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Phytochemical screening, docking study, and *in vitro* assessment of cytotoxicity against MCF7 and 3T3 cell lines of Indonesian sea cucumber *Stichopus* sp.

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ABSTRACT

In this study, phytochemical screening, docking study, and cytotoxicity against MCF7 and 3T3 cell lines of Indonesian sea cucumber *Stichopus* sp. fractions were investigated. The total phenol content and phytochemicals screening of these samples were ascertained by a fraction conventional method. The thiazolyl blue tetrazolium bromide assay was applied to determine the effect of *Stichopus* sp. fractions on cell viability. Phytochemical screening exhibited positive results for alkaloids, steroids, flavonoids, saponins, and triterpenoids. The highest amount of phenol content was discovered in the hexane fraction, and the lowest was in the sediment. A molecular docking study was conducted to examine the interaction between the *Stichopus* sp. chemical compounds and the MMP-9 cancer protein. The results of the *in vitro* assessment of cytotoxicity against the MCF7 and 3T3 cell lines of Indonesian sea cucumber *Stichopus* sp. clearly revealed that all of the fractions are toxic for cancerous MCF7 cell lines and most fractions are not toxic for non-cancerous 3T3 cell lines (normal cell lines). The *in vitro* assessment of cytotoxicity against the MCF7 and 3T3 cell lines showed that *Stichopus* sp. fractions have the ability to be a potential candidate for anticancer drugs.

INTRODUCTION

Golden sea cucumbers, *Stichopus* sp., have been widely used as food and medicinal ingredients in the Middle East and Asia, including Indonesia (Sara *et al*., 2011). As a food ingredient, sea cucumbers have high nutritional value with good-quality protein consisting of a rich amino acid and low-fat profile, which is supported by minerals (Ceesay *et al*., 2019). Current studies have reported that the sea cucumber contains a lot of bioactive compounds that can be used as antitumor, anticancer, antioxidant, antifungal, anticoagulant, antihypertensive, antimicrobial, antithrombotic, anti-inflammatory, and antiangiogenic agents (Adriansyah *et al*., 2014; Bordbar *et al*., 2011; Hawa *et al*., 1999; Lu *et al*., 2010; Tian *et al*., 2005; Zhang *et al*., 2009). Several important bioactivity compounds that have been identified and isolated for this sea cucumber are fucosylated chondroitin sulfate, cerebrosides, sterols, gangliosides, omega-3 fatty acids, sterol glycosides, lectins, frondogenin, heparin, glycosides, and omega-6 (Bordbar *et al*., 2011; Findlay and Daljeet, 1984; Vieira and Mour˜ao, 1988).

Frondoside A, a triterpenoid glycoside compound, is one of the active chemical compounds contained in sea cucumbers that shows a potential to inhibit cancer cell growth through caspase activation in human pancreatic cells, leukemia, and breast cancer (Jin *et al*., 2009; Li *et al*., 2008; Marzouqi *et al*., 2011). Attoub *et al*. (2013) revealed that frondoside A exhibits the decline of growth, lymph node metastasis, and angiogenesis of LNM35 xenograft in athymic mice. Moreover, frondoside A had the capability to inhibit lung tumor growth. In another research finding, Zou *et al*. (2005) declared that cytotoxicity (ED_{50} 0.96–5.0 µg/ml) against 10 human tumor cell lines was obtained from 6 triterpene glycosides isolated from sea cucumber. It is also known that the sea cucumber containing sulfated polysaccharides, fucoidans, has significant anticancer activity (Fitton *et al*., 2015; Malyarenko *et al*., 2017).

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It was reported that the sea cucumber *Stichopus* sp. already contains sulfated polysaccharides, chondroitin sulfate, and sulfated fucan. Evaluation of anticancer activity demonstrated that sulfated fucan carried slight activity against migration of MDA-MB-231 cells *in vitro* and colony formation inhibition of those cell lines (Thinh *et al*., 2018). Based on our information, phytochemical screening, the total phenol and flavonoid content of Indonesian sea cucumber *Stichopus* sp. extract will differ from *Stichopus* and other species. *Stichopus* sp. which is available in Lampung (Indonesia) has never been studied before. For this reason, this study was designed to perform phytochemical screening as well as evaluate the cytotoxicity of sea cucumber extracts against MCF7 cancer cells and 3T3 normal cells. Furthermore, a molecular docking study was carried out to predict the interaction of the chemical constituents of *Stichopus* sp. with the MMP-9 enzyme. MMP-9 has been reported in many types of cancer and the inhibition of MMP 9 could be beneficial in the treatment of various cancer conditions (Paramvir *et al*., 2018).

MATERIALS AND METHODS

Chemicals and reagents

We used Indonesian (Lampung) sea cucumber *Stichopus* sp. All types of solvents and acids (pure analytical grade) were acquired from E. Merck. We obtained other chemicals from E. Merck.

Preparation of samples of *Stichopus* sp.

Sea cucumbers, *Stichopus* sp., were collected from Lampung, Sumatra Island, Indonesia. Fresh and healthy sea cucumbers were separated immediately and packed in polyethylene bags under an incubator in an ice bath. Samples were stored at a cold temperature before further processing. The sea cucumbers were washed with water until clean and then mashed using a blender until they formed a paste. The samples were then frozen at −70°C and then dried using a freeze dryer for 2 days. All *Stichopus* sp. powder samples were packed in closed glass bottles until the extraction process was carried out.

