when touched it feels like sand. After weeding, the weight of Holothurian atra and Holothurian edulis is obtained clean is 3,200 grams which is then cut into small pieces and dried. The drying process lasts for 5 days. The dry weight of H. scabra is 560 grams, meaning the yield is 17.5%. Physical appearance of sea cucumber that is already dry, hard, stiff, brownish, smells a bit fishy, and contains a bit of lime on the surface of the body

4. Result and Discussion

GC-MS analysis of crude extracts of *Holothurian atra* contains 7 active components. Active components include 9-Octadecenoic acid (Z) -, methyl ester, Hexadecanoic acid, methyl ester, Octadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z, Z) -, methyl ester, n-Hexadecanoic acid, Octadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z, Z) -, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z, Z) Acid, Ethyl Ester, and Ethyl Oleate. The highest percentage of peak area is 9-Octadecenoic acid (Z) -, methyl ester. *Holothuria edulis* contains 3 active components, including [1,1'-Bibicyclo [2.2.2] octane] -4-carboxylic acid, 5- (1-bromo-1-methyl-ethyl) -2-methyl-cyclohexanol, and Hexadecanoic acid, ethyl ester (Table 1).

Antioxidant activity in *H. atra* increases with concentration remembering with a regression value of y = 1.0972x + 13,736 and IC50 value 33,514. whereas *H edulis* has antioxidant activity smaller than H. atra with an IC50 value of 41.6828 with a regression value of y = 1.0938x + 4.4072

Table 1. Bioactive compound detected from ethanolic extract of *Holothurian atra* and *Holothurian edulis*

Sample	Compound analyzed	Peak	% Peak area	R.Time	Moleculer weight	Formula
Holothurian Atra	9-Octadecenoic acid (Z)-, methyl ester	8	47.29	20.430	C19H36O2	296
	Hexadecanoic acid, methyl ester	4	28.41	17.936	C17H34O2	270
	Octadecanoic acid, methyl ester	9	5.96	20.804	C19H38O2	298
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	7	4.47	20.330	C19H34O2	294
	n-Hexadecanoic acid	5	3.42	18.472	C16H32O2	256
	Hexadecanoic Acid, Ethyl Ester	6	2.54	18.869	C18H36O2	284
	Ethyl Oleate	10	1.51	21.412	C20H38O2	310
Holothurian edulis	[1,1'-Bibicyclo[2.2.2]octane]-4-carboxylic acid	1	92.11	8.024	C17H26O2	262
	5-(1-bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	2	2.74	18.093	C10H19BrO	234
	Hexadecanoic acid, ethyl ester	8	6.12	18.905	C18H36O2	284

Table 2. Antioxidant Activity Testing Results

Sample	Konsentrasi ppm	Absorbansi	% Inhibisi	Regresi	IC50
Holothurian atra	10		14.4307	y=1.0972x+13.736	33.514
	30		15.7272		
	50		17.982		
	70		18.4329		
	90		18.5636		
Holothurian edulis	10		5.0723	y=1.0938x+4.4072	41.6828
	30		7.4408		
	50		7.5536		
	70		7.4408		
	90		5.0723		

The gas chromatograms of *H. atra* and *H. edulis* confirmed the existence of numerous interesting compounds with different retention times. The compounds were identified through mass spectrometry attached with GC. The identified compounds and their molecular formula, molecular weight, peak area (%) are given in Table 1; nature of the compounds and their biological activities are given in Table 3. Most of the identified compounds have been reported to possess interesting biological activities (Table 3). 9-Octadecenoic acid (Z)-, methyl ester, Hexadecanoic acid, methyl ester, Octadecanoic acid, methyl ester , 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, n-Hexadecanoic acid, and Hexadecanoic Acid, ethyl ester.

Table 3. Activity of Bioactive compound identified in the ethanolic extracts of *Holothuria atra* dan *Holothuria edulis*

Sr. No	Name of compound	Activity	Reference
1	9-Octadecenoic acid (Z)-, methyl ester	antimicrobial activity anticance activity	[9] [10]
2	Hexadecanoic acid, methyl ester	Antibacterial and antifungal Antioxidant, decrease blood cholesterol, anti-inflammatory	[11] [12]
3	Octadecanoic acid, methyl ester	antibacterial	[13]
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anti-inflammatory and antiarthritic	[10]
5	n-Hexadecanoic acid	antioxidant	[14]
6	Hexadecanoic Acid, ethyl ester	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic	[11]

GC-MS analysis of crude extracts of *H. atra* and *H. edulis* revealed presence of several bioactive compounds having strong biological activities such as antibacterial, antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer, antiarthritic, decrease blood cholesterol, hemolytic, and anti-androgenic.

