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ORIGINAL PAPERS

Cytotoxic and Antioxidant Potential of Chloroform Extract of *Holothuria tubulosa* Gmelin, 1791[1]

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Abstract—Natural products are promising anticancer agents and antioxidants. The present study was aimed to evaluate the cytotoxic and antioxidant potential and the contents of phenolic compounds of a chloroform extract from *Holothuria tubulosa* collected from the Gökova Gulf (Muğla, Turkey) for the first time. Cell viability was assessed by the MTT assay. Apoptotic cell death was analyzed by flow cytometry. The antioxidant activity of the extract was determined by the DPPH radical scavenging activity assay, the ABTS radical cation scavenging activity assay, the β-carotene-linoleic acid and FRAP assays and by the phosphomolybdenum method. Total flavonoid and tannin contents in the extract were investigated. Also, the phenolic compounds of the extract were detected by HPLC. The extract showed the potential cytotoxic effect against cancer cells and was capable of inducing apoptosis in A549 and HeLa cells. Overall, the antioxidant capacity of the extract may be evaluated as low. The phenolic compound content of the extract was also found to be low. The most abundant component in the extract was ellagic acid (482.293 μg/g extract) according to the HPLC analysis. Our results may contribute to further studies on the use of *H. tubulosa* for obtaining new anticancer and other therapeutic agents.

Keywords: cancer cell lines, cell viability, antioxidant activity, *Holothuria tubulosa*, HPLC

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INTRODUCTION

Cancer is currently one of the most significant public health challenges [1]. It has been stated that microorganisms, plants, and marine invertebrates may be considered among the natural product sources that can provide anticancer activity thanks to their ability to develop a wide diversity of chemical defense mechanisms [41].

Due to the developing biomedical technologies, the compounds obtained from natural products have become the focus of pharmacological studies conducted to develop new drugs. The marine environment, which represents an important source of biodiversity, provides an opportunity to obtain novel anticancer remedies with new mechanisms of action in the treatment of cancer and find pioneering compounds [11, 21].

Free radicals that may occur during vital activities or as a result of environmental factors such as smoke, pollution, or radiation are highly active due to their unpaired electrons and can react with vital molecules to create a toxic effect. Antioxidants neutralize free radicals produced in biological systems, protecting the cell from negative effects [35, 36].

Sea cucumbers from the class Holothuroidea (phylum Echinodermata) are known as echinoderms that form dense populations in warm shallow waters [11]. Sea cucumbers produce secondary metabolites with unique bioactive properties in order to adapt to new environmental conditions [34] and contain different compounds such as triterpene glycosides, glycosaminoglycans, peptides, lectins, and glycosphingolipids. It has been stated that various activities of sea cucumbers such as anticancer, anti-angiogenic, antimicrobial and anti-inflammatory can be attributed to these compounds that are found in them in distinguishable amounts [11, 19, 21]. Different studies indicated the effects of secondary metabolites from different sea cucumber species on various cancer cells [19, 21, 24]. Additionally, the antioxidant effects of sea cucumbers were shown [3, 22, 39].

Holothuria tubulosa Gmelin, 1791 is one of the commercial species in Turkey [7]. Only a limited number of studies on the anti-cancer and antioxidant

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potentials of sea cucumber species from Turkey are available in the literature. For instance, one of the studies determined the potential cytotoxic and apoptotic effects and phenolic compound contents of aqueous and methanolic extracts of *H. tubulosa* collected from the Gökova Gulf [2]. Another study investigated the antioxidant and antimicrobial effects of acetonitrile/trifluoroacetic acid, methanol/water, and methanol extracts obtained from *H. tubulosa* [22]. Various studies demonstrated that the solvents used for extraction had an important role in the biological activities tested [18, 42]. Random collection and screening of samples are among fundamental approaches to identifying new bioactive compounds from natural sources [23]. The composition of secondary metabolites changes depending on environmental factors such as climate, soil structure, altitude, and ecological conditions [26]. Similarly, gathering various samples from rich ecosystems and testing them for any biological activity may provide a new compound to be isolated since organisms living in a species-rich environment need to develop their defense and competition mechanisms [23]. The present study is the first attempt to consider the potential cytotoxic and antioxidant effects and the contents of the phenolic compounds of a chloroform extract from *H. tubulosa* (HTC) collected from the Gökova Gulf (Muğla), Aegean Sea. In addition, the apoptotic effects of an HTC extract on A549 and HeLa cells were investigated as well.