Extraction procedure

The *stichopus* sp. Powder (127.9 g) was macerated in methanol (2.5 l) until complete extraction and evaporated to obtain a crude methanol extract. The crude extract was partitioned in stages, starting from nonpolar to polar solvents. The solvents used were hexane, chloroform, ethyl acetate, butanol, acetone, and methanol. The six fractions obtained from this stratified partitioning process (hexane fraction, chloroform fraction, ethyl acetate fraction, butanol fraction, acetone fraction, and methanol fraction) were used for phytochemical screening and cytotoxicity assessment.

Phytochemical screening

The stock solutions of all fractions were prepared by dissolving a 1 g sample in 100 ml of their solvent. These stock solutions were used for phytochemical screening following the Harborne (1998) and Kokate (1997) method. The phytochemical screening was accomplished to observe the presence of alkaloids, saponins, steroids, tannins, and terpenoids qualitatively. In addition, the Folin-Ciocalteu method was conducted to ascertain the total phenol compound content quantitatively, applying gallic acid as a standard (Chen *et al*., 2015).

Identification of the chemical compounds of Stichopus sp.

All the samples of *Stichopus* sp. fractions were weighed to as much as 10 mg to identify chemical compounds using quadrupole time-of-flight liquid chromatography-mass spectrometry (QTOF LCMS-MS) and gas chromatography-mass spectrometry (GC-MS) which are supported by the library database on the instrument.

Assessment of cytotoxicity

Cytotoxicity screening

Cell viability studies were conducted in breast cancer (MCF7) cell lines and fibroblast (NIH/3T3) cell lines as normal cells. The cell lines were grown in Dulbecco's Modified Eagle Medium high glucose enriched with 10% (v/v) fetal bovine serum and 1% penicillin-streptomycin at 37°C in a CO_2 incubator. Confluent cultures were harvested by trypsin and diluted in media to prepare a cell suspension. The centrifugation was applied to the cell suspension at 1,500 rpm for 5 minutes. A sample of cells was counted by using a hemocytometer. After calculating the cells, MCF7 and NIH/3T3 were seeded into 96-well plates at a density of 1×10^4 cells per well. The cell lines were cultured in a CO_2 incubator for 24 hours.

Thiazolyl blue tetrazolium bromide (MTT) assays

The effect of *Stichopus* sp. extract on cell viability was determined using the MTT test. After the incubation period of MCF7 and NIH/3T3, the extracts dissolved in dimethyl sulfoxide (DMSO) (final concentrations of 50, 100, and 200 µg/ml) were placed into the well plates and put in a CO_2 incubator for another 24 hours. Each sample was replicated three times. At the expiration of the 24 hours treatment period, supernatants were removed from the wells. Formerly, 90 µl of serum-free medium and 10 µl of MTT solution (5 mg/ml) were put into each well. The well plates were stored in a humidified 5% CO_2 incubator for 3 hours at 37°C. Then, the medium and MTT solution were discarded, and 100 µl of DMSO was put into the well plates. The optical density (OD) was determined at 570 nm on a multiwell spectrophotometer (Varioskan Flash, Thermo Fisher Scientific), and the cell viability was demonstrated using the following formula:

$$\text{Cell Viability (\%)} = \frac{\text{(Mean OD)}}{\text{(Control OD)}} \text{ x } 100\ \%$$

By plotting cell survival (%) versus log concentration (µg/ml) in the GraphPad Prism software, the sample concentrations that decrease the viability of the intact cells by 50% (IC_{50}) can be counted.

Morphological analysis

The cell lines treated with *Stichopus* sp. extract were observed by a light microscope (Olympus, Japan) to recognize morphological alterations of cells. The photographs were taken at ×20 magnification. This observation was accomplished to determine the changes (such as ballooning of the cells) induced by the samples.

Molecular docking of the chemical constituents of *Stichopus* sp.

Molecular docking was carried out using the AutoDock 4.2 software program. The interaction visualization of the molecular docking results was used by the Discovery Studio program. A molecular docking study was carried out to understand the interaction mode of cancer protein binding with ligands. The receptor used is an α-estrogen receptor taken from the Protein Data Bank (PDB) with PDB ID 4WZV, and external validation has been carried out (Liu *et al*., 2015).

RESULTS AND DISCUSSION

Study of phytochemical screening

The dried powder *of Stichopus* sp. sea cucumbers was obtained by drying the sea cucumber paste using a freeze dryer for 2 days. The dried samples of sea cucumbers were macerated using methanol, and the solvent was evaporated until the sea cucumber extract was obtained. The crude extract was partitioned in stages, starting from nonpolar to polar solvents. The solvents used were hexane, chloroform, ethyl acetate, butanol, acetone, and methanol. The six fractions obtained from this stratified partitioning process were used for phytochemical screening and cytotoxicity assessment. The stratified partition of *Stichopus* sp. sea cucumbers extract can be seen in Figure 1.

