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Research Article

Phytochemical analysis, antioxidant, and anticancer activities of three brown algae from the Persian Gulf

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Abstract

Marine seaweeds are sources of bioactive compounds such as novel anticancer components. This study investigated the phytochemical composition, antioxidant, and anticancer activities of aqueous and methanolic extracts of three brown algae (*Sargassum obtusifolium*, *Padina gymnospora*, and *Cystoseira indica*) from the Persian Gulf. The contents of total polyphenols, total flavonoids, and total carbohydrates were determined. The antioxidant activity of the extracts was determined by DPPH method. The cytotoxicity of extracts was evaluated using the MTT assay on breast cancer (MCF7) and lung cancer (A549) cell lines. The aqueous extracts had higher levels of polyphenols, flavonoids, and carbohydrates (ranging from 125±11 to 202±19 mg/g) than methanolic extracts (ranging from 43.8±3.4 to 76.6±6.9 mg/g). At 50 µg/mL, the inhibition of DPPH radical ranging from 40.9±0.6 to 62.3±1.4% with an IC_{50} ranged from 60.9±0.5 to 30.4±0.4 µg/mL. The MTT cell proliferation assay confirmed a significant reduction in MCF7 cell line viability in the methanolic extracts at 390 µg/mL and 190 µg/mL compared to the control group. The methanolic extract of *S. obtusifolium* showed the highest selectivity index on MCF7 and A549 cells (1.16±0.19 and 1.77±0.16 respectively), despite containing lower levels of phytochemicals than the aqueous extracts. The findings suggest that the methanolic extract of *S. obtusifolium* exhibits selective cytotoxicity against MCF7 cells. Also, the aqueous extract of *C. indica* shows an adequate antioxidant index, usable in nutritional science.

Introduction

Cancer remains a formidable challenge in contemporary healthcare, driving extensive research efforts toward effective treatments (Dyshlovoy and Honecker, 2019). While conventional approaches like surgery, chemotherapy, and radiotherapy are the mainstays for cancer treatment, their limitations and adverse effects underscore the need for alternative therapies (Abbas and Rehman, 2018). On the other hand, Natural products have been introduced as unique chemotherapy drugs (Newman and Cragg, 2012; Newman and Cragg, 2016) and they have emerged as promising sources of novel compounds with therapeutic potential, offering efficacy with reduced side effects (Ameri *et al*., 2017; Bhagwat *et al*., 2024). Notably, marine organisms have become a focal point in the search for bioactive compounds, with the ocean serving as a vast reservoir of pharmacologically active molecules (Rajabiyan *et al*., 2021). Among marine organisms, algae have garnered attention for their traditional use in cancer treatment, particularly in Asian countries (Emtyazjoo *et al*., 2023).

Brown algae, in particular, have emerged as a rich source of bioactive compounds, including polyphenols and sulfated polysaccharides, renowned for their potent antioxidant activity (Newman and Cragg, 2012). Recent research indicates that brown algae exhibit antiproliferative, apoptotic, and anti-angiogenic activity. They also demonstrate cytotoxic effects *in vitro* and *in vivo* (Salamat *et al*., 2022; Van Alstyne and Borgen, 2024).

Some of the anticancer secondary metabolites found in brown algae are polyphenols, including phenolic acids, phlorotannins, bromophenols, and flavonoids (Cotas *et al*., 2020; Chung and Champagne, 2008). Another group of macromolecules that has recently attracted the attention of researchers is polysaccharides. The primary polysaccharides extracted from seaweed are fucoidan and laminarin from brown algae (Alboofetileh *et al*., 2023), carrageenan from red algae (Khotimchenko *et al*., 2020), and Ulvan from green algae. Sulfated polysaccharides have significant properties such as reducing the proliferation of cancer cells, controlling the inflammatory process, and exhibiting antioxidant activity (Fidelis *et al*., 2010).

The Persian Gulf harbors a diverse array of green, brown, and red algae species, offering a rich source of marine biodiversity and potential bioactive compounds (Pirian *et al*., 2020; Farasat *et al.,* 2023). There are over 250 species of algae in the Persian Gulf (Piri *et al.*, 2016). Given the diverse array of algae species inhabiting the Persian Gulf, there exists considerable potential for novel bioactive compounds yet to be explored (Moayyed *et al*., 2023; Sadeghi *et al*., 2024; Sadeghi *et al*., 2024). The current study aimed to evaluate the phytochemical screening of aqueous and methanolic extracts of three algae (*S. obtusifolium*, *P. gymnospora*, and *C. indica*) from the Persian Gulf and evaluate their antioxidant and cytotoxic activities against cancer cell lines.

Material and methods

Materials

Research-grade materials used in the experiments were procured from various

suppliers. Aluminum chloride, concentrated sulfuric acid, the Folin-Ciocalteu reagent, sodium carbonate, phenol, dimethyl sulphoxide, methanol, and gallic acid were obtained from Merck, Germany. The standard glucose, quercetin, and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) were purchased from Sigma-Aldrich, USA. Fetal bovine serum (FBS) was obtained from Gibco, USA. DMEM medium and Penicillin-streptomycin were purchased from Bioidea, Iran. The MCF7 breast cancer, A549 lung cancer, and VERO normal cell lines were obtained from the Cell Bank of Type Culture Collection of the Persian Gulf Marine Biotechnology Research Center, Marine Stem Cell Laboratory, Bushehr University of Medical Science, Iran. Folin-Ciocalteu method for TPC determination is based on the oxidation of phenolic compounds in the presence of Na_2CO_3.

Brown algae collection

Three Brown algae specimens were collected from along the coast of Bushehr in the Persian Gulf, Iran in April and May 2020. A voucher specimen of each alga (A2109231AP for *S. obtusifolium*, A2828612AP for *P. gymnospora* and A2408717AP for *C. indica*) was deposited for future reference in the Marine Pharmaceutical Science Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and was botanically identified by dr. Golfakhrabadi. The collected samples were rinsed with seawater and then distilled water. Mud and epiphytes were removed and air-dried in the dark place at room temperature for a week.