The presence of antioxidant activity in the extracts of *H. arta* and *H. edulis* (Table 2), can be seen from the results of GC-MS measurements that the active components contained in both sea cucumbers have activity both as antioxidants and as anticancer. Research conducted by [15] found that sea cucumbers have potential as antioxidants. Antioxidants are compounds that can inhibit the oxidation of other molecules.

5. Conclusion

This study reveals that, *Holothuria atra* dan *Holothuria edulis* may also be considered as an important medicinal plant. In fact, six chemical constituents have been identified from a fraction of the ethanol extract by GC-MS analysis. A high percentage of these compounds is represented by fatty acids and phenol compounds which possess many desirable biological activities. The results, it could be concluded that *Holothurian atra and Holothurian edulis* contains various bioactive compounds have various biological activities and antioxidant activity.

6. Reference

[1] Mangindaan, Remy E.P and Margareth S. P. Lesnussa. (2013). Aktivitas Sitotoksik Dari Ekstrak Bintang Ular (*Ophiomastix annulosa*) Terhadap Perkembangan Awal Embrio Bulu Babi (*Tripneustes gratilla*). *Jurnal Pesisir dan Laut Tropis.* **3 (1):**18-23.

[2] Aziz, A. (1997). Status of sea cucumber fisheries and farming in Indonesia. Pusat penelitian Oseanologi LIPI. Jakarta, XXII (1): 9-19 .

[3] Dahuri, R. (2005). Menggali Bahan Baku Obat di dalam Laut. Departemen Perikanan dan Kelautan. *http://www/dkp.go.id* (28 Januari 2012)

[4] Akerina, Febrina Olivia.,Tati Nurhayati., and Ruddy Suwandy (2015) Isolasi dan Karakterisasi Senyawa Antibakteri Dari Bulu Babi. *JPHPI 2015.**18 (1):***61-73

[5] Aprilia, H, A., Delianis P., and Ervia Y. (2012). Uji Toksisitas Ekstrak Kloroform Cangkang dan Duri Landak Laut (*Diadema setosum*) Terhadap Mortalitas *Nauplius Artemia sp. Journal of Marine Research. **1(1):*** 75-8.

[6] Nurjanah S, E Gumbira-Sa'id, K, S., Suprihatin, and Riani, E. (2007). Pengaruh Tepung Teripang Pasir (Holothuria scabra) Terhadap Perilaku Seksual Dan Kadar Testosteron Darah Mencit IPB Bogor.

[7] Aryantina, P, L. (2002). Ekstraksi Komponen Antibakteri dari Teripang dan Pengujian Aktivitasnya sebagai Antibakteria (Skripsi) Institut Pertanian Bogor, Bogor.

[8] Pranoto, E, N., Ma'ruf, W, F., and Pringgenies, D. (2012). Kajian Aktivitas Bioaktif Ekstrak Teripang Pasir (Holothuria scabra) Terhadap Jamur Candida albicans. *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan*, **1(2)**: 1-8

[9] Krishnaveni, M., Dhanalakshmi, R., Nandhini, N. (2014) GC-MS Analysis of Phytochemicals,Fatty Acids and Antimicrobial Potency of Dry Christmas Lima Beans. *International Journal of Pharmaceutical Sciences Review and Research* **27(2)**:63-66.

[10] Singh, R., Chaturvedi, P. (2019). Phytochemical Characterization of Rhizome, Fruit, Leaf and Callus of Rheum emodi Wall. using GC-MS. Pharmacogn J. 2019; 11(3): 617-623

[11] Tulika, T., Agarwal, M. (2017). Phytochemical Screening and GC-MS Analysis of Bioactive Constituents in the Ethanolic Extract of Pistia Stratiotes L. and Eichhornia Crassipes (Mart.) Solms. *Journal of Pharmacognosy and Phytochemistry*; 6(1): 195-206

[12] Hema R., 2S. Kumaravel and 3K. Alagusundaram. (2011). GC/MS Determination of Bioactive Components of Murraya koenigii *Journal of American Science***, 7(1)**

[13] Chandrasekaran, M, Venugopalan V, Krishnan K. (2008). Antimicrobial Activity of Fatty Acid Methyl Esters of Some Members of Chenopodiaceae *Zeitschrift fur Naturforschung C (Z Naturforsch C)* **63(5-6)**:331-6

[14] Prakash, O., Manjul Gondwal and A K Pant. (2011). Essential oils composition and antioxidant activity of water extract from seeds and fruit pulp of Skimmia anquetilia N.P. Taylor & Airy Shaw. *Inian Journal of Natural Products and Resources* Vol. 2(4): 435-441

[15] Rasyid, A., (2012). Identifikasi Senyawa Metabolit Sekunder Serta Uji Aktivitas Antibakteri Dan Antioksidan Ekstrak Metanol Teripang Stichopus hermanii. *Jurnal Ilmu dan Teknologi Kelautan Tropis,* Vol. 4, No. 2: 360-368,