Based on the results of the phytochemical screening of the six fractions of *Stichopus* sp., it showed the presence of alkaloids, flavonoids, steroids, triterpenoids, and saponins. However, tannins were not present in *Stichopus* sp. fractions. Most fractions contain alkaloids. A triterpenoid, steroid, flavonoid, and alkaloid were detected in sea cucumber hexane and chloroform fractions. The ethyl acetate fraction showed the presence of

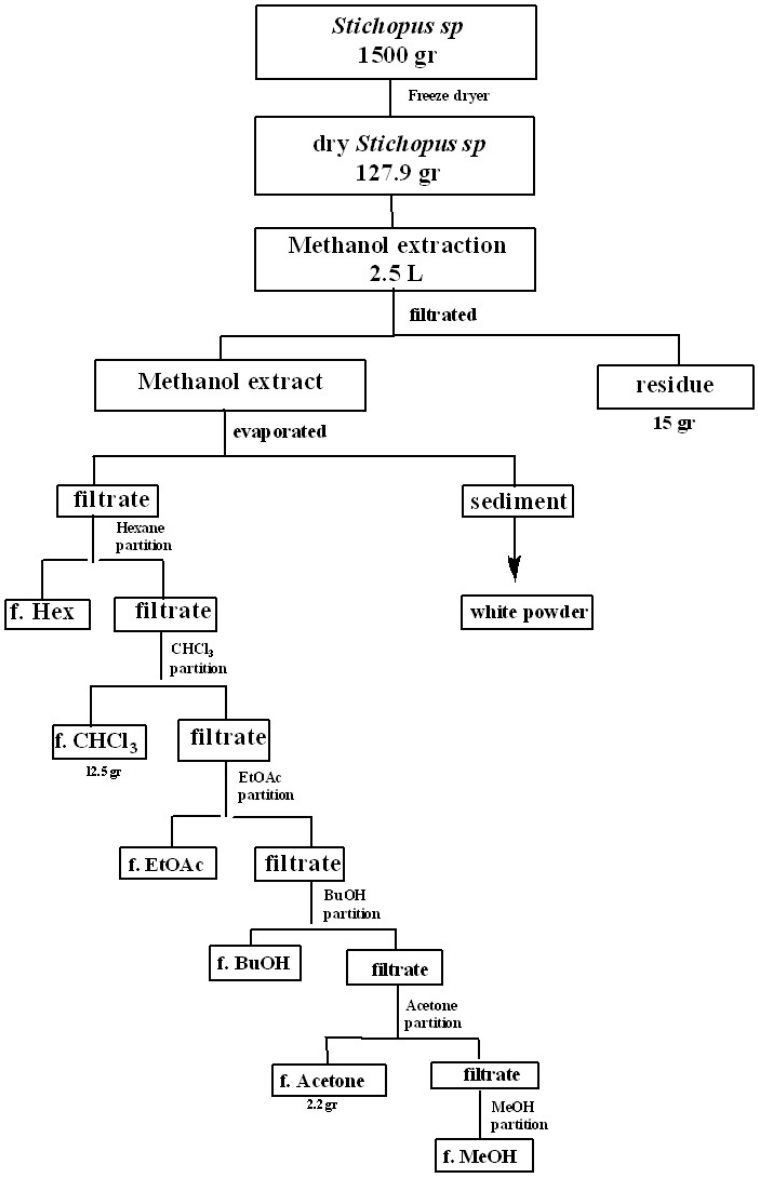


Figure 1. Schematic of fractionation partition samples of *Stichopus* sp.

alkaloid and triterpenoid compounds, butanol fraction contains alkaloids and flavonoids, acetone fraction contains saponins, and methanol fraction contains alkaloid compounds. The results of the phytochemical screening of the *Stichopus* sp. extract can be seen in Table 1.

The total phenol content result of seven samples of *Stichopus* sp. demonstrated by the *Folin-Ciocalteu* method is presented in Table 1. Among the seven samples, the hexane fraction showed the highest amount of phenolic compounds (7.01 mg/g) followed by methanol (5.48 mg/g), butanol (4.33 mg/g), chloroform (3.77 mg/g), acetone (1.67 mg/g), and ethyl acetate (1.05 mg/g).

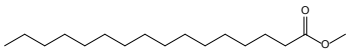
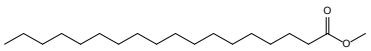
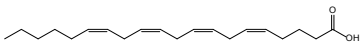
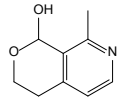
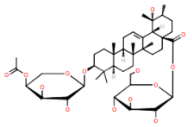
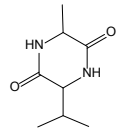
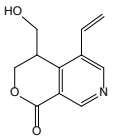
The chemical constituents contained in a fraction are biologically active substances. Almost all the active compounds present in the extract are components of secondary metabolites. These active compounds play important role in biological activity. The interaction of many active compounds in each fraction could be synergistic or antagonistic so that the existence of different active compounds in each fraction show different biological activity. This generates the uniqueness and attractiveness properties in fractions compared to drugs which generally contain only one active compound. In this study, phytochemical screening was carried out on the crude extract of sea cucumber *Stichopus* sp. using hexane, chloroform, ethyl acetate, butanol, acetone,

Table 1. Phytochemical and total phenol screening analysis of *Stichopus* sp. fractions.

