

Cytotoxicity of *Sargassum angustifolium* Partitions against Breast and Cervical Cancer Cell Lines

Abstract

Background: Marine organisms produce a variety of compounds with pharmacological activities including anticancer effects. This study attempt to find cytotoxicity of hexane (HEX), dichloromethane (DCM), and butanol (BUTOH) partitions of *Sargassum angustifolium*. **Materials and Methods:** *S. angustifolium* was collected from Bushehr, a Southwest coastline of Persian Gulf. The plant was extracted by maceration with methanol-ethyl acetate. The extract was evaporated under vacuum and partitioned by Kupchan method to yield HEX, DCM, and BUTOH partitions. The cytotoxic activity of the extract (150, 450, and 900 µg/ml) was investigated against MCF-7 (breast cancer), HeLa (cervical cancer), and human umbilical vein endothelial cells cell lines by mitochondrial tetrazolium test assay after 72 h. **Results:** The cell survivals of HeLa and MCF-7 cell were decreased by increasing the concentration of extracts from 150 µg/ml to 900 µg/ml. The median growth inhibitory concentration value of HEX partition was 71 and 77 µg/ml against HeLa and MCF-7, dichloromethane partition was 36 and 88 µg/ml against HeLa and MCF-7, respectively. BUTOH partition was 25 µg/ml against MCF-7. **Conclusion:** This study reveals that different partitions of *S. angustifolium* have cytotoxic activity against cancer cell lines.

Keywords: Cancer, cytotoxic, Persian Gulf, *Sargassum*

Introduction

Seaweed is a marine macro alga comprised many genres and species. These marine organisms have been traditionally used as food sources and therapeutic agents, especially in some parts of Asia such as Japan, Korea, and China.^[1] Seaweed dietary values are due to the existence of many compounds, such as vitamins, fatty acids, minerals, trace elements, dietary fibers included soluble, insoluble and total dietary fibers, and amino acids.^[1]

According to photosynthetic pigments, seaweed is classified into three major groups; green, brown, and red algae.^[2] They produce several kinds of secondary metabolites such as phlorotannins, fucoidans, alginate, laminaran, and terpenoids that have shown a wide range of biological effects. Different species of *Sargassum* from sargassaceae family have shown antioxidant,^[3] antibacterial,^[4] antifungal,^[5] anticancer,^[6] antitumor,^[7] anti-Herpes,^[8] and antiallergic effects.^[9] hepatoprotective,^[10] antihistamine,^[11,12] anti-inflammatory,^[11] and anticholinergic.^[13]

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Cancer is one of the most important diseases in the world, and cancer chemotherapy is the gold standard of treatment. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells too.^[14]

Some metabolites isolated from the algae, influence proliferation, apoptosis, and cell cycle arrest with different mechanisms. Some mechanisms which are suggested included increasing natural killer cells,^[15] activation of nonspecific immune system,^[16] inhibition of cell growth,^[17] angiogenesis,^[18] and induction of apoptosis.^[19]

Iran has coastal lines about 1260 km along the Persian Gulf and the Oman Sea. More than 250 species of different algae have been identified in this area. Despite the existence of a great extent of marine algae in this region, there are only a few studies on the phytochemical analysis and biological activities of these seaweeds.^[20] This study investigates the cytotoxicity of the seaweed *Sargassum angustifolium* and its different partitions.

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Materials and Methods

Authentication of plant material

S. angustifolium was collected in autumn 2015 from Bushehr, a Southwest coastline of Persian Gulf. Voucher specimens were made and deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences (Code: 2662) and were identified by the Agricultural and Natural Resources Research Center of Bushehr.

Preparation of the extracts

The algae were air-dried in the shade at room temperature and ground to a powder with a Philip's mill and then extracted by maceration with methanol-ethyl acetate (1:1) at room temperature. The extract was evaporated under vacuum and partitioned by Kupchan method to yield hexane (HEX), dichloromethane (DCM), butanol (BUTOH), and water partitions.^[21]

The partitions were subjected to cytotoxic test.