MATERIALS AND METHODS

Collection and Extraction of Holothuria tubulosa

H. tubulosa species were collected from the Gökova Gulf (Aegean Sea) in Muğla, Turkey, by Prof. Dr. Ali Türker. To summarize, after removing internal organs, samples were washed with tap and deionized water. About 10 g of each sliced and dried sea cucumbers' bodies were extracted with 100 mL of chloroform (Merck, USA) at 30°C for 8 h at least 3 times. Later on, chloroform was evaporated by rotary evaporation (IKA, RV 10, USA). The crude extract was kept frozen in a refrigerator at −20°C in the dark. The extract was dissolved in 10% DMSO (Applichem, Germany) to achieve a stock solution, and the final concentration of DMSO did not exceed 1% in the assays.

Cell Cultures and MTT Assay

Human lung adenocarcinoma (A549), human cervix adenocarcinoma (HeLa), human breast adenocarcinoma (MCF-7), human prostate adenocarcinoma (PC-3), and human embryonic kidney (HEK293) cell lines were provided by the American Type Culture Collection (ATCC). The cell lines were maintained in the RPMI 1640 medium containing stable L-glutamine (Biochrom, Germany), 10% fetal bovine serum (FBS) (Biochrom, Germany), 100 units/mL penicillin, and 100 μg/mL streptomycin sulphate (Biochrom, Germany), at 37°C and 5% CO_2 in a conditioned incubator.

For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [29], cells at a density of 4×10^3 cells/well were seeded onto 96-well plates. After 24 h of cell culturing, eight different concentrations of the HTC extract (from 0.0078 to 1 mg/mL) were added to the cells at 24, 48, and 72 h. The control groups were untreated cells. Following incubation, the medium was discharged from each well. Then 100 μL of fresh medium and 10 μL of the MTT solution (5 mg/mL) were added to each well, respectively. After further 4 h incubation and discarding the medium, the formazan crystals formed by viable cells were dissolved by the addition of 100 μL DMSO. The absorbance (Abs) at 540 nm was detected with a microplate reader (Thermo Scientific, Multiscan FC, USA). The results were expressed in terms of the percentage of cell viability through the equation of Moongkarndi et al. [28]. IC_{50} (the concentration causing mortality of 50% of the cell population tested) values were also calculated based on the results of the MTT assay for each incubation time.

Flow Cytometry Analysis for Apoptotic Cell Death

The apoptotic effect of the HTC extract at 0.25–1 mg/mL on the A549 and HeLa cell lines for 48 h was investigated by Annexin V-FITC/propidium iodide staining using a commercial kit (BioVision, 4F15K01010, USA) protocol. Untreated cells were considered as control. Each cell suspension was analyzed by flow cytometry (BD FACSCanto A, BD Biosciences) using the BD FACSDiva software v6.13.

Antioxidant Activity Assays

DPPH radical scavenging activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability was detected by the method of Turan and Mammadov [43]. The radical scavenging activity was expressed as percentage of inhibition of DPPH radical. Approximate IC_{50} value (concentration of the sample to scavenge 50% of the DPPH radicals) of both BHA (butylated hydroxyanisole) used as the positive control and the extract was determined from the graph plotted against mg/mL concentrations using the percentage of scavenging activity obtained.

ABTS radical cation scavenging activity: ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity was evaluated according to the protocol described by Re et al. [37]. The ABTS radical cation scavenging activity was indicated as percentage. Approximate IC_{50} value (concentration of the sample to scavenge 50% of ABTS radical activity) was calculated as specified in the DPPH activity test.

β-Carotene-linoleic acid assay: The assay was based on the method of Amarowicz et al. [4]. In brief, 2 mg β-carotene, 1 mL chloroform, 20 μL linoleic acid, and 200 μL Tween 20 were mixed, and then chloroform was removed on a rotary evaporator. After that, the remaining mixture was supplemented with 100 mL distilled water. A 24 mL portion of this β-carotene-linoleic acid solution was mixed with 1 mL of sample solution. The initial absorbances at 470 nm were read. The absorbances were again measured for 120 min every 30 min, after incubation at 50°C. The result was determined using the formula of Amarowicz et al. [4].