Extraction

Hydroalcoholic and aqueous extractions were conducted using methanol 70% (Merck, Germany) and distilled water as solvent respectively. Twenty grams of powdered samples were mixed with solvents in a ratio of 1:6 (w/v). The extraction process lasted three days at room temperature in a dark environment with shaking at 100 rpm. The solvent evaporated after collecting the supernatant by centrifugation and the extract evaporated and lyophilized (Guedes *et al*., 2013; Gunasekaran *et al*., 2017).

Phytochemical analysis

Phytochemical analysis of the lyophilized extracts encompassed the determination of total phenolic, flavonoid, and carbohydrate contents. All determinations were measured in triplicate.

Qualitative evaluation of total polyphenols

The total polyphenol content of the extracts was determined by absorption spectroscopy using the Folin-Ciocalteu method based on Gallic acid as standard (López *et al*., 2011). Calibration curves were derived using various concentrations of Gallic acid dissolved in methanol. Briefly, to determine the calibration curve, 20 mg of Gallic acid was dissolved in 100 mL of 50% methanol and then diluted to concentrations of 100, 50, and 25 µg/mL. Next, 100 µL of each concentration or diluted standard or extract (100 µg/mL) was added to 0.5 mL of Folin-Ciocalteu reagent and 1 mL of 20% sodium carbonate. The solution was mixed and kept in the dark for one hour. Absorbance readings were performed at

765 nm. Each sample was measured in triplicate.

Qualitative evaluation of total flavonoids

Total flavonoid content was assessed using the aluminum chloride method, based on forming a flavonoid-aluminum complex. At first, methanolic quercetin standard solutions were prepared at various concentrations (25, 50, 75, 100 µg/mL). Next, 100 µL of standard solutions or 0.2 mL of the sample (containing 0.2 mg of each tested extract), 0.2 mL of aluminum chloride solution, and 0.1 mL of 33% aqueous acetic acid were added to a tube and stirred well. Then ethanol (90%) was added to reach a volume of 5 mL and stored at room temperature for 30 minutes. Light absorption was measured at a wavelength of 414 nm, and a standard solution curve was generated (Hassan *et al*., 2013). All the tests were carried out in triplicate.

Qualitative evaluation of total carbohydrates

The total carbohydrate content of methanolic and aqueous extracts was determined by the Dubois method (DuBois *et al*., 1956). Standard glucose solution (100 µg/mL) was prepared by dissolving 10 mg of glucose in 100 mL of deionized aqueous and then diluted to concentrations of 75, 50, and 25 µg/mL. Next, 100 µL of the standard glucose solutions or samples (containing 0.2 mg of each tested extract) were added to 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid and shaken for 30 minutes. The absorption of all the tubes was read by a spectrophotometer at 490 nm to draw a standard curve of glucose. For each sample, three replications were conducted, and the average value was used as a reference total for the carbohydrate content of the sample.

Evaluation of antioxidant activity by DPPH method

The antioxidant activity of methanol and aqueous extracts of *S. obtusifolium, P. gymnospora, and C. indica* were evaluated by the DPPH method using 2,2-diphenyl-1-picryl-hydrazyl-hydrate based on reported procedure (Grujić *et al*., 2014). The reference standard was ascorbic acid and all analyses were carried out in triplicate. Sample of ascorbic acid prepared in serial concentration by dilution method in methanol (50, 40, 30, 20, and 10 µg/mL). In this assay, 3 mL of 0.1 mM DPPH solution was mixed with 0.1 mL of extract solution (50 µg/mL) or prepared standard solutions and the contents were shaken rapidly and intensively for 15 seconds. They were put in a dark space at room temperature for 30 minutes to read absorption by a UV-visible spectrophotometer at 517 nm. A similar procedure was performed for the methanol solvent as the blank. The percentage of inhibition of DPPH free radicals was measured using the following equation (Palanisamy *et al*., 2017):

$$\text{Inhibition absorbance} = (Control\ absorbance - Sample\ absorbance)/(Control\ absorbance)\times 100$$

IC_{50} was determined from the regression line (Khlifi *et al*., 2011).

Cell culture for in vitro anticancer activity

The extracts were evaluated for their cytotoxic activity *in vitro* on three different cell lines (MCF7, A549, and Vero) using the MTT cell proliferation assay. The cells (15×10^3 cells / well) were cultured in DMEM culture medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Pen/Strep). They were incubated at 37°C with 5% CO_2 and 95% O_2 in an incubator.

MTT Assay

The cell viability was assessed using the MTT method. Aqueous and methanol extracts of *S. obtusifolium*, *P. gymnospora*, and *C. indica* were prepared at serial concentrations using a two-fold serial dilution (6.25 mg/mL, 3.125 mg/mL, 1.56 mg/mL, 780 µg/mL, 390 µg/mL, 190 µg/mL, 97 µg/mL, 48 µg/mL, 24 µg/mL, 12.6 µg/mL). 100 µL of diluted crude extract was added to MCF7, A549, and VERO cells, and the best concentrations were selected. The cells were then incubated for 72 h. After incubation, 10µL of 5 mg/mL MTT was added to each well and incubated for 4 hours. The supernatant was removed, dimethyl sulfoxide (DMSO) was added to all wells, followed by a 20-minute incubation. The absorbance of the plates was read at 573 nm using an ELISA reader.