Cytotoxic test

After providing these partitions at the second step, for cytotoxic screening, we used three cell lines: MCF-7, HeLa, and human umbilical vein endothelial cell (HUVEC). At first, three cell lines were growing in the Dulbecco's modified Eagle's medium cell culture medium which supplemented with 10% fetal bovine serum. Penicillin and streptomycin were added to the media. All cell lines were cultured at 37°C in air/carbon dioxide (95:5) atmosphere.

At the second, the concentration of 150, 450, and 900 µg/ml^[22] from all partitions were tested for each cell line. Samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. The final DMSO concentration used was 1% of total volume of medium in all treatments including the control group.

For mitochondrial tetrazolium test (MTT) assay, 1×10^5 cells/well were plated into 96-well plates and incubated for 24 h before addition of extracts. The plates were allowed to proliferate and reach their exponential phase of growth. The incubation time for each cell line was assigned according to the normal growth curve of that cell line and was determined twice as long as the doubling time of each cell line. And then, 20 µl from all partitions were added to the media.

After 72 h of incubation for these type of cells, 30 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent (5 mg/ml) in phosphate buffered serum was added to each well. The plates were incubated at 37°C for 4 h. At the end of the incubation period, the medium was removed and 100 µl cell culture grade DMSO was added to each well. The formazan salts were quantified by reading the absorbance at 540 nm on a microplate reader.

Cell viability in MTT assays was calculated as a percentage of untreated cells (control value). The cytotoxicity value was presented as the median growth inhibitory concentration (IC_{50}) of the reagents compared to control.

Statistical analysis

All experiments were done in triplicate. Results are expressed as the mean \pm standard error of the mean. Statistical analysis was done using SIGMASTAT™ (Jandel Software, version 12, CA, USA), which was used to perform statistical tests. Analysis of variance followed by the Student–Newman–Keuls test was used to see the differences among the groups. Significance was assumed at 5% level.

Results and Discussion

The cytotoxicity results obtained from the extracts against MCF-7, HeLa, and HUVEC cells were shown in Table 1. Values were modified from those of NCI and Geran *et al.*^[23] as follows: $IC_{50} \leq 20$ µg/ml = highly active, IC_{50} 21–200 µg/ml = moderately active, IC_{50} 201–500 µg/ml = weakly active, and $IC_{50} > 501$ µg/ml = inactive.

Figure 1 shows the MTT test results for different *S. angustifolium* extract partition with different concentrations on the HUVEC cell line after 72 h. We observed a significant reduction in cell viability at the doses of 150, 450, and 900 (µg/ml) of DCM partition, 450 and 900 (µg/ml) of HEX and BUTOH partitions.

The results for different concentrations from *S. angustifolium* partitions on HeLa cell line, after 72 h, are shown in Figure 2. At the base of these results, a significant reduction in cell viability the doses of 450 and 900 (µg/ml) of DCM partition, 150, 450, and 900 (µg/ml) of HEX and 150 and 450 (µg/ml) BUTOH partitions.

