ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 26 (1) (2023): 1–9

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Antioxidant and Anticancer Activities of Sand Sea Cucumber (*Holothuria scabra*) Extracts using Wet Rendering Extraction Method

Nunik Gustini [a,*], Diah Anggraini Wulandari [a], Fauzy Rachman [a], Eris Septiana [a], Siti Irma Rahmawati [a], Gita Syahputra [a], Martha Sari [a], Masteria Yunovilsa Putra [a]

[a] Research Center for Vaccine and Drug, Research Organization for Health, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor Km. 46, Cibinong-Bogor, West Java 16911, Indonesia

* Corresponding author: nunik.gustini@brin.go.id

https://doi.org/10.14710/jksa.26.1.1-9

Article Info

Article history:

Received: 18th April 2022
Revised: 9th September 2022
Accepted: 20th September 2022
Online: 31st January 2023

Keywords:
sea cucumber, *Holothuria scabra*, antioxidant, anticancer, wet rendering

Abstract

Antioxidant and anticancer activities of sand sea cucumber *Holothuria scabra* (dried and fresh) extracts were studied. The highest extraction yield of sea cucumber *H. scabra* (3.9%) was obtained using dried *H. scabra* at 60°C. The highest antioxidant activity was found in fresh *H. scabra* extract at 60°C with an IC_{50} value of 629.89 ± 0.15 µg/mL using the ABTS method, and the highest antioxidant activity by DPPH method was found in dried *H. scabra* extract at 70°C with an IC_{50} value of 32017.18 ± 0.82 µg/mL. The best antioxidant activity based on FRAP and TBARS methods was found in fresh *H. scabra* extracts at 80°C, respectively. The highest total phenol and flavonoid contained in dried *H. scabra* extract were 317.54 ± 8.91 mg GAE/100 g sample and 247.56 ± 11.70 mg QE/100 g sample. *H. scabra* extracts inhibited more than 50% of the growth of the MDA-MB-231 cell line at concentrations of 25 and 50 µg/mL except for dried *H. scabra* extracts at 80°C. Similarly, the extracts showed the highest cytotoxic effect up to 100% at the highest concentration (100 µg/mL) except for dried *H. scabra* extracts at 70°C and 80°C.

1. Introduction

Sea cucumbers are cylindrical invertebrates that live in various seabed habitats, from warm tropical waters to deep cold seas. These marine benthic organisms can be found in seagrass, coral reef, mangrove, and mudflat ecosystems [1, 2]. *Holothuria scabra*, also known as sand sea cucumber (sandfish), belongs to the *Holothuriidae* family, generally having the same anatomy as other sea cucumbers [3]. *H. scabra* is mainly found in tropical areas of the Pacific region and is one of the most important commercial species.

Sea cucumbers have high nutritional value because they contain high protein and low levels of fat [2] and are utilized as a source of functional food. There are more than 50 nutrients contained in sea cucumbers, including amino acids, polyunsaturated fatty acids, vitamins, trace elements, and active substances such as polysaccharides, collagen, saponins, and brain glycosides [4]. Sea cucumbers are used as a traditional and functional food and traditional medicine due to their nutritional value. Bioactive compounds in sea cucumbers, such as peptides, phenolics, glycoproteins, and glycosaminoglycans, have therapeutic properties and medicinal benefits [5]. Various studies have reported that sea cucumber extracts can cure asthma [6], have cytotoxic and anticancer activity [7], are antiproliferative and anti-inflammatory [8], and have antioxidant properties [9] that can inhibit the formation of free radicals in the body.

Free radicals are very active atoms or molecules with an unpaired electron in valence electrons [10]. Free radicals have essential roles in the human body, such as creating energy, cell growth, signaling, and immunization [11]. However, due to their high activity, free radicals can cause various cell and tissue disorders and damage or modify DNA. These disorders can lead to

cell death, gene abnormalities, and cancer. Bioactive compounds that are naturally contained in food and beverages derived from plants, animals, and microbes can inhibit or eliminate free radicals from the body [3]. Besides having antioxidant activity, sea cucumber also has anticancer activity and potentially be an anticancer drug [12]. Anticancer activity of bioactive compounds from sea cucumber through several molecular mechanisms in cancer cells such as cytotoxicity activity [7], induction of apoptosis [13], cell cycle arrest [14], reduction of tumor growth [15], antimetastatic and antiangiogenic [16], and inhibition of drug resistance [17] has been reported.

Maceration and soxhletation are commonly used for extraction; however, both methods involve using many chemical solvents. Antioxidant and anticancer activities of sea cucumber *H. scabra* extracts obtained by soxhletation, maceration, and water infusion have been reported by Wulandari *et al.* [18]. The soxhletation extracts showed potential antioxidant and anticancer activity with IC_{50} of 5.97 ± 0.95 TEAC/mg extract and 10.96 ± 16.27 µg/mL (against human breast cancer MDA-MB-231), respectively. The potent cytotoxic activity against MDA-MB-231 was also demonstrated by maceration extracts with IC_{50} of 12.64 ± 9.97 µg/mL [18]. The research conducted by Althunibat *et al.* [9] and Nobsathian *et al.* [3] also showed the antioxidant activity of *H. scabra* extracts obtained from maceration. However, the antioxidant and anticancer activities of sea cucumber *H. scabra* extracts obtained by the wet rendering extraction method have not been widely reported.

The wet rendering extraction method is carried out by adding the sample with water and then heating it to destroy the cellular structure [19, 20]. Wet rendering extraction has the advantages of easy handling, low cost, and no chemical use throughout the procedure [19]. Therefore, wet rendering can be an alternative environmentally friendly extraction method. This research examined the antioxidant and anticancer activities of *H. scabra* extracts obtained by wet rendering extraction methods using dried and fresh *H. scabra*. The information gained from this study provides an overview of the wet rendering extraction method along with the antioxidant and anticancer activities of the extracts obtained so that further development can be carried out.

2. Materials and Methods

2.1. Sample preparation

The *H. scabra* were obtained from cultivation at Balai Besar Riset Budidaya Laut dan Penyuluhan Perikanan, Badan Riset dan Sumber Daya Manusia (BBRBLPP-BRSDM), Gondol, Bali, Indonesia. The *H. scabra* used were 12-month-olds weighing less than 200 g for each fresh *H. scabra*.