Phosphomolybdenum method: The total antioxidant capacity was also assessed by the phosphomolybdenum method of Berk et al. [10]. The result obtained was expressed as milligram of ascorbic acid equivalent per gram of extract (mg AAE/g extract).

Ferric reducing antioxidant power (FRAP) assay: This assay was carried out by the method of Zengin and Aktumsek [44] with some modifications. Each sample solution was added to FRAP reagent prepared by mixing 0.3 M acetate buffer, 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$. After 30 min incubation, the absorbance at 593 nm was determined. FRAP activity was expressed as milligram of Trolox equivalent per gram of extract (mg TE/g extract).

Determination of Contents

Total flavonoid content: Total flavonoid content of the extract was detected by the method of Aryal et al. [5] with some modifications. A 0.2 mL portion of 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL (1 M) potassium acetate, and 5.6 mL distilled water were added to 1 mL of sample solution. After the mixture was left to settle for 30 min, the absorbance at 415 nm was taken against the blank. The total flavonoid content of the extract was expressed as milligram of quercetin equivalent per gram of extract (mg QE/g extract).

Total tannin content: This assay was carried out by the vanillin method according to the protocol described by Bekir et al. [9]. The total tannin content of the extract was expressed as milligram of catechin equivalent per gram of extract (mg CE/g extract).

Analysis of Phenolic Contents by HPLC

Analysis of phenolic components by High Performance Liquid Chromatography (HPLC) was carried out by the method of Caponio et al. [16] with a slight modification with photodiode array detector (SPDM20A), LC20 AT pump, and a SIL-20ACHT auto sampler. In brief, separations were performed on a C-18 column (250 × 4.6 mm length, 5 μm particle size) with a CTO-10ASVp column oven. Solvent A (3% formic acid) and solvent B (methanol) formed the mobile phase. The sample (0.2 *g*) dissolved in the mobile phase was injected into HPLC after filtration at 0.45 μm. The standards used were 15 compounds listed in Table 3. The quantity of phenolic contents was expressed as μg/g extract.

Data Analysis

The results obtained in the cell viability and antioxidant activity experiments were presented as mean ± standard error of at least three replicates. The data in the cell viability assay were analyzed using one-way ANOVA and the Duncan's post-hoc test ($p < 0.05$) to assess the statistical comparison between the means of each treatment for each incubation time using SPSS software (version 22.0 for Windows, IBM Corp., Armonk, N.Y., USA). IC_{50} values in the cell viability assay were also determined using the GraphPad Prism 7.0 software program (GraphPad Software, Inc., San Diego, CA).

RESULTS

Potential Effects of the Extract on Cell Viability

The effect of HTC extract on cell viability was appraised using the MTT assay at 24, 48, and 72 h (Fig. 1). HTC extract at 0.25–1 mg/mL was observed to decrease the viability rate in all cells tested per incubation time. HTC extract at the concentrations tested on the HeLa, MCF-7, and PC-3 cell lines proved to be effective at each incubation time in comparison to untreated control cells. Cell viability of the HEK293 cell line treated with HTC extract, especially at 1.25 mg/mL and higher concentrations, was generally higher at 24 h than those at 48 and 72 h of incubation as observed in other cells. All concentrations of the extract significantly reduced cell viability in all cells at 72 h. The IC_{50} values (Table 1) determined for cancer cells at 48 h were lower than that of non-cancerous cell line HEK293. In addition, the values suggest that the cytotoxic potential of the extract among cancer cells is higher in MCF-7 and PC-3 cells.