The cell viability was assessed using the MTT method. The cells were seeded for 42 h prior to treatment. Aqueous and methanol extracts of *S. obtusifolium*, *P. gymnospora*, and *C. indica* were prepared at serial concentrations using a two-fold serial dilution (6.25 mg/mL, 3.125 mg/mL, 1.56 mg/mL, 780 µg/mL, 390 µg/mL, 190 µg/mL, 97 µg/mL, 48 µg/mL, 24 µg/mL, 12.6 µg/mL). 100 µL of diluted crude extract was added to MCF7, A549, and VERO cells, and the best concentrations were selected. The cells were then incubated for 72 h. After incubation, 10µL of 5 mg/mL MTT was added to each well and incubated for 4 hours. The supernatant was removed, dimethyl sulfoxide (DMSO) was added to all wells, followed by a 20-minute incubation. The absorbance of the plates was read at 573 nm using an ELISA reader.

Survival percentage and selectivity index

The IC50 values were determined from a sigmoidal dose-response curve of the data generated in GraphPad Prism 8 software (Graph Pad Software Inc. San Diego, California, USA). The percentage of cell viability was calculated using the following equation:

$$Percent\ cell\ viability = (\text{OD treatment})/(\text{OD positive control}) \times 100$$

The selectivity index (SI) is a ratio used to measure the difference between cytotoxicity and anticancer activity. It is calculated by dividing the IC50 of normal cells by the IC50 of cancer cells. The SI indicates the degree of selective

cytotoxicity of an extract. A selectivity index higher than two suggests that the extract possesses selective cytotoxicity. Conversely, SI values lower than two indicate that the extract is a general toxin, meaning that while it exhibits high cytotoxicity toward cancer cell lines, it also affects normal cell lines (Marudhupandi *et al*., 2015; Senthilraja and Kathiresan, 2015):

$$SI=(IC_{50}\ Normal\ cell)/(IC_{50}\ cancer\ cell)$$

Statistical analysis

Statistical analysis and determination of total polyphenols, flavonoids, and carbohydrates was performed using SPSS software. The t-test method was used to analysis of the difference between aqueous and methanolic extracts for each item such as total yield, phenols, flavonoids and carbohydrates. One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests was also used to analysis of the differences in IC50 among the experimental groups. All data were expressed as mean±SD of three independent experiments. The *P*-value of less than 0.05 was considered significant.

Results

Extraction efficiencies

The extraction efficiencies of the brown algae *C. indica*, *S. obtusifolium*, and *P. gymnospora* are presented in Table 1. For all species, aqueous extracts contain higher amount of phenol, flavonoid and carbohydrate compared to methanol extracts, also aqueous extracts have higher percentage yields (12.70 – 26.25%) than methanol extracts (2.50 – 4.27%). The aqueous extract of *S. obtusifolium* demonstrated the highest percentage yield (26.25%), whereas methanolic extract of *P. gymnospora* displayed the lowest efficiency (2.5%). *S. obtusifolium* is the best choice between these six extracts based on their extraction yields and contents, but *P. gymnospora* did not show promising results in this regard (Table 1; Fig. 1).

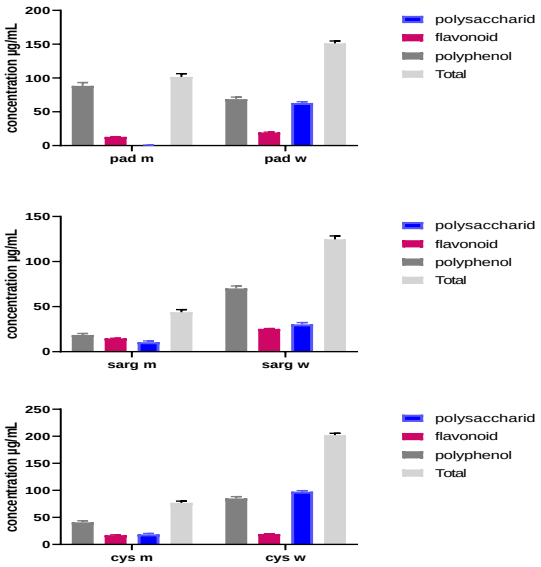

Figure 1: Polyphenol, flavonoid and, carbohydrate content in aqueous and methanolic extract of *S. obtusifolium, P. gymnospora*, *C. indica.*

Phytochemical analysis

Total polyphenols

TPC of the algal extracts was determined based on a standard curve for gallic acid in the range of 25 - 200 µg/mL and the linear calibration curve with y=0.0029x+0.0236; R^2=0.948 (Fig. 2a).

The methanolic extract of *P. gymnospora* exhibited the highest polyphenol content, whereas the methanolic extract of *S. obtusifolium* showed the lowest content of polyphenol, by 88 and 19 mg GAE per 1 g extract respectively (Table 1).

Total flavonoids

A calibration curve for quercetin in the range of 25 µg/mL-100 µg/mL with y=0.011x+0.0275; R^2=0.98 was prepared (Fig. 2b). The amount of flavonoids in extracts was expressed as quercetin equivalents (mg QE/g dry weight of the samples) (Table 1). The highest content of total flavonoids was found in the aqueous extract of *S. obtusifolium*, in contrast, the lowest content of total flavonoids was observed in the methanol extract of *P. gymnospora*, by 25 and 13 mg quercetin per 1 g extract, respectively.

Total carbohydrates

The carbohydrate content was assessed using a glucose calibration curve in the range of 12.5 µg/mL-100 µg/mL with y=0.0029x+0.0235; R^2=1 (Fig. 2c). The aqueous extract of *C. indica* demonstrated the highest carbohydrate content. In contrast, the methanolic extract of *P. gymnospora* exhibited the lowest content of carbohydrates by 97.6 and 0.4 mg glucose per 1 g extract, respectively (Table 1).