2.2. Extraction of *H. scabra*

H. scabra were extracted using wet rendering methods reported by Santativongchai *et al.* [20] with slight modifications. The wet rendering extraction method was conducted by boiling a 100 g sample with distilled water in the water bath with a ratio sample to distilled water of 1:3 at 60, 70, and 80°C for 4 hours, respectively, as depicted in Figure 1.

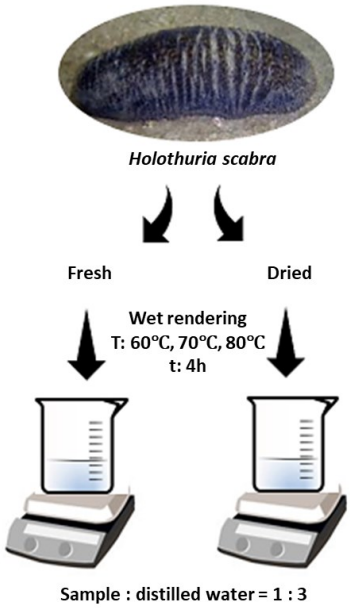

Figure 1. The extraction method of *H. scabra*

2.3. Measuring antioxidant activities by ABTS assay

Approximately 189 mg of potassium peroxydisulfate ($K_2S_2O_8$) (Merck, Germany) was dissolved in 5 mL of distilled water to obtain a 2.45 µM potassium peroxydisulfate ($K_2S_2O_8$) stock solution. A 19.2 mg of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Sigma, Germany) was dissolved in 5 mL of distilled water to make 7 mM of ABTS A solution. The ABTS A solution was then added with 88 µL of potassium peroxydisulfate ($K_2S_2O_8$) solution and incubated for 16 hours at room temperature in a dark room. After incubation, the mixture was added with 155 mL of absolute ethanol (Merck, Germany) to obtain an ABTS solution. The ABTS solution was checked for absorbance at 734 nm until gaining an absorption at 0.700 (the addition of ethanol is required if the absorption is above 0.700). Extracts of *H. scabra* were prepared in different concentrations of 125, 250, 500, 1000, and 2000 µg/mL in absolute ethanol. Approximately 10 µL of each concentration series of extracts was put in a 96-well microplate, and 190 µL of ABTS solution was added. The plate was then incubated for 6 minutes at room temperature in a dark room. The plate was then measured using a microplate reader (Tecan, Switzerland) at 734 nm. All tests were carried out in triplicate [21].

2.4. Measuring antioxidant activity by DPPH assay

Approximately 19.71 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, Germany) was dissolved in 50 mL of absolute methanol (Merck, Germany) to obtain a stock solution of 1 mM DPPH. *H. scabra* extract stock solution was prepared in absolute methanol at 100 mg/mL. A series of 5, 10, 20, 50, and 100 µL of *H. scabra* extract stock solution was added to a 96-well microplate, and 40 µL of DPPH stock solution was added

in each series. Each series was added with absolute methanol until the final volume of the mixture was 200 µL. A blank solution was prepared by adding 40 µL of DPPH stock solution with 160 µL of absolute methanol. The plate was then incubated for 30 minutes at 37°C in a dark room. The plates were then measured using a microplate reader (Tecan, Switzerland) at 517 nm. All tests were performed in triplicate [21]. The scavenging activity was calculated using Equation (1).

$$Free\ radical\ scavenging\ (\%) = \frac{A_o - A_T}{A_o} \times 100 \quad (1)$$

where, A_O is the absorbance of the DPPH solution and A_T is the absorbance of the test sample. The percent scavenging values were plotted against concentration, and regression equations were obtained to calculate the IC_{50} value.

2.5. Determination of ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power (FRAP) assay was determined according to the procedure reported by Zeb and Ullah [22]. FRAP reagent was prepared freshly and then warmed at 37°C. The FRAP reagent consisted of 2.5 mL of 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma, Switzerland) solution in 40 mmol/L hydrochloric acids (HCl) (Merck, Germany), 2.5 mL of 20 mmol/L ferric chlorides ($FeCl_3$) (Merck, Germany) solution and 25 mL of 0.3 mol/L acetate buffer pH 3.6. A total of 40 µL sample supernatant were mixed with 0.2 mL of distilled water and 1.8 mL of FRAP reagent. The absorbance of the mixture was then measured at 593 nm after incubation at 37°C for 10 minutes using a microplate reader (Tecan, Switzerland). All tests were carried out in triplicate. One mmol/L of ferrous sulfate ($FeSO_4$) (Merck, Germany) was used as a standard solution. The final result was the concentration of antioxidants that can reduce ferric equivalent to 1 mmol/L $FeSO_4$.

2.6. Determination of thiobarbituric acid reactive substance (TBARS)

The determination of thiobarbituric acid reactive substance (TBARS) was adapted from Zeb and Ullah [22]. A standard solution of 4.0 mM of thiobarbituric acid (TBA) (Merck, Germany) was prepared by dissolving 57.66 mg of TBA in 100 mL of glacial acetic acid (CH_3COOH) (Merck, Germany). Another standard stock solution of 1 mM malondialdehyde (MDA) (Sigma, Singapore) was prepared by dissolving 31.35 mg of MDA in 100 mL glacial acetic acid. From the stock solution, various concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were prepared to make a calibration curve with a concentration range of 0.1 to 1.0 mM. For extraction of TBARS in sample purpose, 1 g sample was weighed and placed into a 25 mL test tube and 5 mL of the solvent (100% glacial acetic acid or 50% glacial acetic acid in water). About 0.01% of butylated hydroxytoluene (BHT) (Sigma, Singapore) was used to prevent further oxidation of the medium. The sample was then shaken for an hour and filtered. The filtrate was used for analysis. For the analytical procedure, 1 mL of standard MDA solution was taken into a 10 mL test tube and mixed with 1 mL TBA. The mixture was then heated in the water bath at 95°C for 60 minutes, then cooled at room temperature, and the absorbance was measured at 532 nm. The two different samples extract (with 100% glacial acetic acid or 50% glacial acetic acid in water) were prepared. About 1 mL extract of each sample was mixed with 1 mL of TBA reagent, and the next steps followed the procedure above. All tests were performed in triplicate. TBARS was calculated as µM/g of the sample using Equation (2).

$$TBARS\ (\mu M/g) = \frac{Ac \times V}{W} \quad (2)$$

where Ac is the amount determined from the calibration curve, W is the weight of the sample taken, and V is the volume in mL or dilution factor of the total extract prepared.