Potential Apoptotic Effects of the Extract

The apoptotic effect of the extract on A549 and HeLa cells at different concentrations was investigated for 48 h incubation time (Fig. 2). In our analysis, the percentage of apoptotic cells in A549 cells treated with the extract at 1 mg/mL was 73.7%. When the cells were treated with HTC extract at 0.125, 0.25, and 0.5 mg/mL, the percentage of apoptotic cells in A549 cells were found to increase from 3.7% (control) to 9, 15.6, and 25.3%, respectively. As in A549 cells, the percentage of apoptotic HeLa cells increased and the percentage of living cells decreased due to the increasing concentrations of the extract. The percentage of apoptotic HeLa cells exposed to the extract at 0.125, 0.25, 0.5, and 1 mg/mL increased from 4.4% (control) to 14.1, 20.2, 45.3, and 87.5%, respectively. Besides, the flow

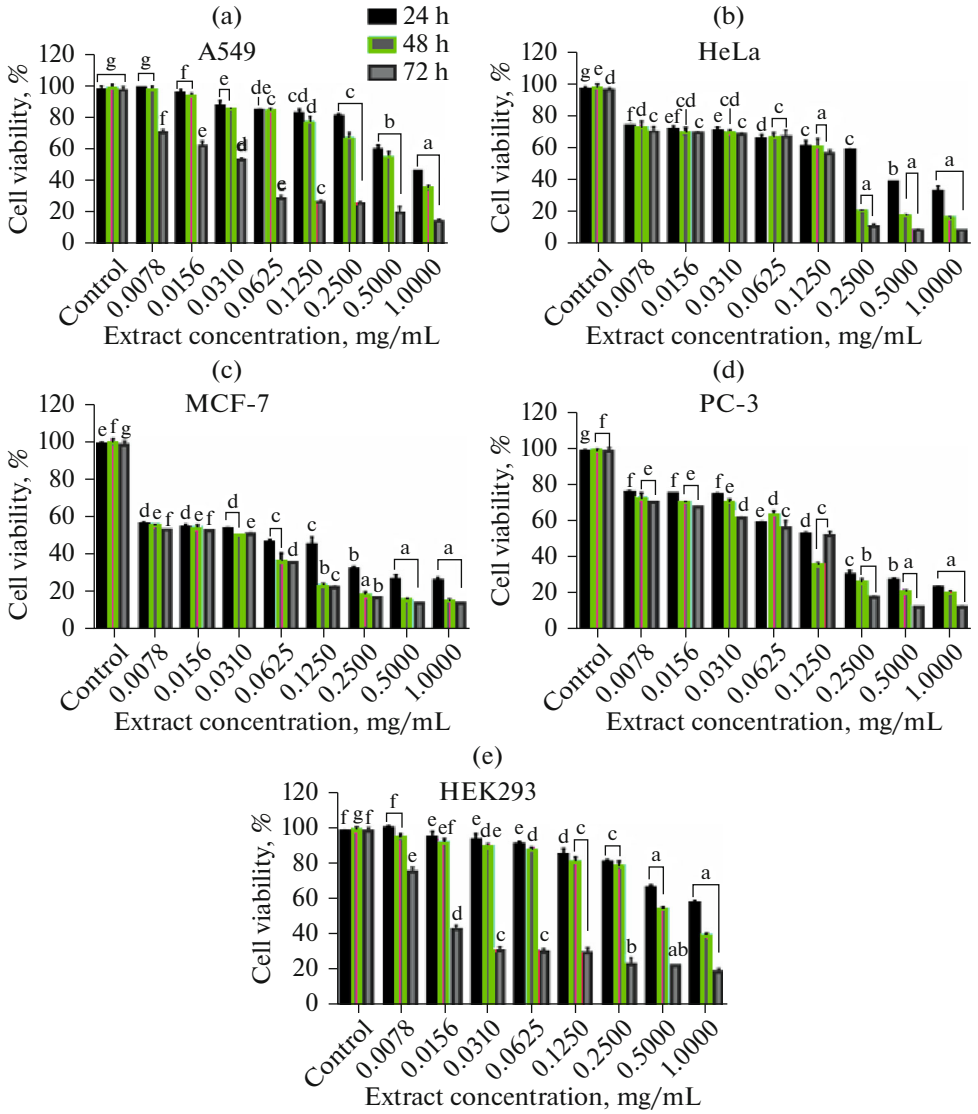

Fig. 1. Concentration and time-dependent effect of HTC extract on A549 (a), HeLa (b), MCF-7 (c), PC-3 (d), and HEK293 (e) cell line viability. Cell viability was evaluated by MTT assay. Data were expressed as mean ± standard error of 3 separate experiments. Different letters represent significant differences ($P<0.05$) according to Duncan's multiple range test.

cytometry analysis showed that both apoptotic A549 and HeLa cells were usually in the late apoptotic phase at 48 h.

