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Research Article

Effect of monsoon variations on the antioxidant contents and antioxidant activity of *Sargassum ilicifolium*

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Abstract

Algae has recently drawn attention due to its strong potential for biological activities as a promising natural antioxidant resource. In this regard, seasonal variations of *Sargassum illicifolium* antioxidant activities were evaluated in the present study. *S. illicifolium* was collected from three coastal sites of the Oman Sea from April to May (spring) 2020 and October to November (autumn) 2020, representing pre- and post-monsoon, respectively. The methanolic extract was used to evaluate its antioxidant activities and properties. Regarding antioxidant activity (DPPH), there is a significant difference for the ferric reducing antioxidant power (FRAP), total flavonoids, total anthocyanin, and β-carotene variables analyzed between different sites during pre- and post-monsoon (p>0.05). Meanwhile, IC50 in pre-monsoon was significantly higher than in post-monsoon (p<0.05). However, statistical analysis revealed a strong positive correlation between IC50 and FRAP content in pre-monsoon; total phenolic and anthocyanin contents positively correlated to β-carotene post-monsoon. This study revealed that the seasonal variations lead to alter the biological activities regarding antioxidant contents in *S. illicifolium*, which need to be considered when considering the seaweed as a commercial antioxidant resource.

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Introduction

The distribution and diversity of marine macroalgae on the Iranian coast of the Oman Sea are prosperous (Mahdi Abkenar *et al*., 2021). More than 83 dominant marine macroalgal species, such as Rhodophyta, Phaeophyta, and Chlorophyta have been recorded in this area (Gharanjik and Mahdi Abkenar, 1999).

Sargassum illicifolium is known as a tropical and sub-tropical species from Phaeophyta (family Sargassaceae and order Fucales), which is found abundantly in the coastal intertidal area of the Oman Sea, Iran (Rohani-Ghadikolaei *et al*., 2012). *S. illicifolium* has several functional bioactive compounds that have created an imminent consequence in the biomedical fields (Marinho *et al*., 2019). In addition, the *S. ilicifolium* extract is well known for antioxidant, anti-inflammatory, antibacterial, and anticancer activities (Taheri *et al*., 2018; Aghajanpoor *et al.*, 2020; Sahragard *et al*., 2021; Yende *et al*., 2021).

Global climate change has an extreme effect on macroalgae species that form habitats, including the extinction of algae populations in marine ecosystems (Wang *et al*., 2021). According to Ramage (1971), a monsoon is traditionally a seasonal reverse wind along with the appropriate change in precipitation. However, it is now used to describe seasonal changes in atmospheric circulation and precipitation associated with the annual latitude oscillation of the intertropical convergence zone between the northern and southern equators (Wang *et al*., 2019).

Algae present seasonal variations in their composition, e.g., proteins and lipids (Marinho *et al.*, 2015a, b; Marinho *et al*., 2019), which may also be the case of antioxidants, and this needs to be considered when evaluating their potential as a source of antioxidant compounds and capacity. Meanwhile, it is reported that the seasonal pattern of the pigment concentration generally is lower in summer and higher in winter; it has been reported for many seaweed species and is related to changes in the availability of light and nutritional concentration in seawater (Stengel and Dring, 1998; Schmid *et al*., 2017).

The present study aimed to evaluate seasonal variation in the content of antioxidant activity and properties based on standard assays of *S. illicifolium.* It was collected at three stations at Tang, Chabahar, and Gowatr sites on the Oman Sea coast (Iran) from April to May (spring) and October to November (autumn) 2020, each representing pre- and post-monsoon, respectively.

Materials and methods

Seaweeds collection

Samples of *S. illicifolium* specimens were collected during low tide times (depending on the tide table obtained from www.tideforecast. com) by 2020 for two seasons: April to May (pre-monsoon) and October to November (post-monsoon), representing the autumn and spring, respectively, from three different locations on the northern coasts of the Oman Sea, Iran (Tang: latitude, 25° 14′ 49″ N; longitude: 59° 48′ 02″ E, Chabahar Bay: latitude, 25° 14′ 17″ N; longitude: 60° 38′ 57″ E; and Gowatr Bay: latitude, 25° 12′ 59″ N; longitude: 61° 29′ 22″ E). The Offshore

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Fisheries Research Center determined taxonomic algae. The algae were identified by Aleem (1993); they belonged to the family of *S. illicifolium* (Turner) from Phaeophyta (Fig. 1).