2.7. Determination of total phenolic content

The measurement of total phenolic content (TPC) using the Folin-Ciocalteu reagent (Sigma, Germany) refers to Sekhon-Loodu and Rupasinghe [23]. As a standard curve, the gallic acid (Sigma, Germany) solutions were prepared in distilled water with a concentration series of 6.25, 12.5, 25, 50, and 100 µg/mL. *H. scabra* extracts were dissolved in dimethyl sulfoxide (DMSO) (Merck, Germany): water in 1:10 (v/v) at 1 mg/mL. Approximately 20 µL of extracts, gallic acid, and 100 µL of 0.2 N Folin-Ciocalteu reagent were added to the 96-well microplate. After 5 minutes of incubation, 80 µL of 7.5% sodium carbonate (Na_2CO_3) (dissolved in water) (Merck, Germany) was added to the microplate and then incubated in a dark place at room temperature for 2 hours. The absorbance was measured at 760 nm using a microplate reader (Tecan, Switzerland). TPC values are expressed as mg gallic acid equivalents (GAE) per 100 g of dried extracts. All tests were performed in triplicate.

2.8. Determination of total flavonoid content

The measurement of total flavonoid content (TFC) using aluminum chloride colorimetric assay refers to Chatatikun and Chiabchalard [24]. The quercetin (Sigma, Germany) solutions used as a standard curve were prepared in absolute ethanol (Merck, Germany) with a series of concentrations of 0.5, 1, 2.5, 5, 10, and 25 µg/mL. Approximately 50 µL of 1 mg/mL of *H. scabra* extracts and quercetin for the standard curve, 10 µL of 10% aluminum chloride ($AlCl_3$) (Merck, Germany) solution, and 150 µL of absolute ethanol (Merck, Germany) were added to the 96-well microplate. As much as 10 µL of 1 M (approximately 8.2 g/ 100 mL) sodium acetate (CH_3COONa) (Merck, Germany) solution was added to the microplate. The mixture was shaken for a few seconds and incubated at room temperature in a dark room for 40 minutes, and the absorbance was then measured at 415 nm using a microplate reader (Tecan, Switzerland). TFC values are as mg of quercetin equivalents (QE) per 100 g of dried extracts. All tests were carried out in triplicate.

2.9. Sulforhodamine B (SRB) assay

The cytotoxic activity of *H. scabra* extracts was investigated by sulforhodamine B (SRB) assay adapted from Vichai and Kirtikara [25]. The cytotoxicity of *H. scabra* extracts was tested on the MDA-MB-231 breast cancer cell line. Doxorubicin (Sigma Aldrich, Germany) was used as the positive control. Seed cells at an initial concentration of 3 × 10^3 cell/well in 150 µL of Dulbecco's Modified Eagle Medium (DMEM) (Sigma Aldrich, USA) containing 10% v/v fetal bovine serum (FBS) (Sigma Aldrich, USA) were prepared. The seed cells were allowed to stand for 24 hours to get attached to the plates. *H. scabra* extracts were applied to the plate at various concentrations (0, 5, 12.5, 25, and 50 µg/mL), then incubated for 48 hours. A 50 µL of 10% cold trichloroacetic acid (TCA) (Merck, Germany) was added to the plates and left at 4°C for an hour to fix the cells, and then the plates were washed with distilled water. A 50 µL of 0.4% SRB (Sigma Aldrich, USA) dissolved in 1% acetic acid (Merck, Germany) was used to stain the plates for 30 minutes at room temperature. The plates were then washed with 1% acetic acid and air-dried. A 100 µL/well of 10 M tris base (Sigma Aldrich, USA) (pH 10.5) was used to solubilize the dye, then the optical density (OD) of each well was measured at 570 nm using a microplate reader (Tecan, Switzerland) [26].

3. Results and Discussion

3.1. The yield of *H. scabra* extract

In general, extraction yields were better when using dried *H. scabra* compared with a fresh sample. Figure 2 showed that an increase in temperature resulted in a decreased extraction yield for both dried and fresh *H. scabra* respectively. The highest extraction yields were obtained at 60°C for dried and fresh *H. scabra* with values of 3.9% and 2.42%, respectively. In contrast, the lowest extraction yields were obtained at the highest extraction temperature (80°C) for both dried (3.72%) and fresh (1.6%) *H. scabra*, respectively.

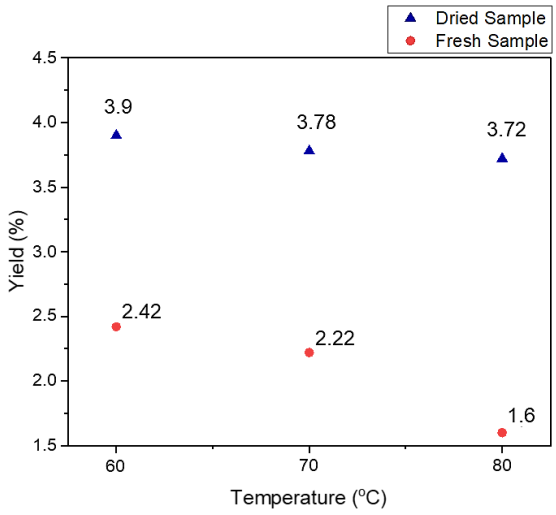

Figure 2. Extraction yield of *H. scabra*

3.2. Antioxidant activities by ABTS, DPPH, FRAP, and TBARS MDA assay

Antioxidant assay of *H. scabra* extracts using ABTS methods showed that the best antioxidant activity was fresh *H. scabra* extract at 60°C with an IC_{50} value of 629.89 ± 0.15 µg/mL (Table 1). The IC_{50} value of antioxidant activity in other fresh *H. scabra* extracts at 70°C and 80°C were 870.26 ± 0.37 and 1029.12 ± 0.76 µg/mL respectively. In the dried *H. scabra* extracts at 60, 70, and 80°C, the IC_{50} value of antioxidant activity were 983.58 ± 0.36, 1065.23 ± 0.29, and 1051.73 ± 0.45 µg/mL, respectively.