Antioxidant Activity of the Extract

The results of the antioxidant activity of HTC are provided in Table 2. The DPPH free radical scavenging ability was 5.825 ± 0.060% for HTC extract at 1 mg/mL and 34.399 ± 0.085% for BHA at 0.1 mg/mL. The ABTS radical scavenging activity of HTC extract and BHA were 12.96 ± 0.370% at 0.25 mg/mL and 92.939 ± 0.081% at 0.15 mg/mL, respectively. As can be seen in Table 2, the HTC extract has a low antioxidant capacity compared to the positive control BHA in the three tests (DPPH free radical scavenging activity, ABTS radical cation scavenging activity, and β-carotene/linoleic acid method). Antioxidant activities of HTC extract were estimated by phosphomolybdenum method and FRAP assay as well. Data in Table 2 represent calibration values using ascorbic acid and Trolox, respectively, and the results were expressed as mg AAE/g extract and mg TE/g extract.

Total Content of Flavonoid and Tannins in the Extract

The total content of flavonoids in terms of quercetin equivalent (mg QE/g) in HTC extract was 8.5 ± 0.038 mg QE/g extract, while the total content of tannins in terms of catechin equivalent was 9.668 ± 0.058 mg CE/g extract.

Table 1. Approximate IC_{50} values (μg/mL) of HTC extract for the cell lines

IC_{50}, μg/mL	24 h	48 h	72 h
A549	709.7	493.4	35.62
HeLa	253.7	176.6	153.4
MCF-7	151.6	65.72	46.84
PC-3	132.2	110.9	106.9
HEK293	–*	644.9	26.04

*In the HEK293 cell line exposed to the tested HTC extracts, the IC_{50} value could not be calculated for this incubation time since the cell viability was over 50% at 24 h.

Phenolic Compounds of the Extract

The contents of phenolic compounds found in HTC extract analyzed by the HPLC method are provided in Table 3. The contents of 8 phenolic compounds among 15 standards were determined in HTC extract. The compound with the highest content was ellagic acid. It was followed by caffeic acid, 2,5-dihydroxy benzoic acid, gallic acid, naringin, and epicatechin, respectively (Table 3). Chlorogenic acid and 4-dihydroxy benzoic acid were detected in low amounts. However, such compounds as 3,4-dihydroxy benzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, rutin, cinnamic acid, and quercetin were not detected in HTC extract.

DISCUSSION

Different organisms generate diverse metabolites. Unique metabolites with anticancer activities produced by marine organisms attract special attention of pharmaceutical industries [8, 30]. Sea cucumbers are valued for their nutritional properties, as well as for their therapeutic and pharmaceutical potential [21]. In the present study, an MTT assay was carried out to investigate the potential cytotoxic effect of HTC extract depending on different concentrations and incubation times. HTC extract exhibited cytotoxicity to all cancer cell lines examined. The decreases in IC_{50} value reflect the increases in cytotoxic potential [31]. When IC_{50} values were obtained separately at 24 and 48 h, it was evident that HTC extract had generally more cytotoxic effects on MCF-7 and PC-3 cancer cell lines than on A549 and HeLa cancer cell lines. In addition, when compared to a non-cancerous cell line, HEK293, the potential cytotoxicity of HTC extract towards all cancer cells tested at 24 h and in MCF-7, PC-3, and HeLa cells at 48 h was remarkable. In other words, HTC extract exhibited selective cytotoxicity towards specified cancer cells compared to the non-cancerous cell line HEK293, especially after treatment for 24 and 48 h. However, the treatment of HEK293 cells with the extract for 72 h was not selective, and it caused approximately 60% cytotoxicity even at the concentration of 15.6 μg/mL. In general, HTC extract showed significant cytotoxicity towards all the cell lines tested after 72 h treatment compared to untreated cells. Compared to the IC_{50} values reported by Alper and Güneş, the cytotoxic potential of HTC extract against MCF-7 and PC-3 cell lines at 24 and 48 h was higher than that of *H. tubulosa* aqueous (HTS) extract (the IC_{50} values at 24 and 48 h were 228.5 and 176.7 μg/mL, respectively, for MCF-7 cells; 282.6 and 177.2 μg/mL, respectively, for PC-3 cells) and that of *H. tubulosa* methanolic (HTM) extract (the IC_{50} values at 24 and 48 h were 267.6 and 221.5 μg/mL, respectively, for MCF-7 cells; 282.3 and 257.2 μg/mL, respectively, for PC-3 cells). In addition, IC_{50} values varied at 72 h depending on the cell line and extract [2]. It has been reported in other studies that the differences in the polarity of the solvents selected for extraction could result in variations in the level of the compounds in the extract, which eventually might lead to differences in its biological activities [13, 32, 42]. Unlike the previous work [2], the different effects of the extracts on cell viability and also differences in calculated IC_{50} values may be due to the different polarities of the solvents used in the studies. In a previous study, Luparello et al. [25] examined the effects of total and filtered aqueous extracts from the coelomic fluid of the *H. tubulosa* collected in the Gulf of Palermo (Sicily, Italy) against MDA-MB231 (breast cancer) cell line, and, according to the authors, both total and fractionated extracts were able to inhibit the cell viability. Various studies using different cell lines have also shown that another species of the genus *Holothuria* has cytotoxic effects. Two compounds obtained from *H. impatiens*, one known (Bivittosid D) and the other new (Impatienside A), have been reported to have *in vitro* cytotoxic effects against seven