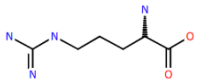
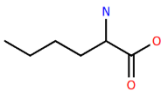
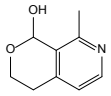
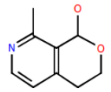
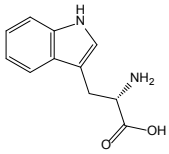
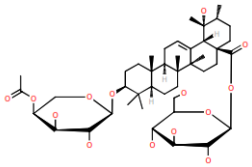
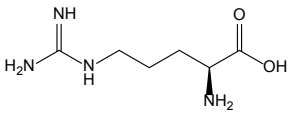
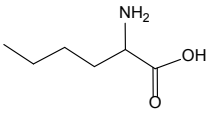
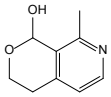
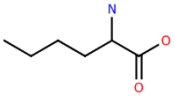
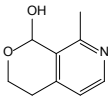
Biochemicals	Solvent						
	Hexane	$CHCl_3$	EtOAc	BuOH	Acetone	MeOH	Sediment
Alkaloids	+	+	+	+	−	+	+
Steroids	+	+	−	−	−	−	+
Flavonoids	+	+	−	+	−	−	−
Saponins	−	+	−	−	+		−
Tannins	−	−	−	−	−	−	−
Triterpenoids	+	+	+	−	−	−	−
Total Phenol content (mg/g)	7.01	3.77	1.05	4.33	1.67	5.48	nd

+: presence; −: absent; nd: not detected.

Table 2. Chemical constituent of *Stichopus* sp. based on GCMS and QTOF-LCMS/MS.

No	Fraction	Compound	Formula	Observed *m/z*	Observed RT (minute)	Chemical structure
1.	Hexane	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270.5	19.55	
		Methyl stearat	$C_{19}H_{38}O_2$	298.5	21.49	
		Arachidonic acid	$C_{20}H_{32}O_2$	304.5	22.68	
2.	Chloroform	Gentiatibetine	$C_9H_{11}NO_2$	166.0856	2.25	
		Cornutaside B	$C_{43}H_{68}O_{14}$	809.4699	8.17	
3.	Ethylacetate	Cyvlo (Ala-Val)	$C_8H_{14}N_2O_2$	171.1121	3.78	
		Gentianamine	$C_{11}H_{11}NO_3$	206.0806	4.68	

Continued

No	Fraction	Compound	Formula	Observed *m/z*	Observed RT (minute)	Chemical structure
4.	Butanol	Arginine	$C_6H_{14}N_4O_2$	175.1183	1.18	
		Norleucine	$C_6H_{13}NO_2$	132.1013	1.38	
		Gentiatibetine	$C_9H_{11}NO_2$	166.0856	2.24	
						
5.	Acetone	Gentiatibetine	$C_9H_{11}NO_2$	166.0856	2.25	
		Triptophane	$C_{11}H_{12}N_2O_2$	205.0964	3.19	
		Cornutaside B	$C_{43}H_{68}O_{14}$	809.4695	8.17	
6.	Methanol	Arginine	$C_6H_{14}N_4O_2$	175.1183	1.22	
		Norleucine	$C_6H_{13}NO_2$	132.1013	1.39	
		Gentiatibetine	$C_9H_{11}NO_2$	166.0855	2.25	
						
7.	Sediment	Norleucine	$C_6H_{13}NO_2$	132.1015	1.39	
		Gentiatibetine	$C_9H_{11}NO_2$	166.0857	2.25	

and methanol as solvents. Screening results indicated that the *Stichopus* sp. fractions contained alkaloids, steroids, flavonoids, saponins, and triterpenoids.

All the samples of *Stichopus* sp. fractions were identified chemical compounds using QTOF LCMS-MS and GC-MS which are supported by the library database on the instrument. The chemical compounds that have been identified from the fractions of *Stichopus* sp. are hexane (palmitic acid methyl ester, methyl stearate, and arachidonic acid), chloroform (gentiatibetine, cornutaside B), ethyl acetate (cyclo (Ala-Val), gentianamine), butanol (arginine, norleucine, and gentiatibetine), acetone (gentiatibetine, tryptophan, and cornutaside B), and methanol (arginine, norleucine, and gentiatibetine) and sediment (norleucine, gentiatibetine). The chemical constituents of *Stichopus* sp. based on GC-MS and QTOF LCMS-MS were shown in Table 2.