Table 1. Antioxidant activities of *H. scabra* extracts by ABTS, DPPH, FRAP, and TBARS MDA assay

Wet Rendering		ABTS	DPPH	FRAP	TBARS
H. scabra	Temperature (°C)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	µM Fe_2SO_4 eq/g sample*	nM MDA eq/mg extract*
Dried	60	983.58 ± 0.36	33820.42 ± 1.93	5765.81 ± 14.0	536.77 ± 0.014
Dried	70	1065.23 ± 0.29	32017.18 ± 0.82	5168.4 ± 8.0	455.22 ± 0.12
Dried	80	1051.73 ± 0.45	52250.17 ± 2.48	5688.648 ± 21.0	488.0 ± 0.13
Fresh	60	629.89 ± 0.15	49068.14 ± 2.41	3600.806 ± 13.0	239.39 ± 0.000
Fresh	70	870.26 ± 0.37	38531.89 ± 0.6	2979.029 ± 7.0	129.27 ± 0.01
Fresh	80	1029.12 ± 0.76	45004.03 ± 3.15	2776.889 ± 3.0	92.903 ± 0.01

*Concentration of 1000 µg/mL

The best antioxidant activity measured by the DPPH method was found in dried *H. scabra* extract at 70°C with an IC_{50} value of 32017.18 ± 0.82 µg/mL, while in other dried *H. scabra* extract at 60 and 80°C were 33820.42 ± 1.93 and 52250.17 ± 2.48 µg/mL, respectively. The IC_{50} value of fresh *H. scabra* extracts at 60, 70, and 80°C were 49068.14 ± 2.41, 38531.89 ± 0.6, and 45004.03 ± 3.15 µg/mL, respectively. Murniasih *et al.* [27] reported that the DPPH scavenging activities of *H. scabra* extract in low concentrations (< 1 mg/mL) were under 10%, while ascorbic acid was higher than 50%. The study by Nobsathian *et al.* [3] revealed that the whole body of sea cucumber extract had an IC_{50} value of 33.77 ± 0.24 mg/mL. In contrast, the active components of *H. scabra*, Friedelin, 3-Hydroxybenzaldehyde, and 4-Hydroxybenzaldehyde had IC_{50} values of 14.63 ± 0.01, 14.62 ± 0.01 and 14.78 ± 0.11 mg/mL, respectively. Althunibat *et al.* [9] also reported that the antioxidant activity of *H. scabra* extracts at a 50 mg/mL concentration was 77.46%. This difference in antioxidant value is influenced by the type of sea cucumber [9], the extraction method, and the solvent used [28].

The result of antioxidant activity of dried and fresh *H. scabra* extracts measured by FRAP and TBARS MDA methods showed that the highest antioxidant activity was found in fresh *H. scabra* extracts at 80°C with the value of 2776.889 ± 3.0 µM Fe^{2+}/g and 92.903 ± 0.01 nM/mg respectively. The antioxidant activity based on FRAP and TBARS MDA methods has a similar trend; the

antioxidant activity for dried extracts increased at 70°C and then decreased at 80°C. Meanwhile, for fresh *H. scabra* extracts, antioxidant activity (based on FRAP and TBARS methods) increased with increasing extraction temperature. However, according to the ABTS method, the antioxidant activity of fresh sea cucumber extracts decreased with increasing temperature.

The results of this study indicated the effect of temperatures on antioxidant activity. Réblová [29] reported that increasing temperature (from 80 to 150°C) caused a decrease in the antioxidant activity of δ- tocopherol in pork lard. Khatun *et al.* [30] suggested that the decrease in antioxidant activity that occurred in sea cucumber extract after heating was caused by the destruction of the active components, causing coagulation and reducing free radical scavenging activity. Decreasing antioxidant activity can also be caused by the decomposition of antioxidant compounds into other forms [31]. However, decreasing antioxidant activity with increasing temperature cannot be generalized [32]. For example, sunflower oil has a higher antioxidant activity of caffeic and sinapic acids at 90°C than at 22°C [33].

3.3. Total phenolic and flavonoid content

Phenolic compounds, which are secondary metabolites, play a role in preventing the oxidation process. One or more hydroxyl groups of phenolic compounds that are directly bonded to the aromatic ring can affect the stability between the bond of oxygen and hydrogen atoms in the hydroxyl group, causing phenolic compounds to have antioxidant properties [34]. Both simple phenolic compounds and polyphenols have a high correlation to the antioxidants of the extracts [35]. The higher the total phenol content, the better the antioxidant activity of the extract. The highest total phenolic and flavonoid content were found in dried *H. scabra* extracts at 80°C, which were 317.54 ± 8.91 mg GAE/100 g sample and 247.56 ± 11.70 mg QE/100 g sample (Table 2), respectively.

Table 2. Total phenolic and flavonoid content of *H. scabra* extracts

Wet Rendering		Total phenolic (mg GAE/100 g sample)	Total flavonoid (mg QE/100 g sample)
H. scabra	Temperature (°C)		
Dried	60	229.96 ± 6.91	120.98 ± 17.09
Dried	70	211.79 ± 6.20	49.17 ± 6.79
Dried	80	317.54 ± 8.91	247.56 ± 11.70
Fresh	60	242.46 ± 9.41	112.19 ± 8.01
Fresh	70	234.64 ± 3.71	53.68 ± 1.20
Fresh	80	214.21 ± 4.03	38.58 ± 4.61

The total phenolic content of dried *H. scabra* extracts at 60 and 70°C were 229.96 ± 6.91 and 211.79 ± 6.20 mg GAE/100 g sample, respectively. Whereas the total phenolic content of fresh *H. scabra* extracts at 60, 70, and 80°C were 242.46 ± 9.41, 234.64 ± 3.71, and 214.21 ± 4.03 mg GAE/100 g sample, respectively. The total phenolic and flavonoid content in dried *H. scabra* extracts decreased at 70°C and then increased at 80°C. While the total phenolic and flavonoid content in fresh *H. scabra* extracts decreased with increasing temperature. The effect of temperature on antioxidant activity was also reported by Réblová [32] that the highest antioxidant activity of phenolic acid was obtained at 90°C, and the value continued to decrease with increasing temperature up to 150°C.