Table 2. Antioxidant activity of HTC extract and BHA

Sample	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)	β-Carotene-linoleic acid (%) (120')*	Phosphomolybdenum assay (mg AAE/g extract)	FRAP assay (mg TE/g extract)
HTC	8.968 ± 0.012	0.890 ± 0.003	20.14 ± 1.155	32.511 ± 0.222	30.406 ± 0.180
BHA	0.140 ± 0.002	0.086 ± 0.001	93.50 ± 0.813	–**	–**

* Results for 1 mg/mL at 120 min.
** These analyses have not been performed for BHA.

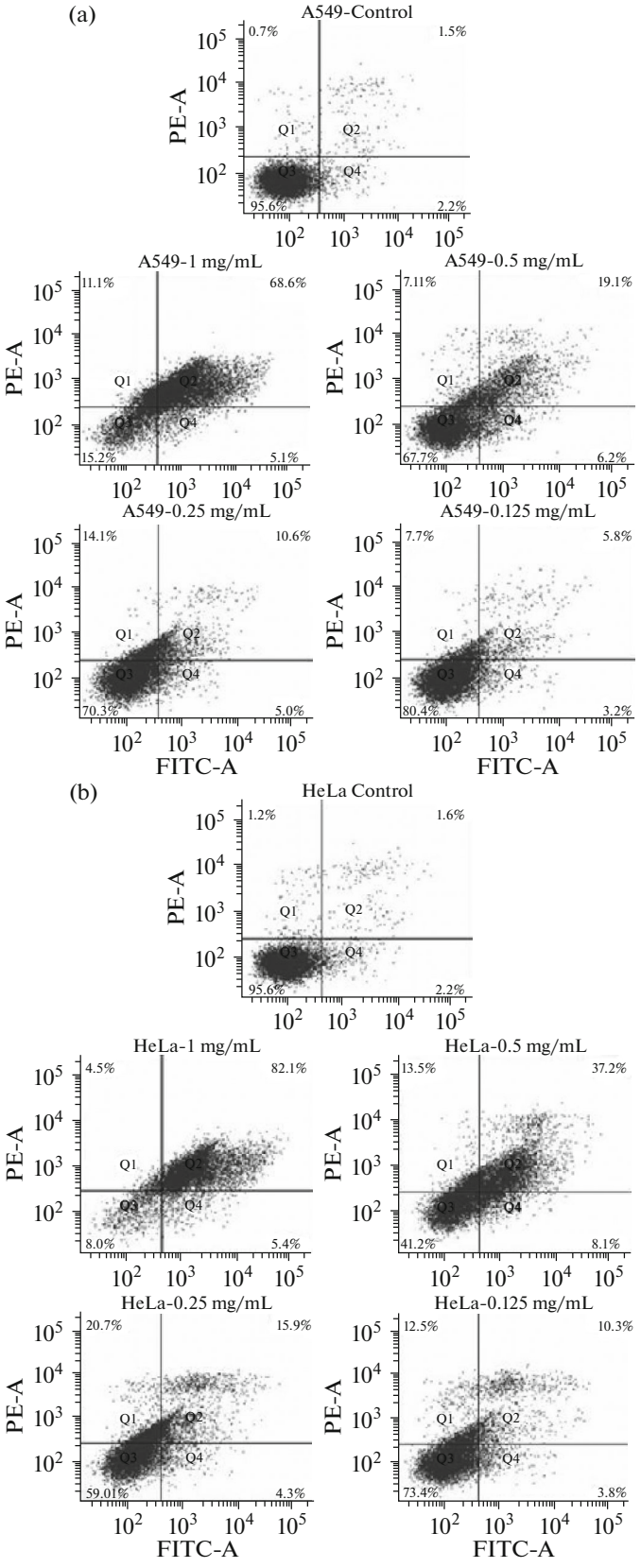

Fig. 2. Potential apoptotic effect of HTC extract on A549 (a) and HeLa (b) cells. Cells were separately treated with HTC extract at 1, 0.5, 0.25, and 0.125 mg/mL for 48 h. Untreated cells used as control. Cells were analyzed by flow cytometry and distributed into 4 quadrants: viable cells (Q3), early apoptotic cells (Q4), late apoptotic cells (Q2), and necrotic cells (Q1).

Table 3. Content (μg/g extract) of phenolic compounds in HTC extract

No.	Phenolic compounds	RT* (min)	HTC Extract (μg/g)
1	Gallic acid	6.8	5.158
2	3,4 Dihydroxybenzoic acid	10.7	–
3	4-Hydroxybenzoic acid	15.7	0.411
4	2,5-Dihydroxybenzoic acid	17.2	29.413
5	Chlorogenic acid	18.2	0.577
6	Vanillic acid	19.2	–
7	Epicatechin	21.3	2.265
8	Caffeic acid	22.7	35.523
9	*p*-Coumaric acid	26.1	–
10	Ferulic acid	30.1	–
11	Rutin	45.6	–
12	Ellagic acid	47.7	482.393
13	Naringin	49.7	3.996
14	Cinnamic acid	68.8	–
15	Quercetin	71.1	–

*RT, retention time.

different human tumor cells, with IC_{50} values of these two compounds varying between 0.37 and 2.75 μg/mL [40]. The methanol extract of *H. atra* from the Indian Ocean has been reported to show antiproliferative activity towards HeLa and MCF-7 cell lines [14]. Mashjoor and Yousefzadi [27] have demonstrated a cytotoxic effect of *n*-hexane, ethyl acetate, and