Research Article

Bioactive Compounds, Antioxidant and Anticancer Activities of Sea Cucumber (*Holothuria atra*) Extract from Talaud Islands, Indonesia

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Abstract | The Talaud Islands are located in North Sulawesi Province, which is the center of marine biodiversity in Indonesia because it is located at the center of the world's coral triangle. One of the marine biota that has the potential to produce bioactive compounds that can be used as raw materials for medicines is the sea cucumber. The purpose of this study was to determine the antioxidant activity, anticancer cytotoxic activity, and inventory the bioactive compound profile of sea cucumber extract *Holothuria atra* from the Talaud Islands. Sea cucumber extract was obtained by maceration using 95% ethanol. Determination of the profile of bioactive compounds using gas chromatography-mass spectrometry (GC-MS). Antioxidant activity was analyzed using the DPPH method by determining the IC50 value, a lower IC50 value indicates greater effectiveness of the compound as a free radical scavenger. Cytotoxic activity using MCF-7 (breast cancer cells). The results showed that the ethanol extract of *Holothuria atra* from the Talaud Islands contained 35 compounds identified by GC–MS analysis, with four principal components, including *Cholesterol isocaproate; Cyclopropane, 1-ethenyl-2-hexenyl-, [1.alpha.,2.beta.(E)]-(.+/-.)-; Undecanoic acid, 2-methyl-, methyl ester; 4-Octanol, 7-methyl-*, these compounds act as antioxidants and anticancer agents. Very strong antioxidant activity with IC50 = 10.089 µg/mL using the DPPH method. The ethanol extract of *Holothuria atra* from the Talaud Islands also showed moderate cytotoxic activity as an anticancer in MCF-7 cells with an IC50 value of 160.40 µg/mL. This study supports the development of nutraceuticals and pharmaceuticals.

Keywords | Anticancer, Antioxidant, Bioactive compounds, GC-MS analysis, *Holothuria atra* extract, MCF-7 breast cancer cells

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INTRODUCTION

Indonesia is an archipelago known as a mega-biodiversity country due to its high biodiversity (von Rintelen *et al.*, 2017). Marine resources have various benefits, including as a source of food and pharmaceuticals. The Talaud Islands are located in North Sulawesi Province, which is the center of marine biodiversity in Indonesia, as it is located at the center of the world's coral triangle. One of the potential and economically important marine resources is the sea

cucumber. The economic value of sea cucumbers is as a source of functional food (Hartati *et al*., 2017; Langdal *et al*., 2022), antioxidants (Hossain *et al*., 2022), and antibacterial (Rasyid *et al*., 2021), as anti inflammatory and anticancer (Wargasetia *et al*., 2023).

Antioxidants are compounds that play a role in the inhibiting oxidation, where inhibition is carried out by capturing free radicals (Munteanu and Apetrei, 2021). Free radicals are molecules that cause instability and are reactive, resulting in cell damage, impaired cell function, and even cell death. A free radical is a molecule that has one or more unpaired electrons. Cancer, stroke, heart disease, and premature aging are caused by the presence of free radicals in the body (Caiati *et al*., 2023). To protect the body from free radicals, the body produces antioxidant compounds. Antioxidants are compounds that can slow down or prevent damage caused by free radicals by inhibiting the activity of free radicals or breaking the chain of oxidation reactions induced by free radicals (Tumilaar *et al*., 2024). In experimental animal studies, sea cucumber extracts have been shown to enhance the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, indicating an increase in antioxidant defense mechanisms (Jahani *et al*., 2025; Zhang *et al*., 2025).

Secondary metabolites commonly produced by sea cucumbers are triterpene saponins, also known as triterpene glycosides, which have been demonstrated to exhibit antioxidant effects in various in vitro systems. Saponins show DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity, ferric reducing power, and total antioxidant capacity (Khotimchenko, 2018). Furthermore, isolated saponin complexes exhibit a broad spectrum of cytotoxic effects against various cell types under in vitro conditions, particularly several human cancer cell lines, including hepatoma cancer, epidermoid carcinoma, MCF-7, SK-Mel2, and others (Hoang *et al*., 2020).

Cancer is one of the most complex diseases and is ranked as the leading cause of death worldwide (Bray *et al*., 2024). Cancer occurs due to abnormal cell growth. Breast cancer is a malignant tumor that arises from uncontrollably growing and developing breast cells. MCF-7 cells are human breast cancer cells with estrogen, progesterone, and glucocorticoid receptors and are one of the breast cancer cell models. These cells have also been reported to exhibit resistance to certain chemotherapeutic agents, such doxorubicin (Buxant *et al*., 2017; Prekovic *et al*., 2023). Therapeutic effectiveness in breast cancer is still a challenge; in addition, chemotherapy drugs are nonselective, so they can affect normal cells and cause side effects.