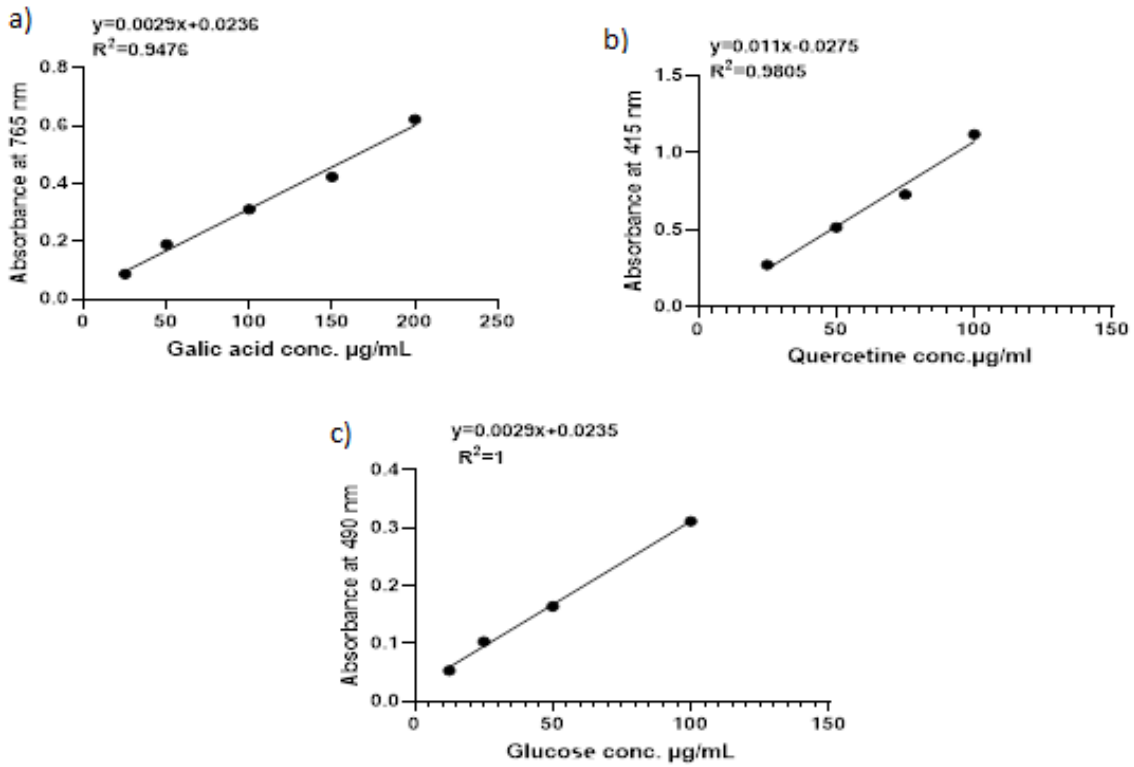

Figure 2: Standard calibration curves of a) Gallic acid, b) Quercetin and c) Glucose.

Table 1: Percentage yield and phytochemical data of brown algae extracts.

Algae species	Solvent Of Extraction	Percentage Yield (% w/w)	Total Phenol (mg GAE[a]±S)	Total Flavonoid (mg QE[b]±SD)	Total carbohydrates (mg Glucose[c]±SD)
Cystoseira Indica	Water	21.35±2.71	85.14±2.68	19.34±0.50	97.59±1.59
	Methanol (70%)	3.67±0.40	40.83±2.54	17.26±0.40	18.57±1.44
Sargassum Obtusifolium	Water	26.25±2.55	70.24±2.20	25.32±0.32	30.27±1.70
	Methanol (70%)	4.27±0.43	18.54±1.31	14.75±0.16	10.55±1.07
Padina Gymnospora	Water	12.70±1.17	68.69±2.48	19.41±0.46	63.11±1.40
	Methanol(70%)	2.50±0.29	18.34±4.00	12.95±0.19	20.39 ±0.24

[a]miligram Gallic Acid Equivalent (GAE) per gram extract, [b]miligram Quercetin Equivalent (QE) per gram extract, [c]mg glucose per gram extract.

Antioxidant activity

The DPPH free radical scavenging of *C. indica* and *P. gymnospora* aqueous extracts were 62.3±1.4 and 59.0±0.9 %, respectively (Table 2). These extracts were introduced as the most influential radical scavengers among studied extracts.

Table 2: DPPH radical scavenging activity of aqueous and methanolic extracts.

Sample	Inhibition % of DPPH radical (mean±SD) in various concentrations (µg/mL)					IC_{50} (µg/mL)
	10	20	30	40	50	
Ascorbic acid	15.0±0.4	28.8±0.2	39.3±0.9	50.4±0.4	62.3±0.1	39.6±0.6
Methanolic *C.indica*	4.2±0.3	7.7±0.5	15.7±0.4	31.0±0.5	47.1±0.8	56.5±0.6
Methanolic *S.obtusifolium*	6.0±0.7	9.5±0.4	13.4±0.5	34.2±0.7	40.9±0.6	60.9±0.5
Methanolic *P.gymnospora*	34.9±1.2	41.3±0.7	41.8±0.8	45.9±0.9	46.8±1.0	57.7±0.7
Aqueous *C.indica*	37.0±1.2	44.6±0.9	45.3±0.9	59.5±0.8	62.3±1.4	30.4±0.4
Aqueous *S.obtusifolium*	32.1±0.8	40.7±1.3	44.2±0.7	46.4±0.9	51.9±0.3	45.3±0.6
Aqueous *P.gymnospora*	26.8±0.7	40.5±0.8	44.9±0.5	53.3±0.5	59.0±0.9	36.6±0.7

The IC_{50} values of the two mentioned extracts were 36.6±0.7 and 30.4±0.4 µg/mL, indicating their superiority over Ascorbic acid (IC_{50}: 39.2±0.6 µg/mL) in this property. Also, based on the results, the IC_{50} of all aqueous extracts show higher radical scavenging ability compared to methanolic extracts and comparable to Ascorbic acid as an antioxidant, this is likely due to the water solubility of phenolic compounds, which have potent DPPH radical inhibitory effects (Gil, Tomás-Barberán *et al*., 2000).