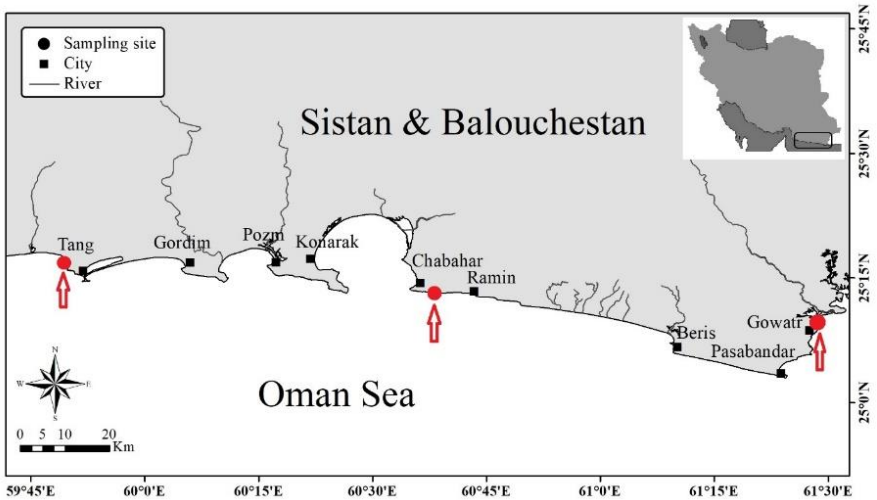


Figure 1: Sampling sites of *Sargassum illicifolium* in different locations of the Oman Sea, Iran.

Preparation of algae methanolic extract

After collection, the algae were taken to the laboratory in plastic bags containing seawater to avoid evaporation. Epiphytic and foreign materials were removed by washing first with seawater and then with distilled water to separate possible contaminants. Dry samples have been prepared by drying fresh algae in the air at room temperature for seven days and storing them in plastic bags for further analysis (González del Val *et al*., 2001).

Sargassum ilicifolium (≈1 kg) was washed with water to remove suspended materials, salts, epiphytes, and microorganisms and dried at room temperature, kept in a dark room and then, ground into a fine powder. Then 20 g of powdered samples were added to 10 mL of 80% methanol and stored at 37°C for 6 h in an orbital shaker incubator (AXYOS, Australia) and centrifugation (Kubota, KN-70, HD8292, Japan) at 1500×RPM for 15 minutes. After 72 h, they were getting centrifuged agent extract. Finally, the extract obtained was filtered with WHATMAN No. 1 filter paper and concentrated to dry to produce a rough extract residue. The extract was diluted with solvents (50 mg/mL) and stored at 4°C for further analysis (Chidambararajan *et al*., 2019) (Fig. 2).



Figure 2: Brown macroalgae *Sargassum illicifolium* collected from different locations in the Oman Sea, Iran.

Antioxidant activity (AOA)

The AOA of *S. illicifolium* extract was determined by hydrogen atom transfer

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(HAD) (DDPH) and electron transfer (ET) (FRAB) methods (Heavisides *et al*., 2018).

2, 2-diphenyl-1-picrylhydrazyl (DPPH %) radical scavenging ability

The DPPH scavenging activity assay of *S. illicifolium* extract was studied following Duan *et al.* (2006). In short, 1 mL of 0.15 mM DPPH was added to a different extract dilution (1, 10, 100, and 1000 µg/mL). Then, the reaction mixture was incubated in a dark room for 30 min. Its absorbance was measured at 517 nm using a spectrophotometer (ELISA Reader Epock, USA). DPPH (%) free radical referral activity is calculated using the following equation (Chidambarajan *et al*., 2019):

$$Scavenging\ activity\ (\%) = \frac{A_{Sample} - A_{Control}}{A_{Control}} \times 100$$

The half-maximal inhibitory concentration (IC50) was calculated by linear regression analysis and expressed as a mean of three determinations. IC50 has been used as an index to compare the AOA of individuals. Ascorbic acid has been used as a positive control (1, 10, 100, and 1000 µg/mL), and reducing power has been expressed as equivalents of mg ascorbic acid (AAE) per mL of dry weight of the sample (DW).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined using the Oyazu (1986) modification method. Briefly, 1 mL of *S. illicifolium* extract in different concentrations (0.1, 0.5, 1 and 2 mg/mL) was added to 1 mL of 0.2 M phosphate buffer (PSB, pH=6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, and 1 mL of 10% TCA was added to this reaction mixture. An aliquot of 1 mL from the incubation mixture was mixed with 1 mL of distilled water and 0.2 mL of 0.1% ferric chloride in the test tube. Absorbance was measured at 700 nm. Gallic acid was used as a standard power and reduction was expressed as equivalent to mg of gallic acid (GAE) per g of dry weight sample (DW).