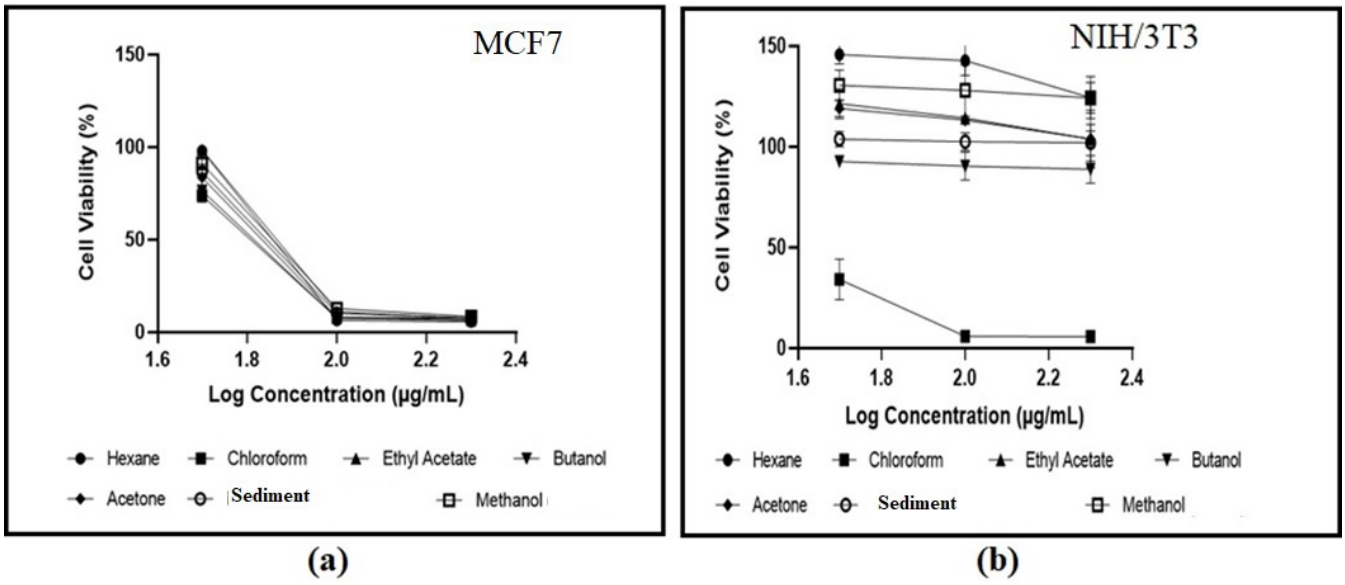


Figure 2. Effect of *Stichopus* sp fractions at different concentrations on (a) MCF7 cell lines and (b) NIH/3T3 cell lines

Table 3. IC_{50} of samples of *Stichopus* sp. fractions.

Fraction	MCF7 IC_{50} (µg/ml)	NIH/3T3 IC_{50} (µg/ml)
Hexane	81.28	>1,000
Chloroform	65.31	18.19
Ethyl acetate	84.53	>1,000
Butanol	67.45	>1,000
Acetone	73.45	>1,000
Methanol	80.91	>1,000
Sediment	76.71	>1,000

***In vitro* assessment of cytotoxicity against MCF7 and 3T3 cell lines of Indonesian sea cucumber *Stichopus* sp.**

The effects of seven samples on the viability of the cancerous MCF7 cell lines and normal NIH/3T3 cell lines evaluated by the MTT assay with various concentrations of the fractions (50, 100, and 200 µg/ml) were demonstrated in Figure 2. The fractions have different effects against the MCF7 cell lines and NIH/3T3 cell lines. The cell viability of MCF7 breast cancer cell lines treated with the fractions was found between 73% and 98% at a concentration of 50 µg/ml and showed a significant reduction at concentrations of 100 µg/ml (6%–12% cell viability) and 200 µg/ml (5%–8% cell viability). Nonetheless, almost all of the fractions on the NIH/3T3 cell lines exhibited a very high viability rate at 50 µg/ml (93%–146%), 100 µg/ml (90%–143%), and 200 µg/ml (87%–124%). The chloroform fraction has the lowest cell viability rate (34% cell viability at a concentration of 50 µg/ml and 5%–6% cell viability at concentrations of 100 and 200 µg/ml) against the NIH/3T3 cell lines compared to other fractions.

As shown in Table 3, all of the samples of *Stichopus* sp. fractions showed moderate cytotoxicity against the MCF7 cell lines with an IC_{50} value of 65–85 µg/ml. However, most fractions of *Stichopus* sp. exhibited no cytotoxicity against the NIH/ 3T3 cell achieving >1,000 µg/ml as an IC_{50} value. Interestingly, the chloroform fraction exhibited high cytotoxicity against NIH/3T3 with an IC_{50} value of 18.19 µg/ml. The criteria used to classify the cytotoxicity were determined as reported by the National Cancer Institute and the Geran protocol, IC_{50} < 20 µg/ml = high cytotoxicity, IC_{50} range between 21 and 200 µg/ml = moderate cytotoxicity, IC_{50} range between 201 and 500 µg/ml = weak cytotoxicity, and IC_{50} > 501 µg/ml = no cytotoxicity (Geran *et al.*, 1972; Nguyen *et al.*, 2020).

According to morphological observation at a concentration of 200 µg/ml, seven samples treated on MCF7 (Fig. 3a–g) promoted morphological changes (such as shrinking of cells and ballooning of cells) and presented signs that cells disengage from the surface, indicating cell death. In addition, Figure 4 shows the morphological untreated NIH/3T3 cell line, and Figure 4a–g exhibit treated NIH/3T3 cell lines. Six samples (Fig. 4a and c–g) provided no significant alteration compared to untreated NIH/3T3 cell lines (Fig. 4). There were a lot of cell attachments in those six samples' figures. However, the chloroform fraction (Fig. 4b) treated on NIH/3T3 generated more cell detachment, more shrinking cells, and ballooning cells (rounded shape) compared to other samples. The rounded morphology is shown by arrowheads.

The results of the *in vitro* assessment of cytotoxicity against the MCF7 and 3T3 cell lines of Indonesian sea cucumber *Stichopus* sp. clearly revealed that all of the samples are toxic for cancerous MCF7 cell lines and most samples are not toxic for