Cytotoxicity

All extracts of the three brown algae studied, except the methanolic extract of *S. obtusifolium*, showed a significant reduction in the survival of cancer cells and normal cells. The methanolic extracts of *S. obtusifolium* at concentrations of 190 µg/mL and 390 µg/mL effectively killed breast cancer cells, with no significant reduction in normal cells (p<0.05; Fig. 3). The cytotoxicity of methanol and aqueous extracts from *S. obtusifolium*, *P. gymnospora, and C. indica* against MCF-7,

A549, and Vero cells was assessed to determine the IC_{50} of the algae extracts (Table 3). The extracts exhibited IC_{50} values ranging from 19 µg/mL to 1790 µg/mL in MCF7 and 63.70 µg/mL to 1610 µg/mL in A549. The cytotoxic results indicate that all extracts effectively inhibited cell growth in the cell lines, particularly in MCF7 and A549 (Figs. 3 and 4).

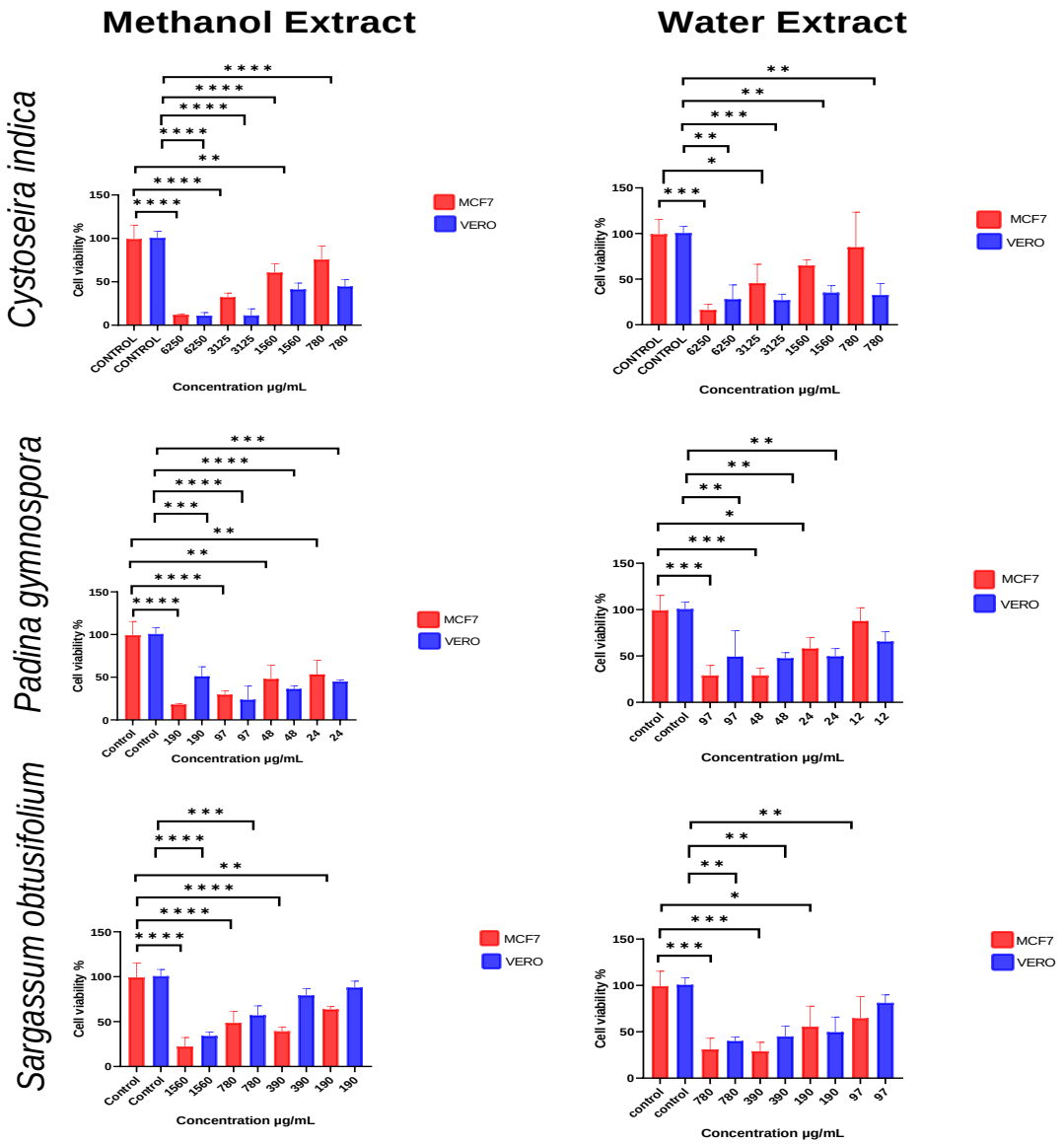

Figure 3: The cell viability percentages of MCF7 cells treated with aqueous and methanolic extracts from three brown algae. *p<0.05, ** p<0.01, *** p<0.001, and **** p<0.001 indicates statistically significant differences between groups.

Selectivity index

The selectivity index values varied among the extracts, with the methanol extract of *S. obtusifolium* exhibiting the highest selectivity index on MCF7 cells. Conversely, the aqueous extract of *C. indica* showed the lowest selectivity index on MCF7 cells. Additionally, the aqueous

extract of *S. obtusifolium* demonstrated the highest selectivity index on A549 cells, while the methanolic extract of *P. gymnospora* exhibited the lowest selectivity index (Table 3). These findings suggest the potential of the methanol extract of *S. obtusifolium* for further investigation.