Total phenolic and flavonoid content

The total phenolic content (TPC) of *S. illicifolium* extract was assessed by the Folin-Ciocalteu method (Cagalj *et al*., 2021) using a UV spectrophotometer (Shimadzu, UV-160A, Japan) with minor modification. Briefly, 20 µL of *S. illicifolium* extract (1 mg/mL) reacted with 1.50 mL of distilled water and 125 µL of 10% Folin-Ciocalteu reagent. The solution was mixed, and after one minute, 375 µL of 20% sodium carbonate solution and 475 µL of distilled water were added. The mixture was left in the dark for 2 h at room temperature. Absorbance was read at 720 nm using a spectrophotometer. The standard curve (0-500 mg/l) was prepared using gallic acid for the calculation of phenolic content (y = 0.004-x, r2 = 0.9999). The TPC was expressed as mg of gallic acid equivalents (GAE) per g of dry weight sample (DW).

The total content of flavonoids (TFC) in *S. illicifolium* extract was assessed by the aluminum chloride colorimetric method described by Cagalj *et al.* (2021). Briefly, 250 µL of *S. illicifolium* extract (1 mg/mL)

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was mixed with 750 µL of 96% ethanol, 50 µL of 10% (w/v) aluminum chloride, 50 µL 1M sodium acetate, and 1400 µL distilled water. The mixture was stored for 30 minutes at room temperature. Absorbance was read at 415 nm using a spectrophotometer. The standard calibration curve (0-100 µg /mL) was plotted using a quercetin solution (Y=0.0076-0.0042, r2 = 0.9412). TFC is the equivalent of mg quercetin (QE) per g of dry weight sample (DW).

Total anthocyanin content

The total anthocyanin content has been measured using a differential protocol spectrophotometric pH, as Heavisides *et al*. (2018) described, with minor modifications. Briefly, 0.5 mL of *S. illicifolium* extract mixed with 3.5 mL of 0.025 M potassium chloride buffer; pH=1. The mixture was stirred and left at room temperature for 15 minutes. Then, the absorbance was measured at 515 and 700 nm against distilled water as a blank.

Then, the extract was combined with 3.5 mL of 0.025 M sodium acetate buffer (pH=4.5). Once again, the mixture was stirred and left at room temperature for 15 minutes. The absorbance of the mixture was measured at the same wavelengths (512 and 700 nm). The total anthocyanin content has been calculated using the following equation:

$$TAC\ (mgC-3-GE/100g\ DW) = (A \times MW \times 1000/(\varepsilon \times C))$$

Where, A is absorbance= (Aλ515–Aλ700) pH 1.0–(A515–A700) pH 4.5; MW is molecular weight for cyanidin-3-glucoside=484.2; DF is the factor of the extract; ε is the molar absorptivity of cyanidin-3-glucoside=24825; C is the concentration of the buffer in mg/mL=0.025. The result was expressed as milligrams of cyanidin-3-glucoside equivalents (mg C-3-GE) per 100 grams of the dry weight of the sample (DW).

β-carotene content

The quantification of the β-carotene was carried out following the methods of Eijckelhoff and Dekker (1995) with minor modifications. Briefly, 20 g of *S. illicifolium* crude extract was dissolved in 10 mL of 85% acetone-hexane and centrifuged at 3000 rpm for 15 minutes at 4°C. Then, the absorbance was measured at 453, 505, 645, and 663 nm using a UV spectrophotometer (Shimadzu, UV -160A, Japan). The contents of the β-carotene have been calculated according to the following equations and expressed as mg per g of dry weight (DW):

$$\beta - Carotene\ (mg/100g\ DW) = 0{,}216A_{663} - 1{,}22A_{645} - 0{,}304A_{505} + 0{,}452A_{453}$$

Statistical analysis

All statistical analyses were conducted by SPSS Software (New York, USA) version 16.0 in triplicate. Shapiro-Wilk and Levene's tests were used to check the normality and homogeneity of variance,

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respectively. To determine whether there are differences between means, ANOVA analysis and Duncan's multiple range test (DMRT) as a post hoc test are applied to correlate the potential of antioxidants in the samples. In addition, the comparison between monitored factors and locations in pre and post-monsoon with independent sample T-tests.

