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Original Article

Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf

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Abstract

Sea algae are widely consumed in the world. There are several seaweeds including brown algae which are authorized for human consumption. These plants contain important phytochemical constituents and have various potential biological activities. The present study investigated the presence of phytochemical constituents and total phenolic quantity of the seaweeds *Sargassum angustifolium*, *Sargassum oligocystum* and *Sargassum boveanum*. Cytotoxicity of seaweeds was tested against HT-29, HeLa and MCF-7 cell lines. Antioxidant potential of these 3 *Sargassum* species was also analyzed. Cytotoxicity was characterized by IC_{50} of human cancer cell lines using sulforhodamine assay. Antioxidant activities were evaluated using 2,2-diphenyl-1- picrylhydrazil. The analysis revealed that tannins, saponins, sterols and triterpenes were the most abundant compounds in these *Sargassum* species while cyanogenic and cardiac glycosides were the least ones. *Sargassum angustifolium* had the highest content of total phenolics (0.061 mg/g) and showed the highest antioxidant activity (IC_{50} = 0.231). Cytotoxic results showed that all species could inhibit cell growth effectively, especially MCF-7 cell line (IC_{50} = 67.3, 56.9, 60.4 for *S. oligocystum*, *S. angustifolium* and *S. boveanum* respectively). Considerable phytochemicals and moderate cytotoxic activity of *S. angustifolium, S. oligocystum* and *S. boveanum* make them appropriate candidate for further studies and identification of their bioactive principles.

Keywords: Antioxidant; Cytotoxic; *Sargassun angustifolium*; *Sargassum oligocystum*; *Sargassum boveanum*; Seaweed

INTRODUCTION

Seaweeds are fresh sources of bioactive compounds with immense medicinal potential which have attracted the attention of pharmaceutical industries (1,2). They usually grow in all seas except in the Polar Regions and produce biologically active compounds. They are used as human food especially by coastal populations (3). There are many reports on the antibacterial (4), antifungal (5), antiviral (6), anti-inflammatory (7), antidiabetic (8), antioxidant (9) and cytotoxic activities of seaweeds (10).

Genus of *Sargassum* is widely distributed in the temperate and tropical oceans of the world. There are numerous reports on their secondary metabolites and biological activities (11). They usually contain terpenoids that exhibits biological activities such as cell toxicity, antioxidant activity, vasodilatory effects, induction of larval settlement of hydrozoan and inhibition of acetylcholine-esterase (12,13).

Different kinds of radicals are generated in the normal metabolic activities and sometimes the antioxidant capacity of the body is inadequate to cope with them. Therefore, there is a growing interest on the discovery of natural antioxidants because they reduce the risk of developing chronic disease such as cancer and also phytochemicals are generally safer than synthetic chemicals (14).

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 (15). 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

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activities of these seaweeds. In the current study in addition to phytochemical screening of three *Sargassum* extracts, their antioxidant activity and cytotoxic potential were investigated.

MATERIAL AND METHODS

Authentication of plant material

The seaweeds were collected in 2012 from the Persian Gulf coasts of Iran close to Bushehr Province. They were identified by Agricultural and Natural Resources Research Center of Bushehr and their voucher specimens coded as 2662 for *S. angustifolium*, 2663 for *S. oligocystum*, and 2664 for *S. boveanum* were deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences (Isfahan, Iran).

Preparation of the extracts

The plant samples were cut into small pieces, completely air-dried, and stored in glass containers until extraction. About 100 g of the dried plant material was macerated for five consecutive days with methanol. The extracts were filtered through 2 layers of cotton fabric and evaporated at room temperature under reduced pressure. Dried residues were stored in clean vials until phytochemical and cytotoxic screening (16).

Phytochemical screening

Tests for phytochemical constituents including alkaloids, steroid and triterpenes, anthraquinones, flavonoids, saponins, cyanogenic glycosides, cardiac glycosides and tannins followed the methods described previously (17).