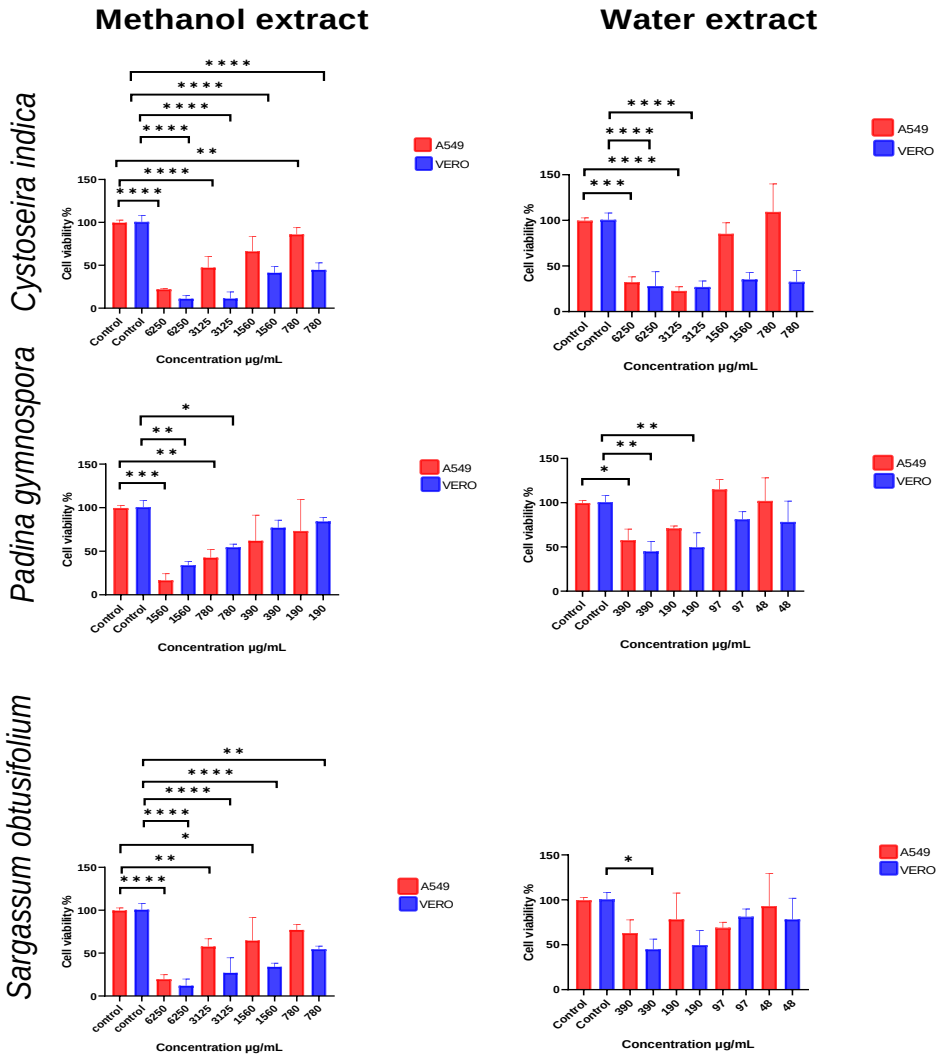

Figure 4: The cell viability percentages of A549 cells treated with aqueous and methanolic extracts from three brown algae. *p<0.05, ** p<0.01, *** p<0.001, and **** p<0.001 indicates statistically significant differences between groups.

Table 3: Cytotoxicity evaluations of aqueous and methanol extracts of *Sargassum obtusifolium*, *Padina gymnospora*, and *Cystoseira indica* on MCF7 and A549 cancer cell lines.

Brown algae (extract solvent)	IC_{50}* (µg/mL)		Selectivity Index	IC_{50} (µg/mL)		Selectivity index
	MCF7	VERO		A549	VERO	
C. indica (methanol)	1410	570	0.40	1600	570	0.35
S. obtusifolium (methanol)	168.00	455.00	2.70	1600	455.00	0.28
P. gymnospora (methanol)	20.30	10.90	0.53	330	10.90	0.03
C. indica (aqueous)	1790	80	0.04	1610	80	0.04
S. obtusifolium (aqueous)	97.10	113.00	1.16	63.70	113.00	1.77
P. gymnospora (aqueous)	19.00	9.60	0.50	181.80	9.60	0.05

*IC_{50}: The half maximal inhibitory concentration.

Discussion

Marine organisms are crucial as sources of bioactive compounds with potential as novel anticancer drugs (Karthikeyan *et al*., 2022). Seaweeds as a rich source of bioactive compounds have shown potential in fighting cancer, making them a cost-effective and safe source for medicinal and pharmacological applications due to their natural antioxidant content (Cotas *et al*., 2021). Brown algae are rich in polysaccharides and polyphenols, and known for their antioxidant and cytotoxic effects against cancer cells.

This study investigated the aqueous and methanolic maceration extraction of bioactive compounds from three different Persian Gulf brown algae. The total amounts of polyphenols, flavonoids, and carbohydrates in brown algae extracts have been determined. The order of species extracts based on the total amount of extracted phytochemicals is: *C. indica* (aqueous)> *P. gymnospora* (aqueous)> *S. obtusifolium* (aqueous)> *P. gymnospora* (methanolic)> *C. indica* (methanolic)> *S. obtusifolium* (methanolic). The findings revealed significant variations in quantity of polyphenols, flavonoids, and carbohydrates among the extracts. Each type of brown algae contains a unique combination of compounds, and extracts from different species of brown algae also vary in their total compound content (Jimenez-Lopez *et al*., 2021). Specifically, the aqueous extracts exhibited higher levels of these bioactive compounds than the methanolic extracts, suggesting that water is more effective for extracting these bioactive compounds. This is consistent with some studies indicating that aqueous extracts of algae contain a higher amount of phytochemicals such as polyphenolic, flavonoid, and polysaccharide compared to alcoholic extracts (Tian *et al*., 2011; Matou *et al*., 2023). Increasing the total compounds containing polyphenolic, flavonoid, and polysaccharide has been found to have a more significant effect on cancer cells due to increased antioxidant and cytotoxicity properties (Zhu, 2018).