Nobsathian *et al.* [3] reported that the total phenol from the crude extract of *H. scabra* was 30.52 ± 0.21 GAE/g sample, while Althunibat *et al.* [9] said that total phenol in *H. scabra* was 4.85 ± 0.004 GAE/g sample. The total phenol of *H. scabra* was lower than other types of wild sea cucumbers, including *B. vitiensis*, *S. cf. quadrifasciatus*, *P. graeffei*, and *H. atra,* with total phenol values of 21.08 ± 0.49, 14.325 ± 0.21, 14.033 ± 0.33, and 10.67 ± 0.12 mg GAE/g sample, respectively [36]. The increasing extraction temperature affected the total phenol and flavonoid content in the *H. Scabra* extracts. The heating process can cause phenolic compounds to undergo reactions such as degradation, oxidation or polymerization [37]. In addition, phenolic compounds are thermosensitive substances, allowing hydrolysis and percentage reduction at high temperatures [38].

3.4. Anticancer activity of *H. scabra*

The cytotoxic activity of *H. scabra* extracts against the MDA-MB-231 breast cancer cell line was investigated using the SRB test with the final concentrations of 25, 50, and 100 µg/mL, respectively, at different temperatures. The anticancer activity of *H. scabra* extracts against MDA-MB-231 can be seen in Table 3.

Table 3. Anticancer activity of *H. scabra* extract toward MDA-MB-231 (Breast cancer cell line)

Wet Rendering		Growth Inhibition (%) – MDA-MB-231		
H. scabra	Temp. (°C)	25 µg/mL	50 µg/mL	100 µg/mL
Dried	60	69.05 ± 13.03	98.51 ± 16.68	101.84 ± 13.89
Dried	70	58.56 ± 8.70	61.20 ± 5.37	73.87 ± 0.25
Dried	80	30.13 ± 8.10	49.93 ± 7.08	63.11 ± 2.17
Fresh	60	79.12 ± 6.04	88.77 ± 1.54	104.32 ± 2.80
Fresh	70	77.04 ± 2.68	86.36 ± 4.45	100.29 ± 0.26
Fresh	80	72.21 ± 10.60	84.89 ± 9.74	104.11 ± 1.43

At the concentration of 25 and 50 µg/mL, the results showed that *H. scabra* extracts could inhibit more than 50% of MDA-MB-231 cell line growth except for dried *H. scabra* extracts at 80°C. While the highest cytotoxic effect up to 100% showed at a concentration of 100 µg/mL, except for dried *H. scabra* extracts at 70 and 80°C with the inhibition of 73.87 ± 0.25% and 63.11 ± 2.17%, respectively. Several studies showed that sea cucumbers have an impressive profile of unique bioactive molecules and medicinal potential for anticancer and antiproliferative [22, 23, 24]. Mashjoor and Yousefzadi [39] showed that organic extracts of gonad and cuvierian tubules of *H. scabra* organs did not show any antiproliferative activity on MCF-7 cells with increasing the concentration of the extracts. However, body wall and intestine extracts of *H. scabra* showed antiproliferative activity on MCF-7 with the lethal concentration of 50% (LC_{50}) of 182.5 ± 12.41 and 109.9 ±

8.36 µg/mL of n-hexane extracts, respectively, 166.85 ± 12.36 and 61.7 ± 3.21 µg/mL of ethyl acetate extracts respectively, and >250 µg/mL of methanol extracts. Furthermore, Mashjoor *et al.* [40] stated that body wall and intestine extracts of *H. scabra* showed cytotoxic activity on HeLa cells with LC_{50} of 116.5 ± 9.92 and 59.3 ± 2.41 µg/mL of hexane extracts, respectively, 101.11 ± 6.48 and 60.3 ± 3.24 µg/mL of ethyl acetate extracts respectively, 142.3 ± 9.41 and 120.2 ± 8.68 µg/mL of methanol extracts respectively.

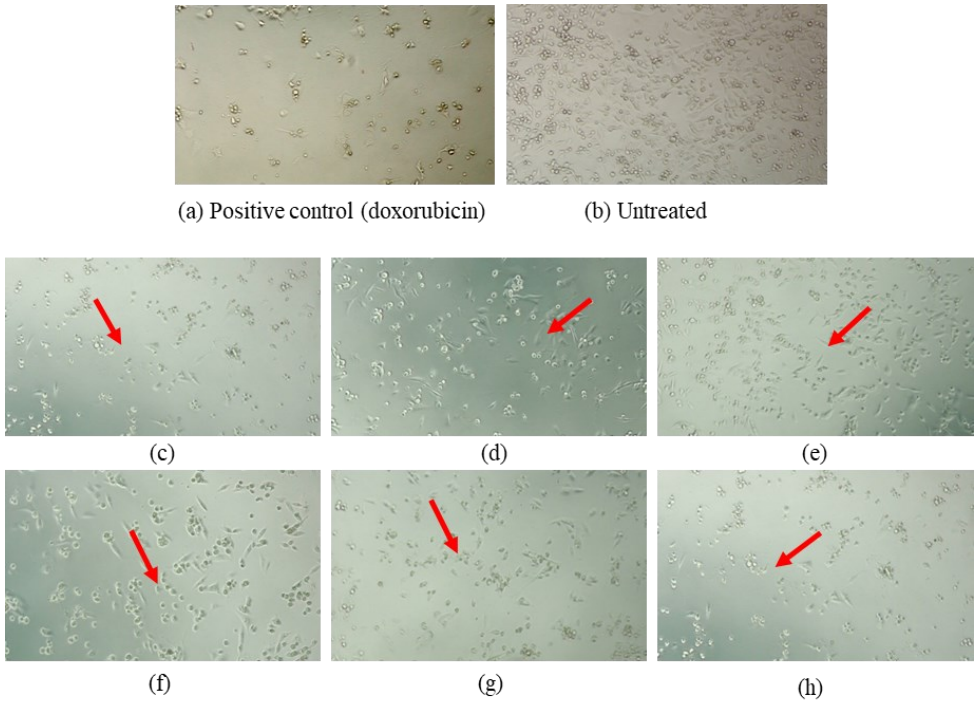

Figure 3. Morphologies of MDA-MB-231 cell line treated with *H. scabra* extracts at a concentration of 50 µg/mL a) positive control (doxorubicin), b) untreated, c) dried extract at 60°C, d) dried extract at 70°C, e) dried extract at 80°C, f) fresh extract at 60°C, g) fresh extract at 70°C, h) fresh extract at 80°C. The arrow showed the damaged cell