One of the marine biota with the potential to produce bioactive compounds for pharmaceutical applications is the sea cucumber (*Holothuria atra*), a marine invertebrate belonging to the phylum Echinodermata (Santosa *et al*., 2020). *Holothuria atra* from the Talaud Islands has an average body length of 32 cm with a weight of 702 grams, dark black in color, with the body surface covered by small and dense papillae on the dorsal part; its body shape is elongated and cylindrical, thick-fleshed, living in sandy substrates (Manampiring *et al*., 2025), which are slow-moving animals, thus requiring a defense mechanism (Rattu *et al*., 2024). The defense mechanisms of *Holothuria atra* occur mechanically and chemically. The chemical defense mechanism is carried out by producing secondary metabolite compounds. The secondary metabolites produced are expected to provide benefits as sources of antioxidants and anticancer agents. Various species of sea cucumbers contain diverse bioactive compounds with strong antioxidant and anticancer activities, making them potentially useful as sources of biopharmaceuticals (Misgiati *et al*., 2024). However, there has been no research on the potential of sea cucumbers from the Talaud Islands as antioxidants and anticancer agents, nor on the compounds present in extracts of the sea cucumber species *Holothuria atra*.

The purpose of this study was to determine antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, anticancer activity using MCF-7 breast cancer cells, and inventory the components of bioactive compounds from sea cucumber extract *Holothuria atra* from the Talaud Islands.

MATERIALS AND METHODS

Study area

Sea cucumber samples of *Holothuria atra* (Figure 1) were collected from the waters of the Talaud archipelago, Indonesia. Sampling was conducted using the perpendicular transect method at stations in the waters of the Talaud Islands (3°50'13.9"N; 126°47'59.3"E, 3°50'43.1"N; 126°41'41.4"E, 3°57'54.4"N; 126°43'51.1"E, 3°38'05.4"N; 126°51'39.3"E) (Figure 2). Specimen collection at each transect was conducted using a quadrat of 10x20 m, in shallow waters, including sandy, sandy–muddy substrates, and seagrass bed areas. These sites were not influenced by human activities. The research was conducted from June to August 2025. Extraction stages were carried out at the Biology Laboratory of Universitas Negeri Manado, antioxidant analysis at the LPPT Laboratory of Gadjah Mada University, anticancer analysis, and compound content analysis at the Central Laboratory of Padjajaran University.

Figure 1: Sea cucumber Holothuria atra from the Talaud Islands.

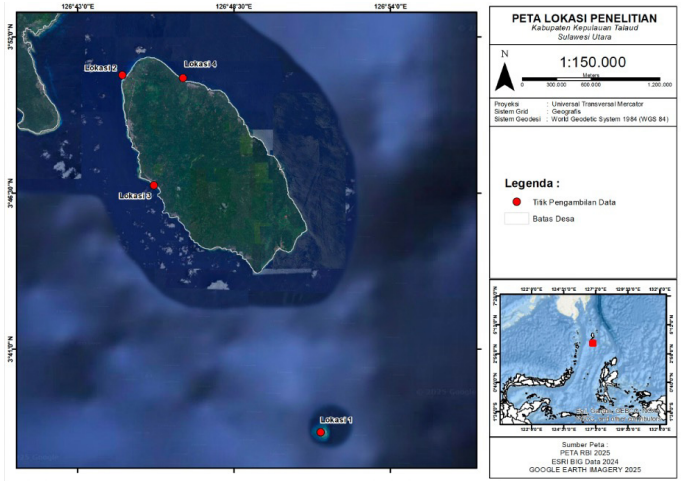

Figure 2: Research location map.

Extraction

The extraction stage refers to the research of Misgiati *et al.* (2024), which is modified, starting with the preparation of sea cucumber (*Holothuria atra*) material, then the viscera are removed, cleaned, and dried. Muscle samples were cut into pieces with a size <1 cm. The maceration process was carried out using a polar solvent, namely 95% ethanol, in a ratio of 1:4 with occasional agitation using a shaker at 180 rpm. The maceration lasted for 2 x 48 hours. The extraction results were then filtered with Whatman filter paper. The next process was evaporation with a vacuum rotary evaporator using a temperature of 40° C, resulting in a viscous extract.

GC-MS analysis (gas chromatography-mass spectrometry)

The compound analysis of *Holothuria atra* was conducted using the GC–MS method, referring to Wakoli *et al.* (2024) with modifications, using MassHunter GC/MS Acquisition 10.0.368 (Agilent Technologies, Inc.), time set for 61.5 minutes with a temperature of 280 °C, detector 240 °C, and column 325 °C. The carrier gas used was helium at a constant flow rate of 1 ml/min. The identification process using GC-MS tools produces several bioactive compounds. It can be seen from the chromatogram peak that the identification of chromatography and mass spectrometry (MS) data is seen from the mass spectrum, with each molecular weight of bioactive compounds.