The present study underscores the importance of Persian Gulf brown algae, as valuable sources of bioactive compounds with potential antioxidant properties. In terms of antioxidant activity, the studied seaweed extracts can be ranked in decreasing order as follows: *C. indica* (aqueous), *P. gymnospora* (aqueous), *S. obtusifolium* (aqueous), *C. indica* (methanolic), *P. gymnospora* (methanolic), *S. obtusifolium* (methanolic). A comparison between studied aqueous and methanolic extracts revealed that aqueous extraction generally led to higher levels of bioactive compounds, promoting greater antioxidant capacity in the studied brown algae species. The aqueous extract of *P. gymnospora* showed higher antioxidant activity than its methanolic extract, and the same trend is observed for *S. obtusifolium* and *C. indica*. The highest antioxidant activity was observed in the aqueous extract of *C. indica* (strong antioxidant), while the lowest was found in the methanolic extract of *S. obtusifolium* (moderate antioxidant). A compound is known to be a powerful antioxidant compound if the IC50 value is <10 µg/mL, strong if the IC50 value ranges from 10-50 µg/mL, while moderate if the IC50 value ranges from 50-100 µg/mL, it is weak if the IC50 value ranges between 100-

250 µg/mL and is inactive when the IC50 value is above 250 µg/mL (Rajabiyan *et al.*, 2023). The aqueous extract of *C. indica* showed the highest levels of total carbohydrates and polyphenols, correlating with its superior antioxidant activity. Generally, all studied aqueous extracts showed higher antioxidant activity than the methanolic extracts. The higher antioxidant capacity in aqueous extracts may be attributed to the higher levels of phenolic, polysaccharide compounds, and secondary metabolites (Vasanthi *et al*., 2020). The antioxidants present in seaweed can protect humans against cancer (Ganesan *et al*., 2019). The primary components of brown algae are polysaccharides and polyphenols. Polysaccharides like laminarans and fucoidans, as well as polyphenols like phlorotannins, have demonstrated potent antioxidant potential by effectively scavenging radicals in a dose-dependent manner (Heo *et al*., 2005, Sanz-Pintos *et al*., 2017). Our results indicate that the aqueous extracts contain more total phytochemical compared to methanolic extracts, which is why aqueous extraction can enhance the cytotoxicity capacity against MCF7 and A549 cells compared to methanolic extraction (Fig. 5).

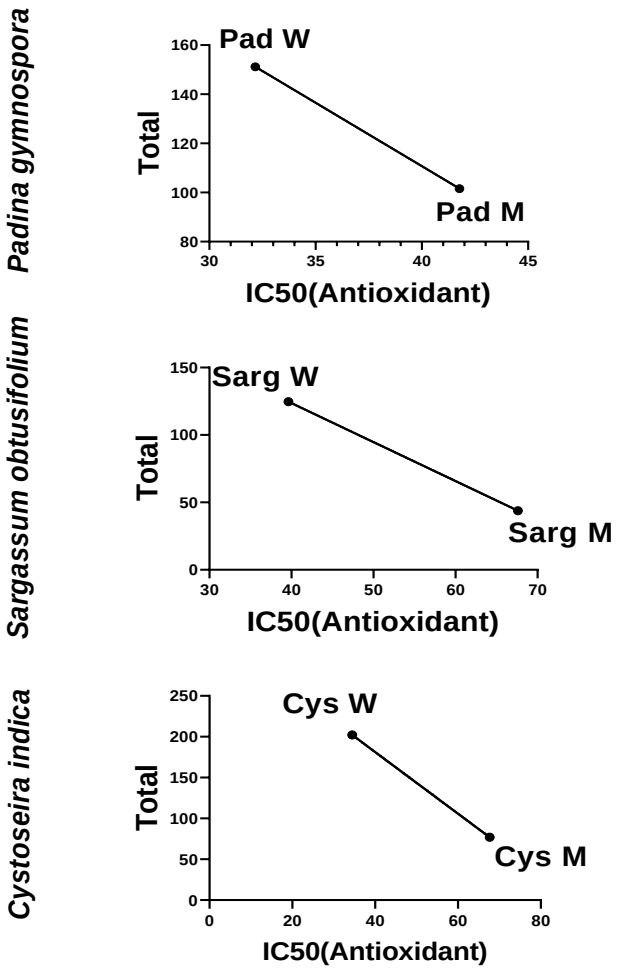

Figure 5: Correlation between the content of total phytochemicals of aqueous extracts of *C. indica, S. obtusifolium, P. gymnospora* compared to methanolic extracts and antioxidant activity.

Previous studies have suggested increasing the total compounds containing polyphenolic, flavonoid, and polysaccharide has been found to have a more significant effect on cancer cells due to increased antioxidant and cytotoxicity properties (Zhu, 2018). In contrast to *P. gymnospora and S. obtusifolium*, there is an inverse correlation between antioxidant activity and cytotoxicity in the aqueous and methanolic extracts of brown algae *C. indica*, as depicted in Figures 4 and 6.