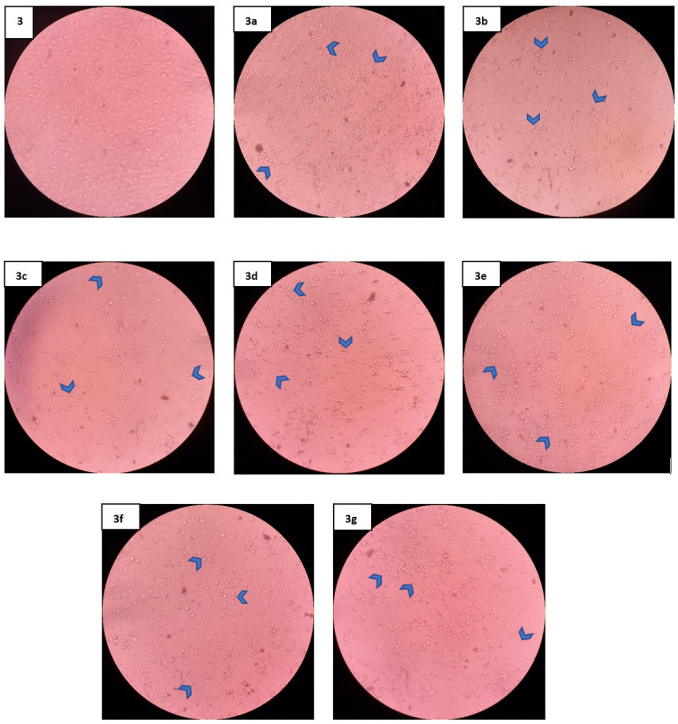


Figure 3. Morphological observation of MCF7 cell lines untreated and treated with the fractions at concentration of 200 µg/mL. Untreated MCF7(3); Hexane fraction (3a); Chloroform fraction (3b); Ethyl Acetate fraction (3c); Butanol fraction (3d); Acetone fraction (3e); Sediment (3f); Methanol fraction (3g). The arrowheads show rounded morphology.

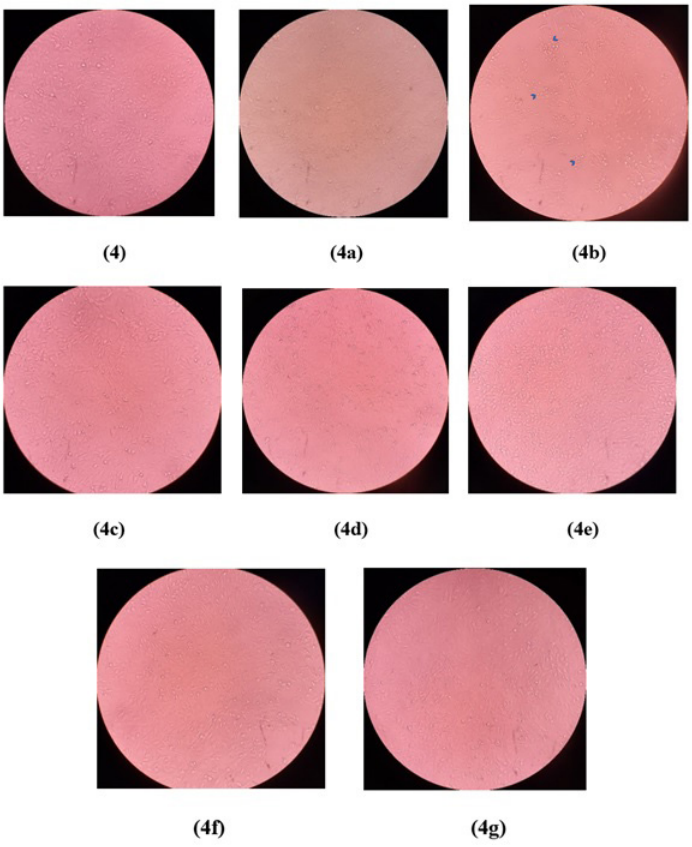
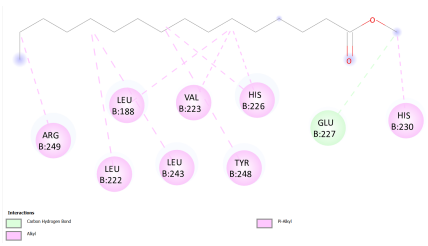
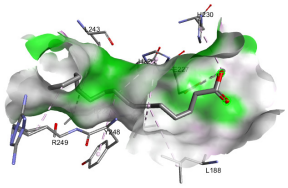
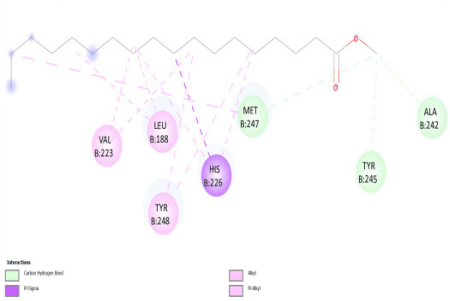