Analysis of antioxidant activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method to determine the ability of the extract to scavange free radicals, follows the method of Gulcin and Alwasel (2023) with modifications. The first stage was the preparation of a control solution; as much as 1 mL of 0.4 mM DPPH solution was added to 4 mL of ethanol. The control solution was subsequently used to determine the maximum wavelength using a UV-Vis spectrophotometer. The wavelength obtained was 517 nm. Then, making the extract stock solution, the control solution that has been made is measured for absorbance at the maximum wavelength to determine the absorbance of the control. The sample used weighed 585.8 mg, the volume of solution was 25 mL, and the content was 23.432 µg/mL. Samples with a certain volume were added with 1 mL of 0.4 mM DPPH solution, and ethanol was added until the total volume became 5 mL. The detailed composition of the antioxidant test volumes used in the DPPH assay is presented in Table 1. The solution was incubated in a dark room for 30 minutes. The sample solution was then measured for absorbance at the maximum wavelength to determine the absorbance of the sample. Determination of Inhibition Concentration 50 value (IC50), based on % inhibition, namely:

$$\%\ Inhibition = \frac{A\ control - A\ sample}{A\ control}\ 100\%$$

Furthermore, a regression curve was created by plotting the sample concentration vs % Inhibition. The IC50 value was calculated using the regression equation that was made.

Analysis of cytotoxic activity of MCF-7 cells

The workflow carried out is first cell culture that will be used in 96-well plates, then incubated (at 37 °C and 5% CO_2 gas until the percentage of cell growth reaches 70%), cells are treated with samples, and then incubated (for 48 hours at 37 °C and 5% CO_2 gas). Then, the addition of resazurin working reagent to the cells and measurement of absorbance using a multimode reader. The anticancer activity analysis followed the procedures of Kis *et al.* (2022) with modifications.

Table 1: Data collection of antioxidant test volume using the DPPH method

Collection (mL)	Concentration (µg/mL)
0.500	2.343
1.000	4.686
2.000	9.373
3.000	14.059
4.000	18.746

The working procedure carried out, the first stage is the preparation of media, positive controls, and samples, by preparing complete Roswell Park Memorial Institute Medium (RPMI) liquid culture media (containing 10% Fetal Bovine Serum (FBS) and 50 µL/50 mL of antibiotics). The positive control used in this test is Cisplatin, which is then dissolved in the sample with the final concentration as a stock. A solvent that is not toxic to cells is used. Then the antiproliferation assay working solution was prepared, namely Resazurin Sodium Salt-Powder, BioReagent. Furthermore, the cell preparation stage that will be used is at least 70% confluent, then the cells are rinsed twice with 1 mL PBS, added 1 mL Trypsin-EDTA solution, then incubated for 5 minutes so that the cell layer is dispersed, then transferred into a tube containing media, centrifuged at 3000 rpm for 5 minutes, then the supernatant is discarded, then the pellet is dissolved into a tube containing media. Cells were then plated into 96-well plates, and the number and viability of cells were determined by trypan blue exclusion. Cells were resuspended with a final cell density of 170,000 cells/ml in media, and seeded into 96-well plates at 17,000 cells per well, corresponding to 100 µL of cell suspension per well. Then, the cells were incubated for 24 hours (or until cells were confluent, min. 70%) at 37 °C and 5% CO_2 gas. Cells were then treated with samples, positive and negative controls, and incubated for 48 hours. Next, administration of resazurin reagent and absorbance measurement, and media in each well. 9 mL of media in the tube was added to 1 mL of Resazurin Sodium Salt-Powder, BioReagent" (10 µL of reagent for 90 µL of media); then 100 µL of the solution mixture was put into each microplate well and incubated for 1-2 hours until a color change was seen (when entering living cells, Resazurin Sodium Salt reagent will be reduced from the blue compound resazurin without intrinsic fluorescent value to the red and highly fluorescent compound resorufin). The conversion value is proportional to the number of metabolically active cells and can therefore be measured quantitatively. To measure absorbance, absorbance spectra for resazurin and resorufin are used. Furthermore, the absorbance was measured at a wavelength of 570 nm using a multimode reader.

RESULTS AND DISCUSSION

Sea cucumber extract *Holothuria atra*

The extract of *Holothuria atra* from the Talaud Islands is a deep orange color with a thick texture (Figure 3). The extraction yield (%) is the ratio between the weight of the obtained extract and the weight of the simplicia used in the soaking process. The ethanol extract of *Holothuria atra* was carried out by the maceration method with ethanol, showing a good extraction yield, which was 10.534% of the thick extract of 21.068 g. The yield is categorized as good if the value is more than 10% (Indonesian Ministry of Health, 2017). However, it should be noted that extraction yield reflects extraction efficiency rather than the concentration of bioactive compounds, as the extract may also contain non-bioactive constituents. This yield measurement is done by comparing the weight of the extract obtained with the weight of the initial simplicia extracted. Therefore, yield values do not necessarily indicate the abundance of active compounds, and further chemical and biological analyses are required to evaluate the bioactive potential of the extract. The yield of a sample is needed to determine the amount of extract obtained during the extraction process.