Anticancer activities of bioactive compounds from sea cucumber were conducted through several molecular mechanisms in cancer cells, such as cytotoxicity activity, induction of apoptosis, cell cycle arrest, reduction of tumor growth, antimetastatic, antiangiogenic, and inhibition of drug resistance [41]. The potential bioactive compounds derived from sea cucumbers as anticancer include A1 (HA1) and 24-Dehydroechinoside A (DHEA), colochiroside A, cucumarioside A2-2, ds-echinoside A, echinoside A, Frondanol A5, Frondoside A, glycosides 1 and 2, holothurin A, holothurin B, intercedensides A (1), B (2), and C (3), philinopside A, philinopside E, saponin, scabraside D, sphingoid bases, stichoposide C, and stichoposide D [12]. Holothurin A3 and A4 were active compounds produced from *H. scabra* that exhibit cytotoxicity by blocking cancer cell growth with toxic effects in carcinoma [42], epidermoid (KB), and hepatocellular (Hep-G2) [43]. The amount of important active compounds of Holothurin A, Holothurin B, and Holothurin B3 in *H. scabra* methanolic extract were 6.01%, 5.41%, and 6.07%, respectively [44].

In this study, doxorubicin, one of the chemotherapeutic drugs [45], was used as a positive control for cytotoxic activity against the breast cancer MDA-MD-231 cell line. The cytotoxic activity (IC_{50}) of doxorubicin against MDA-MB-231 was 0.92 ± 0.27 µg/mL [18] and is considered potent [46]. Figures 3a and 3b showed the morphology of the MDA-MB-231 cell line using doxorubicin as a positive control and without treatment, respectively. Cell damage caused by doxorubicin was shown in Figure 3a, whereas the untreated cells (3b) did not show any damaged cells.

The changes in morphological structures of the MDA-MB-231 cell line (breast cancer) at a concentration of 50 µg/mL showed in Figure 3. The arrows indicate that MDA MB cells were damaged after the addition of *H. scabra* extracts at a concentration of 50 µg/mL. This damage was similar to the cells that occurred in the positive control (doxorubicin). It was illustrated that *H. scabra* extracts inhibited cell growth and proliferation of the MDA-MB-231 breast cancer cell line.

4. Conclusion

The content of bioactive compounds in sand sea cucumber (*H. scabra*) extracts has potential pharmacological effects. Methods commonly used to obtain *H. scabra* extracts, such as maceration and soxhletation, require large amounts of chemicals as solvents, which is not environmentally friendly. Wet rendering is an alternative extraction method that is environmentally friendly because it does not require chemicals in the process. *H. scabra* extracts obtained using the wet rendering extraction method showed potential anticancer activity against the breast cancer cell line, MDA-MB-231. However, the extracts of *H. scabra* did not show potential antioxidant activity. In addition, the *H. scabra* extracts obtained by the wet rendering method could provide insight into the potential development of anticancer therapeutics.

References

[1] Widianingsih, Muhammad Zaenuri, Sutrisno Anggoro, Hermin Panca Sakti Kusumaningrum, Nutritional value of sea cucumber [*Paracaudina australis* (Semper, 1868)], *Aquatic Procedia*, 7, (2016), 271-276 https://doi.org/10.1016/j.aqpro.2016.07.038

[2] Ying Zhong, Muhammad Ahmad Khan, Fereidoon Shahidi, Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (*Cucumaria frondosa*), *Journal of Agricultural and Food Chemistry*, 55, 4, (2007), 1188-1192 https://doi.org/10.1021/jf063085h

[3] Saksit Nobsathian, Patoomratana Tuchinda, Prasert Sobhon, Yotsawan Tinikul, Jaruwan Poljaroen, Ruchanok Tinikul, Morakot Sroyraya, Tanes Poomton, Supakant Chaichotranunt, An antioxidant activity of the whole body of *Holothuria scabra*, *Chemical and Biological Technologies in Agriculture*, 4, 1, (2017), 1-5 https://doi.org/10.1186/s40538-017-0087-7

[4] Hang Qi, Xiaolin Ji, Shan Liu, Dingding Feng, Xiufang Dong, Baoyu He, Janaswamy Srinivas, Chenxu Yu, Antioxidant and anti-dyslipidemic effects of polysaccharidic extract from sea cucumber processing liquor, *Electronic Journal of Biotechnology*, 28, (2017), 1-6 https://doi.org/10.1016/j.ejbt.2017.04.001

[5] Sara Bordbar, Farooq Anwar, Nazamid Saari, High-value components and bioactives from sea cucumbers for functional foods—a review, *Marine Drugs*, 9, 10, (2011), 1761-1805 https://doi.org/10.3390/md9101761

[6] Peck Loo Kiew, Mashitah Mat Don, Jewel of the seabed: sea cucumbers as nutritional and drug candidates, *International Journal of Food Sciences and Nutrition*, 63, 5, (2012), 616-636 https://doi.org/10.3109/09637486.2011.641944

[7] W. A. J. P. Wijesinghe, You Jin Jeon, Perumal Ramasamy, Mohd Effendy A. Wahid, Charles S. Vairappan, Anticancer activity and mediation of apoptosis in human HL-60 leukaemia cells by edible sea cucumber (*Holothuria edulis*) extract, *Food Chemistry*, 139, 1-4, (2013), 326-331 https://doi.org/10.1016/j.foodchem.2013.01.058

[8] Mike Kareh, Rana El Nahas, Lamis Al-Aaraj, Sara Al-Ghadban, Nataly Naser Al Deen, Najat Saliba, Marwan El-Sabban, Rabih Talhouk, Anti-proliferative and anti-inflammatory activities of the sea cucumber *Holothuria polii* aqueous extract, *SAGE Open Medicine*, 6, (2018), https://doi.org/10.1177/2050312118809541