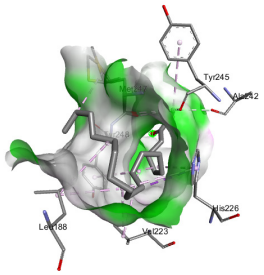
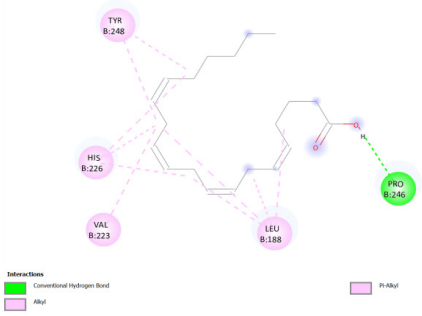
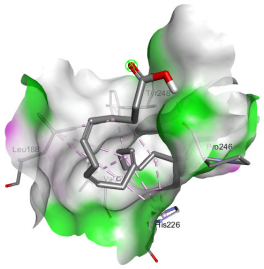
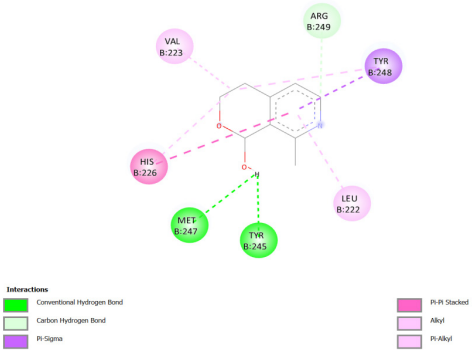
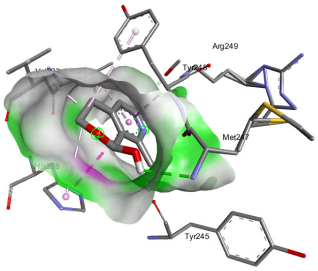
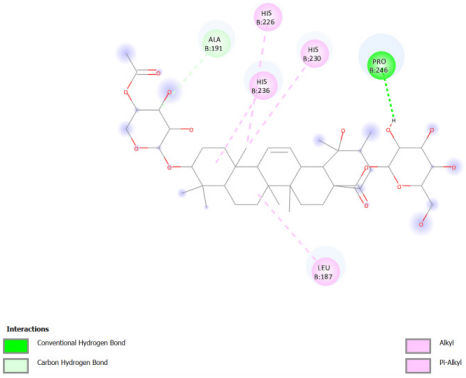
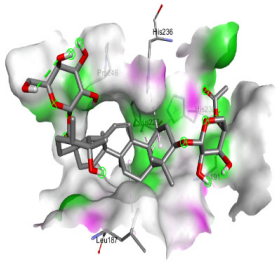
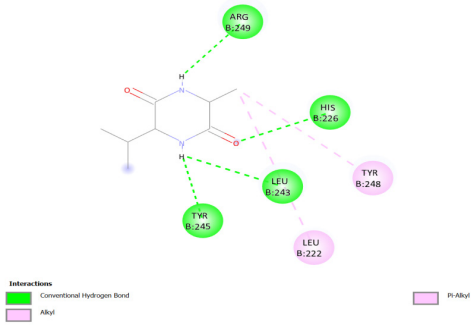
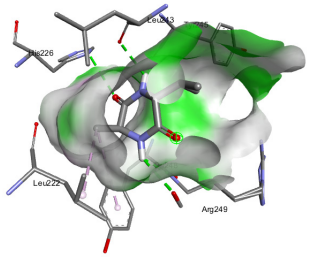
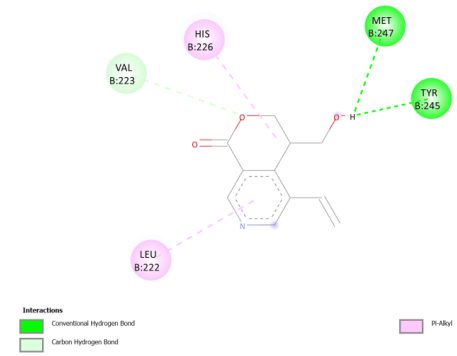
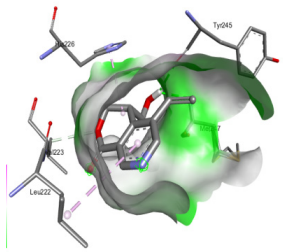
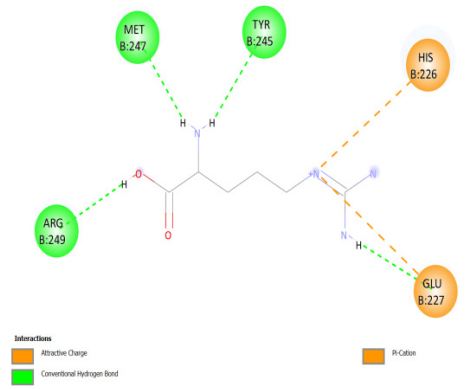
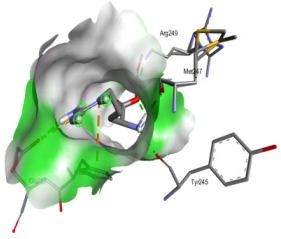