Figure 3: Sea cucumber (Holothuria atra) extract.

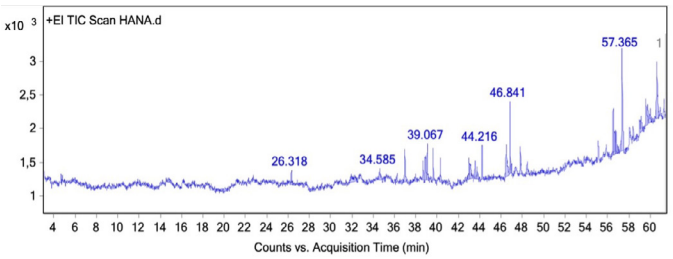

Figure 4: GC-MS Chromatogram of Sea Cucumber Holothuria atra from the Talaud Islands.

Analysis of bioactive compounds with GC-MS

Analysis of *Holothuria atra* sample extract with GC-MS (Gas Chromatography-Mass Spectrometry) obtained a chromatogram that represents the composition of all the constituents of antioxidant and anticancer compounds from the sample. From the chromatogram data, there are many peaks seen in high and large percentages of area, shown in Figure 4. GC-MS results from the chromatogram data show there are four peaks with large percentages, meaning they are the main compounds in the test sample. Table 2 shows 35 main compounds detected in the extract of sea cucumber *Holothuria atra* from the Talaud Islands. Four compounds among them have a large peak height and percent area, namely *Cholesterol isocaproate; Cyclopropane, 1-ethenyl-2-hexenyl-, [1.alpha.,2.beta.(E)]-(.+/-.)-; Undecanoic acid, 2-methyl-, methyl ester; 4-Octanol, 7-methyl-.*

Table 2: Compounds detected in the extract of Holothuria atra from the Talaud Islands.

Peak	Peak area	RT	Area sum %	Height	Width	FWHM	Compound Name
1	316.43	4.677	0.5	116.04	0.086	0.048	Ethane, 1,1'-oxybis[2-methoxy-
2	286.01	4.831	0.46	97.03	0.118	0.037	Semioxamazide
3	390.15	5.969	0.62	70.33	0.151	1.08	2-Formylhistamine
4	1036.9	26.32	1.65	221.14	0.215	0.059	Manganese, bis-[(3- dimethylamino) propylcyclopentadienyl]-
5	2347.8	31.91	3.75	125.32	0.686	0.622	1,2-Ethanediamine, N-ethyl-N'-methyl-
6	1098	34.59	1.75	168.74	0.287	0.069	Mexiletine
7	2829.4	36.95	4.51	489.11	0.244	0.083	Benzenemethanol, .alpha.-[1- (ethylmethylamino)ethyl]-, [R-(R*,S*)]-
8	864.63	37.97	1.38	113	0.287	0.142	Mexiletine
9	1070.8	38.67	1.71	274.2	0.147	0.066	Phthalic acid, 2-methoxyethyl propyl ester
10	1836.3	38.89	2.93	352	0.157	0.083	2,3-Epoxyhexanol
11	1968.2	39.07	3.14	538.62	0.2	0.049	4-Octanol, 7-methyl-
12	1524.9	39.62	2.43	467.18	0.132	0.048	Heptanoic acid, 2-ethyl-
13	1269.3	40.29	2.03	344.26	0.184	0.051	Pentanal
14	1514.5	42.99	2.42	317.74	0.164	0.082	1-Octadecanamine, N-methyl-
15	2133.3	43.09	3.4	220.33	0.308	0.175	4-Trifluoroacetoxyoctane
16	1363.9	43.51	2.18	259.35	0.183	0.091	4-Dodecanol
17	868.1	43.72	1.38	178.92	0.199	0.074	4-Trifluoroacetoxyoctane
18	1841.9	44.22	2.94	497.76	0.151	0.057	Undecanoic acid, 2-methyl-, methyl ester
19	3789	46.48	6.05	468.11	0.354	0.128	1-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic acid, 4,4-dimethyl-2-oxotetrahydrofuran-3-yl ester
20	3682.2	46.84	5.87	1077	0.164	0.05	Cyclopropane, 1-ethenyl-2-hexenyl-, [1.alpha.,2.beta.(E)]-(.+/-.)-
21	395.38	46.98	0.63	140.86	0.089	0.038	R-(-)-Cyclohexylethylamine
22	1910.3	47.82	3.05	407.56	0.215	0.059	2-Pentene, 5-bromo-2,3-dimethyl-
23	1319.5	48.46	2.11	187.8	0.287	0.067	Cyclobutanol
24	172.31	49.08	0.27	80.75	0.082	0.199	sec-Butylamine
25	325.36	49.85	0.52	70.79	0.142	0.202	1-Octadecanamine, N-methyl-
26	348.62	50.47	0.56	95.44	0.164	0.502	Tetrahydrorhombifoline
27	2949.8	56.53	4.71	690.24	0.174	0.063	1-Methylene-2b-hydroxymethyl-3,3-dimethyl- 4b-(3-methylbut-2-enyl)-cyclohexane
28	1381.2	56.7	2.2	340.55	0.113	0.092	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene. 2TMS derivative
29	1676.1	56.77	2.67	359.87	0.152	0.086	Methyl 3-bromo-1-adamantaneacetate
30	636.63	56.92	1.02	126.85	0.161	0.109	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
31	8119.2	57.37	12.95	1530.9	0.252	0.061	Cholesterol isocaproate
32	1580.8	59.14	2.52	226.33	0.23	0.143	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative:
33	2599.7	59.6	4.15	396.25	0.198	0.13	1,4-Bis(trimethylsilyl)benzene
34	1243	60	1.98	200.21	0.228	0.085	1,2-Bis(trimethylsilyl)benzene
35	5989.9	60.63	9.56	852.12	0.295	0.087	Methyl 3-bromo-1-adamantaneacetate