Determination of alkaloids

Powdered specimen of the plants (200 mg) was boiled with 10 ml water and 10 ml of hydrochloric acid on a water bath. Finally it was filtered and its pH was adjusted to about 6-7 with ammonia. One ml of the filtrate was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution). In addition, 1 ml portion was treated similarly with Wagner's reagent (solution of iodine in potassium iodide). Turbidity or colored precipitation with either of these reagents was taken as evidence for the presence of alkaloids (17).

Test for cardiac glycosides

A few drops of the Baljet's reagent (picric acid, ethanol and sodium hydroxide) were added to 2-3 mg of sample. A positive reaction was indicated by orange to deep red color (17).

Test for tannins

Sample (1 g) was boiled with 20 ml distilled water for 5 min in a water bath and filtered while it was hot. Then 1 ml of cool filtrate was diluted to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were added and observed for formation of precipitates and any color change. A bluish-black or brownish-green precipitate indicated the presence of tannins (17).

Test for flavonoids

Powdered sample (1 g) was boiled with 10 ml of distilled water for 5 min and filtered while it was hot. A few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow color which on addition of acid changed to colorless solution depicted the presence of flavonoids (17).

Test for saponins

The extract solution (1 ml) was diluted with distilled water to 20 ml and shaken for 15 min in a graduated cylinder. Development of stable foam suggests the presence of saponins (17).

Test for combined anthraquinones

Powdered sample (1 g) was boiled with 2 ml of 10% hydrochloric acid for 5 min. The mixture was filtered while it was hot and the filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any color change. Rose pink color indicated the presence of anthraquinones (17).

Test for sterols and triterpenes

Three grams of the powdered leaves was placed in a test tube and 10 ml of 50% alcohol was added, the tube was then placed on a water bath and heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. 10 ml of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests.

Liebermann-Burchard's reaction: To test tube I, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated sulfuric acid was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish color in the separation layer indicates the presence of sterols and triterpenes.

Salwoski's test: To test tube II, 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown color at the inter phase indicates the presence of steroidal ring (17).

Determination of total phenolics

The powdered plant material (20 g of each sample) were weighed in to 50 ml flask, sonicated with 30 ml of ethanol 40% for about 30 min and shaken for about 10 min. The extracts were allowed to cool down to room temperature and the flasks were made to volume with extracting solvent.

Preparation of standard

Twenty mg of gallic acid and 30 ml 40% ethanol were added into 50 ml volumetric flask and sonicated until no solid was present in the flask. After cooling down the solution to room temperature, the flask was filled with extracting solvent. The standard solution was diluted several times.

One ml of standard solution transferred to 100 ml volumetric flask with 60-70 high-performance liquid chromatography (HPLC) grade water. The contents swirled to mix. Five ml of Folin-Ciocalteu's phenol reagent was added and mixed again. After 1 min and before 8 min, 15 ml of sodium carbonate solution was added and the time recorded as time zero. The volume was made up to 100 ml exactly with HPLC grade water. The flask stoppered and mixed thoroughly by inverting it several times. After 2 h, the sample was scanned at 550 to 850 nm and maximum absorbance of about 760 nm was recorded. Same solution without the extraction solution was used as the blank solution (18).

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve $y = 1.1771\ x - 0.0252$, $R^2 = 0.9958$ where, y is absorbance at 760 nm and x is total phenolic content in the extracts of different alga expressed in mg/L.

In vitro cytotoxicity assay

The extracts were tested using MCF-7 (human breast adenocarcinoma), HeLa (cervical carcinoma), and HT-29 (human colon adenocarcinoma) cells. The cancer cell lines were bought from Cell Line Service (Germany) and grown in Dulbeccos Modified Eagle Medium (DMEM) (from Invitrogen, USA) supplemented with 10% fetal bovine serum (from Seromed, India). Cells were seeded in 96-well at 3500 cancerous cells/well and 5000 normal cells/well and allowed to adhere for 24 h at 37 °C with 5% CO_2 in a fully humidified incubator. Then 100 µl serially diluted concentrations of samples in medium were dispensed in to the wells of the cell plates and incubated further for 72 h. After removal of the sample medium, the cells were topped up with 200 µl DMEM medium and incubated for 72 h. Afterward cells were fixed with cold 40% trichloroacetic acid at 4 °C for 1 h and washed with tap water. Cell viability was determined by Sulforhodamine assay. The absorbances were measured at 492 nm using a microplate reader (BioTeck, Germany). Percent cell death was calculated relative to the control. The concentration of the extract that inhibited 50% cells growth (IC_{50}) was determined from the graph percent inhibition

against different plant extract concentrations. The cytotoxic activities of all extracts against breast cancer cell lines were labeled according to the National Cancer Institute (NCI, USA) criteria (highly inhibiting activity means IC_{50} ≤20 μg/ml) (19).