methanol extracts from the different organs of *H. scabra*, *H. parva*, and *H. leucospilata* species against MCF-7 and HeLa cell lines. Based on these studies, it may be suggested that the cytotoxic activities observed may be due to different classes of substances in the extracts obtained using different solvents from different tissues of sea cucumbers.

The apoptotic effect of HTC extract on A549 and HeLa cell lines was investigated. The percentage of apoptotic cells treated with the extract was found to increase in a concentration-dependent manner. The results of these findings are comparable with those reported by the previous study [2] where HTS and HTM extracts were able to induce apoptosis in A549 and HeLa cells and the late apoptotic phase was dominant at 48 h. When compared with the previous work [2], HTC extract at 0.5 mg/mL caused a less pronounced increase in the percentage of apoptotic cells in A549 cells at 48 h than HTS and HTM extracts; however, it caused a relatively similar increase in the percentage of apoptotic cells in HeLa cells at 48 h. Different studies have indicated the apoptotic effects of extracts from different sea cucumber species. One of them reported that 73.7% of the T47D (human breast ductal carcinoma) cell population exposed to *H. atra* ethanol extract at 10 μg/mL for 24 h underwent apoptosis, and its induction capacity was confirmed by caspase-3 activation [33]. Sangpairoj et al. [38] stated that ethyl acetate fraction of body wall extracts from *H. scabra* induced apoptosis in human glioblastoma cell lines A172 and U87MG. According to the recent literature, this study was the first to show the apoptotic effect of HTC extract obtained from sea cucumbers collected off Muğla on A549 and HeLa cells.