Gas Chromatography Mass Spectrometry (GC-MS) is a gas chromatography technique used in conjunction with mass spectrometry. Chromatography is able to read compounds with the lowest concentration so that secondary metabolites in sea cucumber extracts are identified with results in the form of chromatograms and mass spectra. All compounds in this study were tentatively identified based on library matching supported by mass spectral fragmentation patterns. Compounds with relative abundance <1% were included for completeness but interpreted with caution due to lower signal intensity. Therefore, only major compounds (≥1% area) are discussed as potential contributors to the observed biological activity.

Antioxidant activity

Measurement of antioxidant activity was determined

by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, starting with the preparation of a blank solution. Making a blank solution aims to determine the amount of absorption by a solution that does not contain the analyte. Then the absorbance was measured and incubated for 30 minutes. The purpose of incubation is to provide time for the reaction between antioxidant compounds and DPPH radicals (Baliyan *et al.*, 2022). The absorbance values and antioxidant activity are presented in Table 3. The measurements were performed in triplicate (biological replicates), using three independently prepared extracts, and the results are expressed as mean ± standard deviation.

$$IC50 = \frac{50 - 11.115}{3.8541} = 10.089\ \mu g/mL$$

Determination of the maximum wavelength of the DPPH standard solution was done by measuring the absorption of the DPPH solution at the maximum absorption wavelength of DPPH at 517 nm. IC50 was determined from linear regression (y = 3.8541x + 11.115; R^2 = 0.9908). The results showed that the IC50 value = 10.089 µg/mL. This IC50 value is the percent inhibition calculated using the regression equation by making the sample content vs % inhibition (Figure 5).

Cytotoxic activity of MCF-7 cells (Anticancer)

The IC50 value in Table 4 and Figure 6 shows the concentration value that produces 50% inhibition of cell proliferation and indicates the potential toxicity of a compound against cells. The IC50 level is calculated from the percentage of dead cells incubated for 24 hours with the addition of the extract. The IC50 value of cisplatin obtained in this assay was 12.95 µg/mL. The test results showed that sea cucumber extract was moderately cytotoxic or slightly active with an IC50 value of 160.4 µg/mL. The IC50 value was determined using nonlinear regression analysis of the dose–response curve, which provides a more accurate estimation by considering all data points despite minor variability at certain concentrations.

The graph of the relationship between extract concentration and mean cell viability can be seen in Figure 6. Table 4 displays the Cytotoxic activity of *Holothuria atra* extract on MCF-7 cells expressed as cell viability (%) or absorbance (mean ± SD). Cell viability was calculated relative to the untreated control (100%), values above 100% indicate increased metabolic activity rather than actual cell proliferation. Slight variations in cell viability at certain concentrations may be attributed to biological variability and the complex composition of the extract. Data are presented as mean ± standard deviation (n = 3). Treatment of ethanol extract of *Holothuria atra* showed a change in color on the well plate (Figure 7), and changes in MCF-7 cell morphology (Figure 8). The top row shows control groups: media + cells (untreated control), DMSO 2% (negative control), and cisplatin (positive control). The second row shows treatment with extract at 1000, 500, 250, and 125 µg/mL, while the third row shows 62.50, 31.25, 15.63, and 7.81 µg/mL. Cells were observed using an inverted microscope at 400x magnification.