DPPH free radical scavenging assay

The free radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay. Sample stock solutions (1.0 mg/ml) of the extracts were diluted to final concentrations of 243, 81, 27, 9, 3 and 1 μg/ml, in ethanol. One ml of a 50 μg/ml DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following equation:

AA% = ((absorbance of the control – absorbance of the sample)/absorbance of the control) × 100

Ethanol (1.0 ml) plus plant extract solutions (2.5 ml) were used as the blank.

DPPH solution (1.0 ml) plus ethanol (2.5 ml) was used as a negative control. The positive controls were ascorbic acid, butylated hydroxyanisole and butylated hydroxytoluene. Assays were carried out in triplicate (20).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Scheffe post hoc test were used for data analysis. All results were expressed as mean ± SD and $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemical constituents

Phytochemical data (Table 1) shows distinct patterns of chemical compositions in constituents of the extracts. The patterns of composition differed considerably in their quantitaties. The results of phytochemical evaluation are shown in Table 1.

This analysis showed the most abundant compounds in *S. angustifolium* were tannins, saponins, sterols and triterpenes fallowed by flavonoids. Cyanogenic and cardiac glycosides were absent in this seaweed. In *S. oligocystum* tannins were the most abundant compounds fallowed by saponins, alkaloids, sterols and triterpenes. Cyanogenic glycosides were not present. Tannins, sterols, triterpenes and saponins were major constituents in *S. boveanum* too.

The amount of total phenol was determined with the Folin-Ciocalteu reagent. Phenolic compounds are a class of antioxidants which act as free radical terminators. Table 2 shows the contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent. The total phenol in selected seaweeds was 0.061, 0.019 and 0.035 mg in 1 g of dry extract in *S. angustifolium, S. oligocystum* and *S. boveanum* respectively.

Table 1. Phytochemical constituents of *Sargassum* species.

Constituents	*S. angustifolium*	*S. oligocystum*	*S. boveanum*
Tannins	+++	+++	++
Alkaloids	+	+	+
Saponins	+++	++	++
Sterols and triterpenes	+++	++	++
Flavonoids	++	+	+
Cyanogenic glycosides	-	-	-
Cardiac glycosides	-	+	-
Anthraquinones	++	++	+

Table 2. Total phenolic content of seaweeds.

Seaweeds	Absorbance at 760 nm	Concentration (mg/g)
S. angustifolium	0.637± 0.001	0.061± 0.0001
S. oligocystum	0.200± 0.004	0.019± 0.00004
S. boveanum	0.390± 0.009	0.035± 0.0001

Table 3. Cytotoxic activities ($IC_{50,}$ μg/ml) of the studied seaweeds on different cell lines**.**

Seaweeds	Cell lines		
	HT-29	**HeLa**	**MCF-7**
S. angustifolium	121.8± 0.09	87.9± 0.02	67.3± 0.14
S. oligocystum	133.9± 0.11	96.7± 0.13	56.9± 0.21
S. boveanum	125.6± 0.21	82.3± 0.18	60.4± 0.16

Table 4. Antioxidant activity (IC_{50}) of the seaweeds**.**

Seaweeds	IC_{50} (mg/ml)
S. angustifolium	0.231± 0.01
S. oligocystum	0.610± 0.04
S. boveanum	0.489± 0.09

Cytotoxic assay

The criteria used to categorize the activity of extracts against MCF-7, HeLa, HT-29 cancer cell lines based on IC_{50} values, were modified from those of NCI and Geran and coworkers (19) as follows: IC_{50} ≤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.