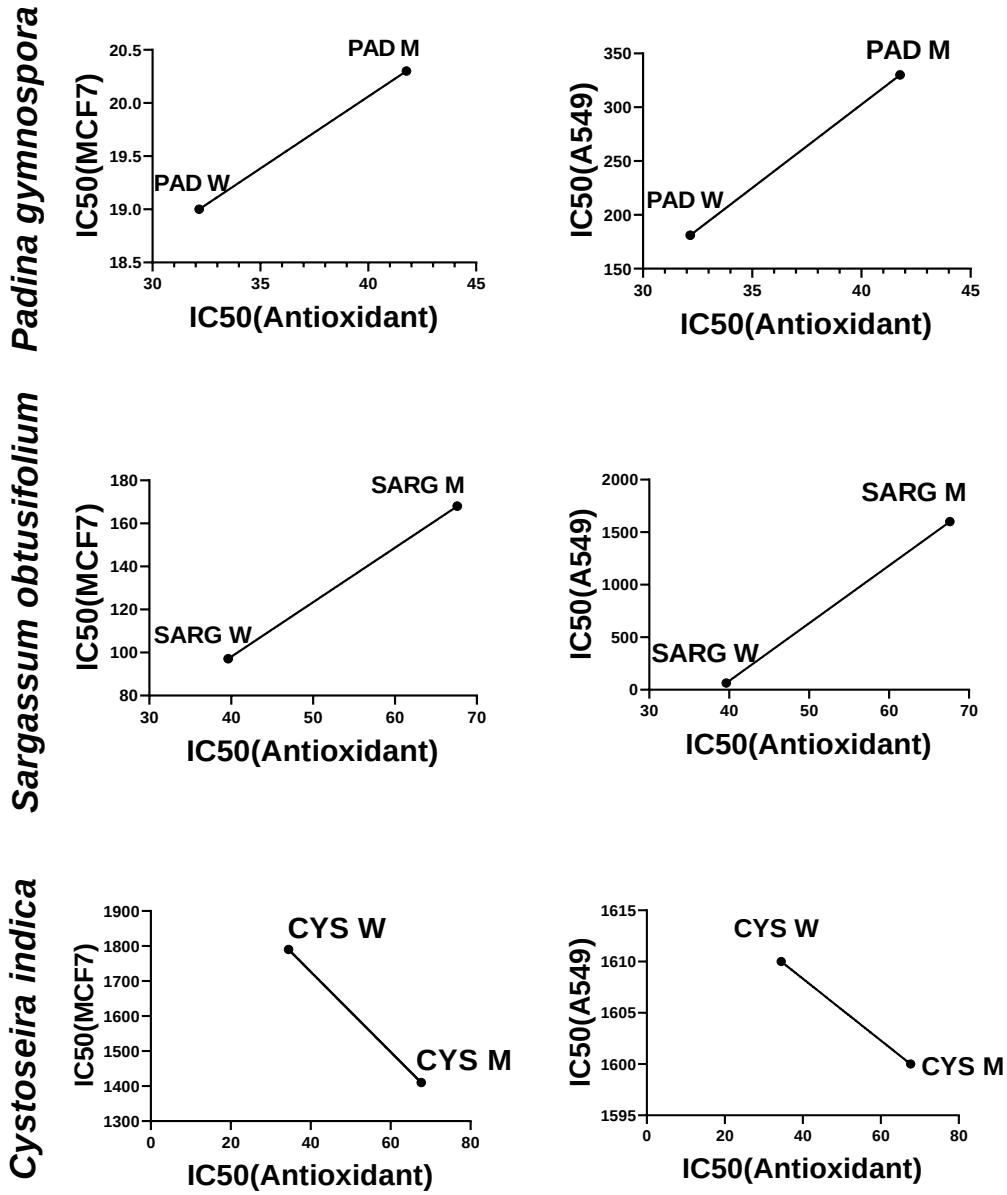

Figure 6: Correlation between the cytotoxicity of aqueous and methanolic extracts of *C. indica, S. obtusifolium, P. gymnospora* with their antioxidant activity.

Previous research has highlighted the synergistic effects of these bioactive compounds (Durgo *et al*., 2013; Liu *et al*., 2018; Zhu, 2018; Dobson *et al*., 2019; Zhang *et al*., 2020). The crude extracts of seaweeds contain various compounds that

have diverse biological effects such as proliferation and anti-proliferation. The antioxidant capacity can have a dual impact, with high levels potentially promoting cancer cell growth by generating ROS on the cancer cell surface (Sadeghi *et al*., 2024). Additionally, these phytochemicals have been shown to inhibit the *in vitro* growth of certain cancer cell lines (Niedzwiecki *et al*., 2016).

Our findings demonstrate that methanolic extracts of *S. obtusifolium* exhibit a high selectivity index compared to other extracts on MCF7 cells, suggesting their potential as a promising complementary therapeutic candidate for breast cancer patients. The selectivity index plays a crucial role in identifying safe anti-cancer drugs. A higher selectivity index ratio indicates that a drug would be more effective and safer during *in vivo* cancer treatment (Lafi *et al*., 2021). An ideal drug would be cytotoxic only at high concentrations and have anticancer activity at low concentrations, resulting in a high selectivity index value (Lichota and Gwozdzinski, 2018; Botteon *et al*., 2021). This study was conducted in vitro and further research is needed to determine the *in vivo* efficacy and safety of the extracts. Because these extracts have potential applications such as in the development of functional foods or nutraceuticals.

Conclusions

Brown algae are rich in polysaccharides and polyphenols, and known for their antioxidant and cytotoxic effects against cancer cells. Aqueous extracts of *P. gymnospora*, *C. indica* contain higher concentrations of polyphenols, flavonoids, and carbohydrates, which cause enhancement in cytotoxic and antioxidant effects. According to antioxidant studies on extracts, the aqueous extract of *C. indica* can be introduced as a powerful antioxidant, comparable with Ascorbic acid. The aqueous extract of *S. obtusifolium* shows promising results for A549 among the studied extracts of *P. gymnospora*, *S. obtusifolium*, and *C. indica*. It's notable, while the aqueous extract of *P. gymnospora* exhibits potent anticancer properties, the methanolic extract of *S. obtusifolium* is considered the safest anticancer agent for MCF7 among studied extracts, based on its selectivity index.

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Conflicts of interest

The authors declare no conflicts of interest.

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