It is stated that the evaluation of antioxidant activity cannot be carried out by only one method, since a single analysis cannot fully reveal all antioxidants [43, 44]. Accordingly, the antioxidant capacity of HTC extract was also studied by different assays. According to the results obtained from the DPPH free radical scavenging activity assay, the ABTS radical cation scavenging activity assay, and the β-carotene/linoleic acid method, the antioxidant capacity of HTC extract was quite low compared to BHA. Various studies have been conducted to search for the antioxidant effects of sea cucumbers. The ABTS radical scavenging activity of the Fraction a (from hydrolysates of the collagen, Fa, Mw < 1 kDa) obtained from *Acaudina molpadioides* was 80% for 2 mg/mL, and its DPPH activity was also 80% for 4 mg/mL. In addition, the EC_{50} (half elimination ratio) value of the Fa for the DPPH scavenging activity was estimated at 1.31 mg/mL [20]. Künili and Çolakoğlu [22] used different solvents for the extraction of *H. tubulosa* in their studies to investigate the antioxidant effects of water-methanol,

methanol, and acetonitrile-trifluoroacetic acid extracts obtained from *H. tubulosa* species collected from the Southern Coasts of Çanakkale Strait in the Marmara Sea. The researchers stated that the antioxidant activities of the extracts at all concentrations were lower than those of the reference antioxidant agents. This result may be considered consistent with the current study due to the low antioxidant capacity of the HTC extract. In contrast, the tegument extract of *H. tubulosa* from the Bizerta Lagoon (Tunisia) showed a potential antioxidant activity [45]. This extract was reported to have a higher antioxidant effect than HTC extract, since the IC_{50} values for ABTS inhibition (25.46 μg/mL) and DPPH inhibition (26.26 μg/mL) for the tegument extract of *H. tubulosa* were lower than those for HTC extract. The total antioxidant capacity of HTC extract was estimated by the phosphomolybdenum method, and the result (Table 2) was found to be similar to that reported previously for a polysaccharide fraction from the red alga *Gracilaria intermedia* which amounted to 28.98 ± 1.86 mg/g EAAsc (equivalent of ascorbic acid) [12]. However, the result was found to be inferior to those obtained from methanol extracts of two marine sponges, *Spongia officinalis* var. *ceylonensis* and *Sigmadocia carnosa* (40.433 ± 0.72188 and 66.33 ± 3.38296 mg/g equivalent of ascorbic acid, respectively) [6]. Furthermore, the present findings obtained through the FRAP assay may be considered important compared to those of FRAP of the extract of five brown seaweeds from Vietnam, which varied between 3.06 and 6.93 mg Trolox equivalents per gram of dried material [17]. The differences in antioxidant activity in the extracts may be due to the polarities of the solvents chosen and the different species used.

In the present study, 8 different phenolic compounds were analyzed, and also a certain level of antioxidant capacity was determined. Among 15 standards, ellagic acid (482.293 μg/g extract) was found to be the most abundant compound in HTC extract. However, Alper and Güneş [2] detected 13 and 12 phenolic compounds in HTS and HTM extract, respectively. They stated that the most abundant phenolic compounds were epicatechin (790.091 μg/g extract) and 2,5-dihydroxy benzoic acid (158.89 μg/g extract) in HTS and HTM extract, respectively. Nine non-volatile phenolic compounds were determined by the HPLC analysis in the mixed (organic/aqueous) extract obtained from *H. atra*; among these compounds, chlorogenic acid had the highest proportion (92.86%) [15]. These data confirm that the phenolic compound content in extracts may differ depending on the solvent selected for extraction.

In conclusion, our results revealed that chloroform extract of *H. tubulosa* from Muğla has a cytotoxic and apoptotic potential against cancer cells, whereas its antioxidant potential is very low. In addition, the phenolic compound content of the extract was found to be low. Our results may contribute to further studies on the use of *H. tubulosa* for obtaining new anticancer agents. Of course, further studies are required to identify bioactive compounds for therapeutic purposes.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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