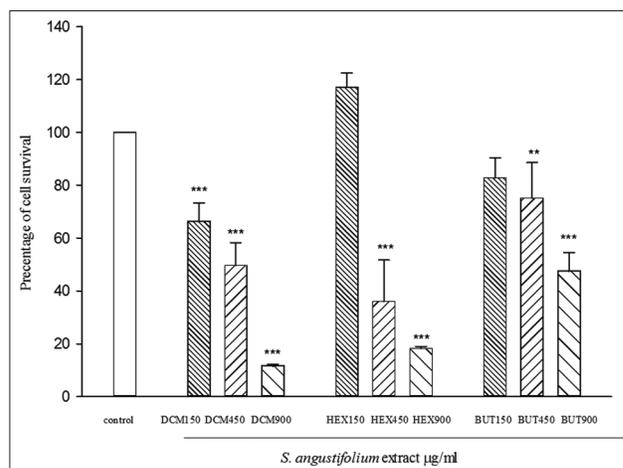


Figure 1: Cytotoxic activity of *Sargassum angustifolium* partitions on human umbilical vein endothelial cell line. Data represent the mean \pm standard error of the mean separate experiments (significant as compared to control, ** $P < 0.01$, * $P < 0.001$). Cells viabilities were assessed by mitochondrial tetrazolium test assay. Cells were incubated for 72 h**

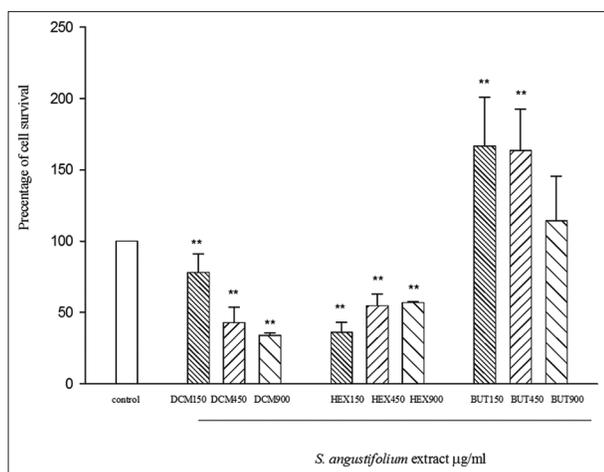


Figure 2: Cytotoxic activity of *Sargassum angustifolium* partitions on HeLa cell line. Data represent the mean \pm standard error of the mean separate experiments (significant as compared to control, $**P < 0.01$). Cells viabilities were assessed by mitochondrial tetrazolium test assay. Cells were incubated for 72 h

Table 1: The median growth inhibitory concentration values ($\mu\text{g/ml}$) of dichloromethane, butanol, and hexane partition fraction of extract of *Sargassum angustifolium*

Sample	IC_{50} ($\mu\text{g/ml}$)-cell lines (MTT assay)		
	MCF-7	HUVEC	HeLa
DCM	36.1773	88.1416	62.5146
HEX	71.266	122.7	134.7
BUTOH	25.2746	23.7122	-

Results are expressed as mean \pm SD. SD: Standard deviation, HEX: Hexane, BUTOH: Butanol, DCM: Dichloromethane, HUVEC: Human umbilical vein endothelial cell, MTT: Mitochondrial tetrazolium test, IC_{50} : The median growth inhibitory concentration

For MCF-7 cell line, after 72 h, results exhibit a significant reduction in cell viability at the doses of 150, 450, and 900 ($\mu\text{g/ml}$) of DCM partition, 450 and 900 ($\mu\text{g/ml}$) of HEX, and 900 ($\mu\text{g/ml}$) BUTOH partitions. These results have been shown in Figure 3.

Nowadays, investigating biological activities and structure elucidation of compounds isolated from marine sources have become the focus of much attention.^[20] Sea algae containing different genres and species produce various secondary metabolites with attractive biological and physiological activities. Several literatures report the potential cytotoxic activity of marine seaweeds.^[24] Our study documents the potential influence of the seaweed *S. angustifolium* extract against HeLa, MCF-7, and HUVEC cell lines using MTT assay. The intensity of HeLa and MCF-7 cell density were decreased by increasing the concentration of extracts from 150 $\mu\text{g/ml}$ to 900 $\mu\text{g/ml}$. This infers the existence of dose-dependent properties of extracts against cancer cell lines which was found effective. The IC_{50} value of HEX, dichloromethane, and BUTOH fractions against HeLa and MCF-7 were 200 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$, respectively. The