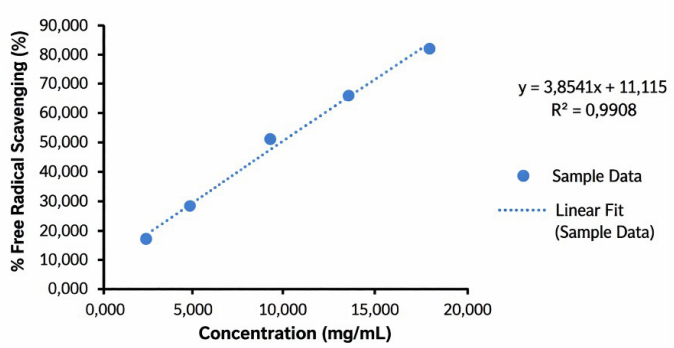

Figure 5: Regression of concentration (µg_mL) vs free radical scavenging (%).

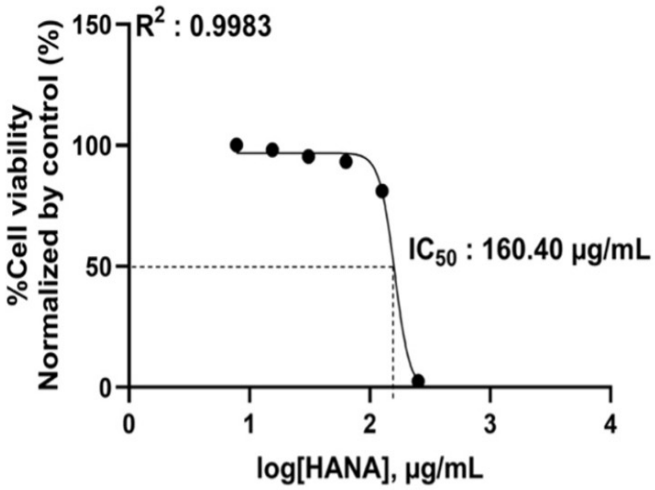

Figure 6: Cytotoxicity test curve of MCF-7 cells.

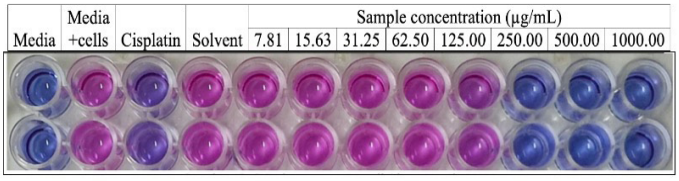

Figure 7: Well plate documentation.

DISCUSSION

The GC-MS chromatogram of the ethanol extract of *Holothuria atra* recorded 35 peaks corresponding to bioactive compounds that were tentatively identified based on comparison with NIST (National Institute of Standards and Technology) and PubChem NCBI (National Library of Medicine) databases supported by mass spectral fragmentation patterns. Previous studies have reported that compounds structurally similar to cholesterol derivatives, fatty acid esters, and alcohol derivatives may exhibit

Table 3: DPPH radical scavenging activity of *Holothuria atra* extract at different concentrations.

Concentration (µg/mL)	Absorbance			% Radical scavenging activity			Average (%)	SD
	I	II	III	I	II	III		
2.343	0.746	0.746	0.745	18.112	18.112	18.222	18.149	0.063
4.686	0.650	0.650	0.650	28.650	28.650	28.650	28.650	0.000
9.373	0.444	0.444	0.443	51.262	51.262	51.372	51.299	0.063
14.059	0.312	0.312	0.312	65.752	65.752	65.752	65.752	0.000
18.746	0.170	0.170	0.169	81.339	81.339	81.449	81.376	0.063

Description: Control absorbance (A_0) used for the calculation of % inhibition was 0.911.

Table 4: Cytotoxic activity of *Holothuria atra* extract on MCF-7 cells expressed as cell viability (%) or absorbance (mean ± SD).

	Media	Media + cells	Cisplatin	Solvent	Sample concentration (µg/mL)							
					7.81	15.63	31.23	62.50	125.00	250.00	500.00	1000.00
Absorbance 570nm	0.5755	0.8813	0.6863	0.8895	0.8829	0.8772	0.8659	0.8590	0.8456	0.6111	0.6024	0.5860
	0.5705	0.8874	0.6891	0.8884	0.8867	0.8787	0.8649	0.8599	0.8461	0.6176	0.6011	0.5898
Absorbance 600nm	0.7043	0.2454	0.5761	0.2317	0.2237	0.2341	0.2417	0.2527	0.3360	0.7291	0.7396	0.7437
	0.7078	0.2432	0.5640	0.2276	0.2235	0.2328	0.2422	0.2538	0.3350	0.7244	0.7353	0.7400
Corrected absorbance (A570 – A600)	-0.1288	0.6359	0.1102	0.6578	0.6591	0.6431	0.6242	0.6063	0.5096	-0.1180	-0.1372	-0.1577
	-0.1373	0.6442	0.1251	0.6608	0.6632	0.6459	0.6227	0.6061	0.5111	-0.1068	-0.1341	-0.1502
% Live cells		99.47	31.46	102.47	102.47	100.40	97.95	95.63	83.12	1.95	-0.54	-3.19
		100.53	33.39	102.68	102.99	100.75	97.76	95.61	83.32	3.39	-0.14	-2.22
Average % Live cells		100.00	32.43	102.49	102.73	100.57	97.85	95.62	83.22	2.67	-0.34	-2.71
SEM		0.53	0.96	0.19	0.26	0.18	0.10	0.01	0.10	0.72	0.20	0.49
Normalization of Data % Live cells		97.57	31.64	100.00	100.24	98.13	95.48	93.30	81.20	2.60	-0.33	-2.64