[9] Osama Y. Althunibat, Ridzwan Bin Hashim, Muhammad Taher, Jamaludin Mohd Daud, Masa-Aki Ikeda, B. I. Zali, In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species, *European Journal of Scientific Research*, 37, 3, (2009), 376-387

[10] Mariusz Gutowski, Sławomir Kowalczyk, A study of free radical chemistry: their role and pathophysiological significance, *Acta Biochimica Polonica*, 60, 1, (2013), 1-16 https://doi.org/10.18388/abp.2013_1944

[11] Barry Halliwell, Antioxidant defence mechanisms: from the beginning to the end (of the beginning), *Free Radical Research*, 31, 4, (1999), 261-272 https://doi.org/10.1080/10715769900300841

[12] Teresa Liliana Wargasetia, Sofy Permana, Widodo, The role of sea cucumber active compound and its derivative as an anti-cancer agent, *Current Pharmacology Reports*, 4, (2018), 27-32 https://doi.org/10.1007/s40495-018-0121-x

[13] Alexandra B. Roginsky, Xian-Zhong Ding, Carl Woodward, Michael B. Ujiki, Brahmchetna Singh, Richard H. Bell Jr, Peter Collin, Thomas E. Adrian, Anti-pancreatic cancer effects of a polar extract from the edible sea cucumber, *Cucumaria frondosa*, *Pancreas*, 39, 5, (2010), 646-652 https://doi.org/10.1097/mpa.0b013e3181c72baf

[14] E. S. Menchinskaya, E. A. Pislyagin, S. N. Kovalchyk, V. N. Davydova, A. S. Silchenko, S. A. Avilov, V. I. Kalinin, D. L. Aminin, Antitumor activity of cucumarioside A2-2, *Chemotherapy*, 59, 3, (2013), 181-191 https://doi.org/10.1159/000354156

[15] Nadia Al Marzouqi, Rabah Iratni, Abderrahim Nemmar, Kholoud Arafat, Mahmood Ahmed Al Sultan, Javed Yasin, Peter Collin, Jan Mester, Thomas E. Adrian, Samir Attoub, Frondoside A inhibits human breast cancer cell survival, migration, invasion and the growth of breast tumor xenografts, *European Journal of Pharmacology*, 668, 1-2, (2011), 25-34 https://doi.org/10.1016/j.ejphar.2011.06.023

[16] Qin Zhao, Zhi-dong Liu, Yong Xue, Jing-feng Wang, Hui Li, Qing-juan Tang, Yu-ming Wang, Ping Dong, Chang-hu Xue, Ds-echinoside A, a new triterpene glycoside derived from sea cucumber, exhibits antimetastatic activity via the inhibition of NF-κB-dependent MMP-9 and VEGF expressions, *Journal of Zhejiang University Science B*, 12, (2011), 534-544 https://doi.org/10.1631/jzus.B1000217

[17] Ekaterina S Menchinskaya, Dmitry L Aminin, Sergey A Avilov, Aleksandra S Silchenko, Pelageya V Andryjashchenko, Vladimir I Kalinin, Valentin A Stonik, Inhibition of tumor cells multidrug resistance by cucumarioside A2-2, frondoside A and their complexes with cholesterol, *Natural Product Communications*, 8, 10, (2013), 1934578X1300801009 https://doi.org/10.1177/1934578X1300801009

[18] Diah Anggraini Wulandari, Nunik Gustini, Tutik Murniasih, Asep Bayu, Martha Sari, Gita Syahputra, Iskandar Azmy Harahap, Abdullah Rasyid, Sari Budi Moria, Siti Irma Rahmawati, Nutritional value and biological activities of sea cucumber *Holothuria scabra* cultured in the open pond system, *Journal of Aquatic Food Product Technology*, 31, 6, (2022), 599-614 https://doi.org/10.1080/10498850.2022.2082902

[19] Nurlaylatul Akmal Mohd Yusoh, Rohaida Che Man, Nurul Aini Mohd Azman, Shalyda Md Shaarani, Siti Kholijah Abdul Mudalip, Siti Zubaidah Sulaiman, Zatul Iffah Mohd Arshad, Recovery of antioxidant from *Decapterus Macarellus* waste using wet rendering method, *Materials Today: Proceedings*, 57, (2022), 1382-1388 https://doi.org/10.1016/j.matpr.2022.03.173

[20] Pitchaya Santativongchai, Wirasak Fungfuang, Visanu Boonyawiwat, Urai Pongchairerk, Phitsanu Tulayakul, Comparison of physicochemical properties and fatty acid composition of crocodile oil (*Crocodylus siamensis*) extracted by using various

extraction methods, *International Journal of Food Properties*, 23, 1, (2020), 1465-1474 https://doi.org/10.1080/10942912.2020.1814324

[21] Prapaporn Jattujan, Pawanrat Chalorak, Tanapan Siangcham, Kant Sangpairoj, Saksit Nobsathian, Tanate Poomtong, Prasert Sobhon, Krai Meemon, *Holothuria scabra* extracts possess anti-oxidant activity and promote stress resistance and lifespan extension in *Caenorhabditis elegans*, *Experimental Gerontology*, 110, (2018), 158-171 https://doi.org/10.1016/j.exger.2018.06.006

[22] Alam Zeb, Fareed Ullah, A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods, *Journal of Analytical Methods in Chemistry*, 2016, (2016), 9412767 https://doi.org/10.1155/2016/9412767

[23] Satvir Sekhon-Loodu, H. P. Vasantha Rupasinghe, Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants, *Frontiers in Nutrition*, 6, 53, (2019), https://doi.org/10.3389/fnut.2019.00053

[24] Moragot Chatatikun, Anchalee Chiabchalard, Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts, *Journal of Chemical and Pharmaceutical Research*, 5, 4, (2013), 97-102

[25] Vanicha Vichai, Kanyawim Kirtikara, Sulforhodamine B colorimetric assay for cytotoxicity screening, *Nature Protocols*, 1, (2006), 1112-1116 https://doi.org/10.1038/nprot.2006.179

[26] Reda F. A. Abdelhameed, Enas E. Eltamany, Dina M. Hal, Amany K. Ibrahim, Asmaa M. AboulMagd, Tarfah Al-Warhi, Khayrya A. Youssif, Adel M. Abd El-kader, Hashim A. Hassanean, Shaimaa Fayez, New cytotoxic cerebrosides from the red sea cucumber *Holothuria spinifera* supported by in-silico studies, *Marine Drugs*, 18, 8, (2020), 405 https://doi.org/10.3390/md18080405