Figure 4. Morphological observation of NIH/3T3 cell lines untreated and treated with the fractions at concentration of 200 µg/mL. Untreated NIH/3T3 (4); Hexane fraction (4a); Chloroform fraction (4b); Ethyl Acetate fraction (4c); Butanol fraction (4d); Acetone fraction (4e); Sediment (4f); Methanol fraction (4g). The arrowheads show rounded morphology.

Table 4. Molecular docking of chemical constituent of *Stichopus* sp.

No	Compound	ΔG kcal/mol	Ki μM	2D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme	3D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme
1.	Palmitic acid methyl ester	−7.68	2.33		
2	Methyl stearat	−8.65	0.460		
3	Arachidonic acid	−7.78	2.00		
4	Gentiatibetine	−7.51	3.12		

Continued

No	Compound	ΔG kcal/mol	Ki μM	2D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme	3D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme
5	Cornutaside B	−6.52	16.68		
6	Cyvlo (Ala-Val)	−7.89	1.63		
7	Gentianamine	−7.37	3.93		
8	Arginine	−6.08	35.03		

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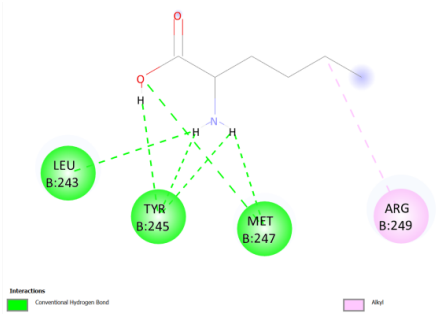
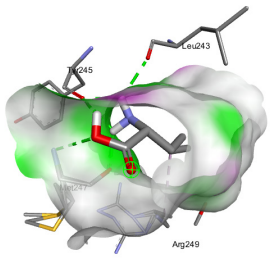
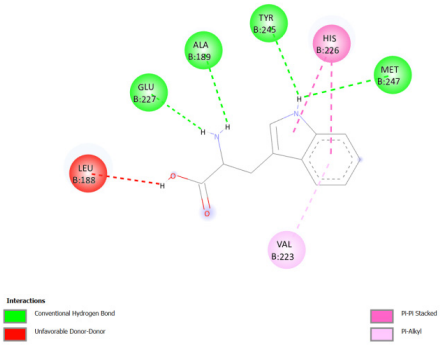
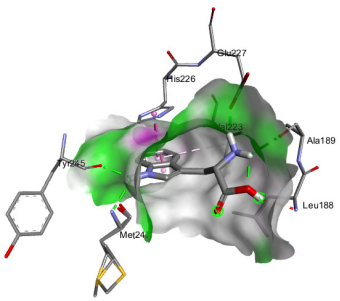
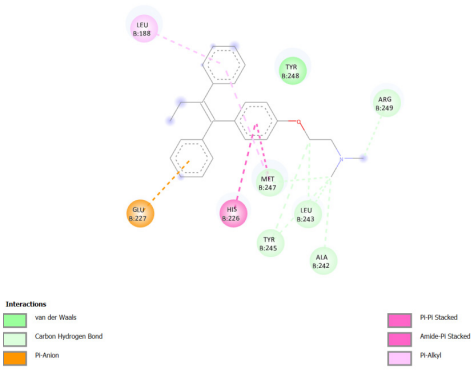
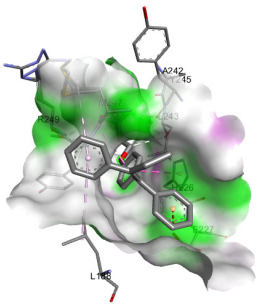
No	Compound	ΔG kcal/mol	Ki μM	2D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme	3D Interaction of chemical constituent of *Stichopus* sp. with 4WZV enzyme
9	Norleucine	−4.73	339.1		
10	Triptophane	−8.65	0.460		
11	Tamoxifen	−11.70	0.002		

noncancerous NIH/3T3 cell lines. Impressively, the chloroform fraction presented higher cytotoxicity in NIH/3T3 cell lines than MCF7 cell lines. Existing phytochemicals play an important role in the cytotoxicity effect. The presence of flavonoid induced sensitivities of cancer cells to cell death (Das *et al*., 2010).

Cepharanthine, liensinine, dauricine, and isoliensinine which are categorized as alkaloid compounds generate autophagy leading to cell death and showed cytotoxic effects specifically towards cancer cells (Law *et al*., 2014). Talib and Mahasneh (2010) reported that flavonoids with alkaloids in *Onobis hirta* showed superior activity to inhibit the growth of cancer cells.

In another study, *Holothuria scabra* and *Cucumaria frondosa* containing triterpene glycosides presented the capability to induce apoptosis in HepG2 cells and have better selective cytotoxicity as a novel antitumor drug (Wang *et al*., 2014). Furthermore, the sphingoid base isolated from *Stichopus variegatus* exhibited a high cytotoxicity effect, inhibited cancer cell growth, and also showed induction of the apoptosis pathway in human colon cancer cells (Janakiram *et al*., 2015).

Molecular docking study

Table 4 shows the interactions of the chemical constituents of *Stichopus* sp. with the amino acid residues involved in 4WZV. The chemical constituents of *Stichopus* sp. include palmitic acid methyl ester, methyl stearate, arachidonic acid, gentiatibetine, cornutaside B, cyclo (Ala-Val), gentianamine, arginine, norleucine, and tryptophan showing the interaction scores of Gibs energy values −7.68, −8.65, −7.78, −7.51, −6.52, −7.89, −7.57, −6.08, −4.73, and −8.65 kcal/mol). Meanwhile, the positive control compound used is tamoxifen, which is indeed used for cancer treatment. The score of Gibs energy interaction of tamoxifen was −11.70 kcal/mol. Unfortunately, for all of the chemical constituents identified in *Stichopus* sp., their inhibitory interaction activity *in silico* was still much lower than that of the tamoxifen as a positive control.

CONCLUSION

The study of phytochemical screening results indicated that the fractions of *Stichopus* sp. contained alkaloids, steroids, flavonoids, saponins, and triterpenoids. The chemical compounds identified in the *Stichopus* sp. fractions were palmitic acid methyl ester, methyl stearate, arachidonic acid, gentiatibetine, cornutaside B, cyclo (Ala-Val), gentianamine, arginine, norleucine, and tryptophan. The *in vitro* assessment of cytotoxicity against the MCF7 and 3T3 cell lines showed that all *Stichopus* sp. fractions are toxic for cancerous MCF7 cell lines and most fractions are not toxic for noncancerous NIH/3T3 cell lines (normal cell lines). The results of the molecular docking study showed moderate activity of the chemical constituents of *Stichopus* sp. compared to tamoxifen as a positive control.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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