Description: The IC50 concentration of Cisplatin used in the test was 43.18 µM = 12.95 µg/mL.

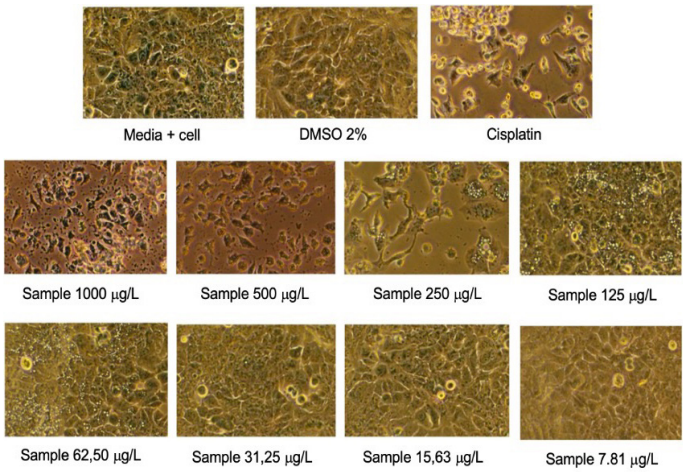

Figure 8: MCF-7 cell morphology after testing with Holothuria atra extract from the Talaud Islands.

biological activities such as antioxidant, antimicrobial, and cytotoxic effects (Salau *et al*., 2023; Radman *et al*., 2023; Peng *et al*., 2024; Martinez *et al*., 2019). These findings suggest that the dominant compounds detected in this study may contribute to the observed bioactivities.

Furthermore, the presence of these compound classes is consistent with previous reports on marine invertebrates, particularly sea cucumbers, which are known to produce diverse secondary metabolites with pharmacological potential. The strong antioxidant activity observed may be associated with the ability of these compounds to act as free radical scavengers or to modulate oxidative stress pathways. Meanwhile, the moderate cytotoxic activity against MCF-7 cells suggests potential anticancer properties, possibly through mechanisms such as membrane disruption, induction of apoptosis, or interference with cellular metabolism. It is important to note that the biological activities observed in this study are likely the result of synergistic or additive interactions among multiple constituents present in the extract, rather than the effect of a single compound.

The DPPH method is used to evaluate the antioxidant activity of compounds present in sea cucumber extracts, so it is widely used to test the ability of compounds that act as electron donors. Based on the results of antioxidant activity measurements on sea cucumber extract *Holothura atra* from the Talaud Islands, it shows that with an increase in solution concentration. The higher the concentration of the solution, the more antioxidant compounds are available

to donate hydrogen or electrons to the DPPH radical. The higher the concentration of the solution, the greater the % free radical capture value obtained.

Previous studies have reported varying antioxidant and cytotoxic activities of *Holothuria atra* depending on geographical origin and extraction conditions. For example, *Holothuria atra* from Lampung, Indonesia, exhibited strong antioxidant activity with an IC_{50} value of 14.22 ± 0.87 µg/mL (Nugroho *et al*., 2022), while samples from Bangladesh showed an IC_{50} value of 88.39 µg/mL (Khandakar *et al*., 2026). In comparison, the extract in the present study demonstrated a lower IC_{50} value (10.089 µg/mL), indicating relatively stronger antioxidant activity. IC50 is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. The smaller the IC50 value obtained, the higher the antioxidant activity of a compound, and vice versa. The IC50 level category states that, if the IC value is <50 ppm, then the compound is a very strong antioxidant, with strong activity for IC 50-100 ppm, moderate activity for IC 100-250 ppm, and weak activity for IC 250-500 ppm (Itam *et al*., 2021; Fatmawati *et al*., 2019). This means that the smaller the IC50 value obtained, the more effective the compound is as a free radical scavenger. The IC50 value (10.089 µg/mL) indicates strong antioxidant activity for a crude extract, although it is generally higher than that of standard antioxidants such as ascorbic acid or BHT. This difference is expected due to the complex composition of crude extracts. The strong antioxidant activity of the extract is likely due to the presence of bioactive compounds that act as free radical scavengers. This activity is probably the result of synergistic interactions among multiple constituents rather than a single compound. Antioxidants are compounds that can inhibit and prevent the oxidation process. It works by stopping free radical reactions from metabolism in the body or from the environment (Andrés *et al*., 2023). Antioxidants are crucial for protecting immune cells from damage caused by free radicals. Leukocyte differentiation tests conducted by Nursid *et al*. (2021) showed that *Holothuria atra* extract enhanced the immune response in test animals, thus offering potential as an immunostimulant.