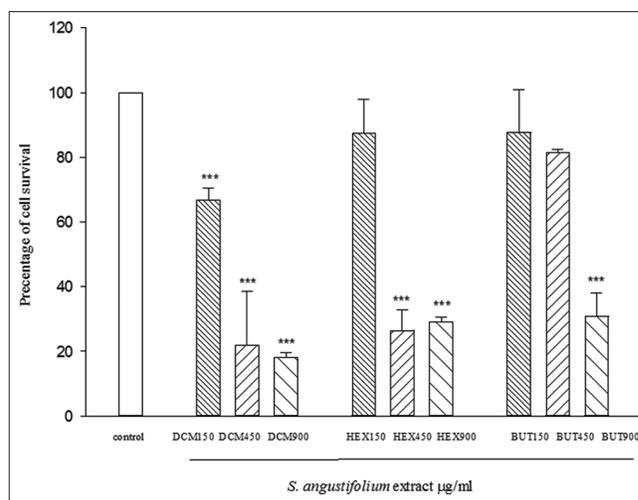


Figure 3: Cytotoxic activity of *Sargassum angustifolium* partitions on MCF-7 cell line. Data represent the mean \pm standard error of the mean separate experiments (significant as compared to control, $**P < 0.01$, $***P < 0.001$). Cells viabilities were assessed by mitochondrial tetrazolium test assay. Cells were incubated for 72 h

study reveals that dichloromethane showed the highest activity, especially against MCF-7 cell line. In addition, partitions were more effective against MCF-7 cell line than HeLa ones.

Marine organisms produce several types of metabolites. Metabolites such as terpenoids, bromophenols, carotene, phlorotannins, sulfated polysaccharides, steroids, and halogenated compounds were isolated and purified from some algae.^[25] Fucoidans are antitumor polysaccharide isolated from *Sargassum polycystum* and some other algae. They seem to exhibit important role against some human cancer cell lines.^[26] Steroids are the other compounds in seaweeds, especially in brown algae with antitumor activity. They have been reported in several *Sargassum* species.^[27] In a study by Tannoury *et al.* both water: ethanol extract and chloroform: ethanol extract of *Sargassum vulgare* showed cytotoxic activity against Jurkat cancer cell line with IC_{50} values of 136.907 $\mu\text{g/ml}$ and 49.056 $\mu\text{g/ml}$, respectively, after 72 h of treatment but another research shows that HEX fraction of *Sargassum thunbergii* was the most cytotoxic partition against HL-60 tumor cell lines. In addition, HEX partition of *S. angustifolium* showed cytotoxicity against T47D and HT-29 cell lines (IC_{50} 166.42 \pm 26.7 and 190.24 \pm 52.8 $\mu\text{g/ml}$), respectively, in the other study. Fucosterol may be the active component in HEX partitions.^[22] Yagdaneh *et al.* have analyzed *S. angustifolium* phytochemically and biologically in a study and reported that tannins, saponins, sterols, and triterpenes were the most abundant compounds in this seaweed. Partitioning pattern of compounds into different solvents depends mainly on their structure. Nonpolar compounds will tend to solvents such as HEX; polar compounds are found in butanolic partition while dichloromethane fraction contains semipolar compounds. We did not investigate fractions

phytochemically, but it seems that semipolar terpenoids and steroids are the main components of dichloromethane fraction. Bioassay-guided isolation of secondary metabolites in dichloromethane fraction is needed to find the active metabolites. In summary, these results show that *S. angustifolium* decreased cell viability in carcinoma.

Conclusion

Different partitions of the seaweed *S.angustifolium* from Persian Gulf, Iran were screened for their cytotoxic analysis by MTT assay against HeLa and MCF-7 cell lines. The cell survivals of both cell lines were decreased by increasing the concentration of extracts. Hexane, dichloromethane and butanol extracts of this seaweed and their active components could emerge as natural and alternative cytotoxic agents or serve as starting points for synthesizing more effective cytotoxic drugs.

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Conflicts of interest

There are no conflicts of interest.

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