The cytotoxicity data for the extracts against MCF-7, HeLa and HT-29 cells are displayed in Table 3. Cytotoxic results showed that all species could inhibit cell growth effectively, especially MCF-7 cell line (IC_{50} were 67.3, 56.9, 60.4 for *S. oligocystum*, *S. angustifolium* and *S. boveanum*, respectively).

Antioxidant activity

The antioxidant activities of the seaweed extracts were measured based on scavenging activity of the stable DPPH free radical. The value of IC_{50} is inversely related to the antioxidant activity. The extracts of three algae were found to have different levels of antioxidant activity. Table 4 shows that *S. angustifolium* indicated the highest antioxidant activity (IC_{50} of 0.231) while *S. oligocystum* showed the lowest one (IC_{50}, 0.610).

DISCUSSION

Sea algae are widely consumed in Japan and there are several seaweeds including brown algae authorized in France for human consumption (21). Moreover, certain algae are used as capsule dosage form in different countries as diet integrator (22). These facts support safety of utilization of the algae extract as natural antioxidant. Based on the results obtained from studies on seaweeds, it has been shown that several algae species can prevent oxidative damage by scavenging free radical and hence able to prevent cancer cell formation (23). Certain algae have long been used in traditional Chinese herbal medicine in the treatment of cancer (24). Many studies have been performed in order to determine the bioactive compounds produced in marine algae. In this field, brown algae of the genus *Sargassum*, (Sargassaceae, Fucales) are known to contain structurally unique secondary metabolites such as plastoquinones (25), chromanols (26) and polysaccharides (27).

In the present research an effort was made to study the similarity and differences amongst three species based on phytochemical analysis and bioactivity. Phytochemical tests revealed many similarities. The only difference in terms of phytochemical analysis of the three plants was the cardiac glycosides which was present only in *S. oligocystum.* The amount of tannins, sterols and triterpenes, saponins and flavonoids were also different. With regard to cytotoxic activities, all species showed higher toxicity against MCF-7 cell lines compared to HT-29 and HeLa cell lines and the results were very similar. Phytochemical and biological results of the current work revealed that these edible seaweeds could be potential candidates in the field of drug development.

The result of the present study showed that the extract of *S. angustifolium*, which contained highest amount of phenolic compounds could exhibit a greater antioxidant activity. *S. angustifolium* is an important species from family Sargasseae. Different

algae of this family have shown antibacterial, cytotoxic, antivirus, antioxidant and antitoxin activities. They seem to have hepatoprotective activity and in pharmacologic studies have reduced blood sugar (28).

The high scavenging property of *S. angustifolium* is related to hydroxyl groups existing in the phenolic compounds. In addition phenolics are strong antibacterial compounds and antibacterial properties of several plants are related to their phenolic contents. So the assessment of antibacterial effect of *S. angustifolium* in future studies is proposed.

In a study by Sadati and colleagues, *S. swartzii* from Asaloye-Niband marine protected area of the Persian Gulf was analyzed for its antioxidant and phenolic contents. The extract was partitioned in different solvents and methanol partition showed the highest phenolic content (0.12 mg/g) and antioxidant activity (73.92 ± 12.3 mmol FeII per 100 g dried plant) that is comparable with our study (29).

Tropical conditions affect phytochemical constituents of the plants especially phenolic content, particularly in marine organisms. In a study by Targete and coworker, a comparison between the total phenolic compounds of algae from different regions of tropical area was demonstrated (30). The results showed that there was significant difference in phenolics of algae related to the climate conditions. Therefore, it is suggested that phenolic content of algae from other regions of Persian Gulf be evaluated and results are compared.

It is the first time that these three *Sargassum* species of Persian Gulf are analyzed for their phytochemical and biological pattern. Further work is necessary to isolate the active principles and elucidate the structure and mode of action of these compounds.

CONCLUSION

The extracts of three *Sargassum* seaweeds from Persian Gulf, Iran were screened for their antioxidant, cytotoxic and phytochemical analysis. Phytochemical and cytotoxic results were similar with few differences in all species. *Sargassum angustifolium* showed the highest antioxidant activity. These seaweed extracts and their active components could emerge as natural and alternative antioxidants or serve as starting points for synthesizing more effective cytotoxic drugs.

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