The cytotoxic properties of a compound can be expressed in IC50 values that are classified into several categories. The United States National Cancer Institute classifies cytotoxic, where IC50 values < 20 µg/mL include high cytotoxic activity, IC50 21-200 µg/mL includes moderate cytotoxic activity, IC50 201-500 µg/mL includes weak cytotoxic, and IC50 > 500 µg/mL has no cytotoxic activity. MCF-7 cells are breast cancer cells (Aniogo *et al*., 2022). Cytotoxic is a substance or compound that can damage normal cells and cancer cells, so it can be used to inhibit the growth of malignant tumor cells and has the potential as an anticancer drug. From Figure 8, it can be seen that MCF-7 cell morphology shows moderate or moderately active cytotoxic activity, indicated by changes in cell morphology, namely inhibition of MCF-7 cancer cell growth. This is due to the presence of compounds that can inhibit the proliferation of MCF-7 cancer cells in sea cucumber extract.

In terms of cytotoxic activity, Holothuria atra extract from the Persian Gulf exhibited potent effects with IC_{50} values ranging from 1.2 to 2.5 µg/mL against HeLa cells (Grauso *et al*., 2019), whereas extracts from the Mentawai Islands showed moderate activity with an IC_{50} value of 41.371 µg/mL against HSC-3 cells (Tasia *et al*., 2025). In contrast, the extract in this study showed an IC_{50} value of 160.4 µg/mL against MCF-7 cells, indicating moderate cytotoxic activity. The IC50 value is an in vitro concentration that can inhibit cancer cell activity by 50%. If the IC50 value obtained is smaller, it means that the compound has the potential to be a good anticancer because only a low dose is needed to inhibit cancer cell proliferation activity by 50% (Berrouet *et al*., 2020). The observed morphological alterations, including cell shrinkage, rounding, detachment, and decreased cell density, suggest the presence of apoptotic-like features. However, as this study employed a resazurin-based cytotoxicity assay combined with morphological observation without the use of apoptosis-specific assays, these changes cannot be conclusively attributed to apoptosis and may also reflect other forms of cell death. The cytotoxic effect observed in this study may involve oxidative stress mediated mechanisms, where increased reactive oxygen species (ROS) can disrupt cellular redox balance and damage essential biomolecules. In addition, mitochondrial dysfunction may contribute through loss of membrane potential and activation of downstream cell death pathways. However, these mechanisms were not directly investigated in this study and require further experimental validation to elucidate the underlying molecular pathways. Apoptosis, or programmed cell death, is a cellular process that occurs in normal and pathological physiological conditions. Induction of apoptosis is one of the mechanisms that contribute importantly to cancer therapy (Chimento *et al*., 2023). Thus, the cytotoxic activity of *Holothuria atra* crude extract from the Talaud archipelago has potential as a breast anticancer. Overall, the results indicate that *Holothuria atra* extract has potential as a source of bioactive compounds with antioxidant and cytotoxic activities; however, further studies are required to confirm its mechanisms and therapeutic relevance.

CONCLUSION

The ethanol extract of the sea cucumber *Holothuria atra* from the Talaud Islands showed very strong antioxidant activity with IC50 = 10.089 µg/mL using the DPPH

method. The ethanol extract of *Holothuria atra* also showed moderate cytotoxic activity as an anticancer in MCF-7 cells with IC50 = 160.40 µg/mL. There are 35 main compounds contained in *Holothuria atra* sea cucumber extract samples using GC-MS; compounds found in the extract act as antioxidants and anticancer agents.

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NOVELTY STATEMENT

The Talaud Islands are located in the world coral triangle, with a high diversity of marine biota, but there has been no research that examines the role of sea cucumbers from the Talaud islands as antioxidants and anticancer using MCF-7 cells (cytotoxic activity of breast cancer cells), as well as compounds contained in Holothuria atra sea cucumber extract from the Talaud islands by GC-MS method.

AUTHOR'S CONTRIBUTION

NM, MYS, and RAM participated in laboratory testing, data analysis, and manuscript preparation. IS and NLIMO contributed to laboratory testing, and data processing. VIYR and FNN contributed to field sampling.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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