[27] Tutik Murniasih, Masteria Yunovilsa Putra, Ratih Pangestuti, Antioxidant capacities of *Holothuria* sea cucumbers, *Annales Bogorienses*, 2015

[28] Jean Mamelona, Emilien Pelletier, Karl Girard-Lalancette, Jean Legault, Salwa Karboune, Selim Kermasha, Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*, *Food Chemistry*, 104, 3, (2007), 1040-1047 https://doi.org/10.1016/j.foodchem.2007.01.016

[29] Zuzana Réblová, The effect of temperature on the antioxidant activity of tocopherols, *European Journal of Lipid Science and Technology*, 108, 10, (2006), 858-863 https://doi.org/10.1002/ejlt.200600091

[30] Mahmuda Khatun, Satomi Eguchi, Tomoko Yamaguchi, Hitoshi Takamura, Teruyoshi Matoba, Effect of thermal treatment on radical-scavenging activity of some spices, *Food Science and Technology Research*, 12, 3, (2006), 178-185 https://doi.org/10.3136/fstr.12.178

[31] Zhihong Cheng, Lan Su, Jeffrey Moore, Kequan Zhou, Marla Luther, Jun-Jie Yin, Liangli Yu, Effects of postharvest treatment and heat stress on availability of wheat antioxidants, *Journal of Agricultural and Food Chemistry*, 54, 15, (2006), 5623-5629 https://doi.org/10.1021/jf060719b

[32] Zuzana Réblová, Effect of temperature on the antioxidant activity of phenolic acids, *Czech Journal of Food Sciences*, 30, 2, (2012), 171-177 http://dx.doi.org/10.17221/57/2011-CJFS

[33] Emma M. Marinova, Nedyalka V. Yanishlieva, Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperatures, *Food Chemistry*, 81, 2, (2003), 189-197 https://doi.org/10.1016/S0308-8146(02)00411-9

[34] Shafira Ananda Djakaria, Irmanida Batubara, Rika Raffiudin, Antioxidant and antibacterial activity of selected indonesian honey against bacteria of acne, *Jurnal Kimia Sains dan Aplikasi*, 23, 8, (2020), 267-275 https://doi.org/10.14710/jksa.23.8.267-275

[35] Mohamad Rafi, Tanti Yulianti Raga Pertiwi, Syaefudin Syaefudin, Determination of Total Phenolic Content and Antioxidant Activity of Six Ornamental Plants, *Jurnal Kimia Sains dan Aplikasi*, 22, 3, (2019), 79-84 https://doi.org/10.14710/jksa.22.3.79-84

[36] Yolanda Avigail, Ervia Yudiati, Delianis Pringgenies, Aktivitas Antioksidan dan Kandungan Total Fenolik Pada Teripang di Perairan Karimunjawa, Jepara, *Journal of Marine Research*, 8, 4, (2019), 346-354 https://doi.org/10.14710/jmr.v8i4.24600

[37] Yahya Maghsoudlou, Mohsen Asghari Ghajari, Sedighe Tavasoli, Effects of heat treatment on the phenolic compounds and antioxidant capacity of quince fruit and its tisane's sensory properties, *Journal of Food Science and Technology*, 56, 5, (2019), 2365-2372 https://doi.org/10.1007/s13197-019-03644-6

[38] Wenjuan Qu, Zhongli Pan, Haile Ma, Extraction modeling and activities of antioxidants from pomegranate marc, *Journal of Food Engineering*, 99, 1, (2010), 16-23 https://doi.org/10.1016/j.jfoodeng.2010.01.020

[39] S. Mashjoor, Morteza Yousefzadi, Cytotoxic effects of three Persian Gulf species of *Holothurians*, *Iranian Journal of Veterinary Research*, 20, 1, (2019), 19-26

[40] Sakineh Mashjoor, Morteza Yousefzadi, Fatemeh Pishevarzad, Assessment of anticancer potential of selected *Holothuria* species, *Indian Journal of Traditional Knowledge*, 18, 2, (2019), 272-280

[41] Teresa Liliana Wargasetia, Widodo, Mechanisms of cancer cell killing by sea cucumber-derived compounds, *Investigational New Drugs*, 35, (2017), 820-826 https://doi.org/10.1007/s10637-017-0505-5

[42] Yong-Xin Li, S. W. A. Himaya, Se-Kwon Kim, Triterpenoids of marine origin as anti-cancer agents, *Molecules*, 18, 7, (2013), 7886-7909 https://doi.org/10.3390/molecules18077886

[43] Charalampos G. Panagos, Derek S. Thomson, Claire Moss, Adam D. Hughes, Maeve S. Kelly, Yan Liu, Wengang Chai, Radhakrishnan Venkatasamy, Domenico Spina, Clive P. Page, Fucosylated chondroitin sulfates from the body wall of the sea cucumber *Holothuria forskali*: Conformation, selectin binding, and biological activity, *Journal of Biological Chemistry*, 289, 41, (2014), 28284-28298 https://doi.org/10.1074/jbc.M114.572297

[44] Teresa Liliana Wargasetia, Hana Ratnawati, Nashi Widodo, Anticancer potential of holothurin A, holothurin B, and holothurin B3 from the sea cucumber *Holothuria scabra*, *AIP Conference Proceedings*, 2020 https://doi.org/10.1063/5.0002552

[45] Oktay Tacar, Pornsak Sriamornsak, Crispin R. Dass, Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems, *Journal of Pharmacy and Pharmacology*, 65, 2, (2013), 157-170 https://doi.org/10.1111/j.2042-7158.2012.01567.x

[46] Muhammad Luqman Nordin, Arifah Abdul Kadir, Zainul Amiruddin Zakaria, Rasedee Abdullah, Muhammad Nazrul Hakim Abdullah, In vitro investigation of cytotoxic and antioxidative activities of *Ardisia crispa* against breast cancer cell lines, MCF-7 and MDA-MB-231, *BMC Complementary and Alternative Medicine*, 18, (2018), 1-10 https://doi.org/10.1186/s12906-018-2153-5