Results

DPPH (%) scavenging activity

Table 1 indicates no significant variation in the scavenging DPPH (%) radicals of methanolic *S. illicifolium* extract tested at 1, 10, 100, and 1000 µg/mL harvested in pre- and post-monsoon among three locations of Tang, Chabahar, and Gowatr gulf (p>0.05).

Table 1: DPPH radical scavenging activity (%DPPH) of 1, 10, 100, and 1000 µg/ml of *Sargassum illicifolium* extracts in different seasons.

Seasons	Concentration (µg/mL)	Stations		
		Tang	Chabahar	Gowatr
Pre- (Spring)	1	48.46±1.37	41.72±1.74	44.9±1.91
	10	50.19±83	58.80±2.01	53.15±0.45
	100	66.28±3.34	57.20±1.15	59.79±0.74
	1000	76.43±1.19	57.96±1.23	61.90±3.01
Post- (autumn)	1	56.19±0.85	53.76±0.99	50.38±1.09
	10	63.27±3.45	61.96±1.36	56.76±2.59
	100	76.98±2.36	66.80±2.78	60.26±2.34
	1000	82.50±2.54	71.12±2.46	61.81±2.19
	Ascorbic acid	55.1±1.00	55.1±1.00	55.1±1.00

*Data were presented as the average of four replicates standard deviation (±SD).

IC50

As shown in Figure 3, The IC50 content of ascorbic acid as a standard antioxidant has shown a significant difference (p< 0.05) between methanolic *S. illicifolium* extract which was harvested in pre-monsoon (85.80±11.40 mg AAE/mL) compared to post- monsoon (46.63±4.65 mg AAE/mL) (sig=0.020; t=9.53; DF=16). Based on the results, the IC50 content between the three locations of Tang, Chabahar, and Gowatr in pre-monsoon was significantly higher than post-monsoon (p>0.05). However, there were no significant differences between samples harvested from studied locations in pre- and post-monsoon, separately (p>0.05). The IC50 content in *S. illicifolium* extract at pre-monsoon ranges from 67.71 to 101.31 mg AAE/mL, whereas in post-monsoon it is found at 41.71 to 54.17 mg AAE/mL.

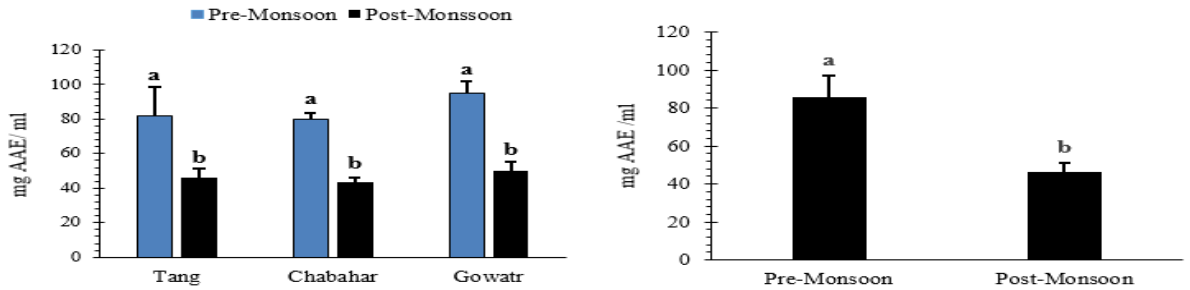


Figure 3: Comparison of the IC50 content of *Sargassum illicifolium* extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea (p>0.05).

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Ferric-reducing power (FRAP) assay

Figure 4 presents the compression of the FRAP content in *S. illicifolium* extract among three locations and seasonal variations on the northern coast of the Oman Sea. According to the results, there was no significant difference found between *S. illicifolium* extract which was harvested in pre- (0.62±0.15 mg GAE/g DW) compared to post- (1.21±0.11 mg GAE/g DW) monsoon (sig=0.28; t=-9.45; DF=14.65). Based on the results, the FRAP content between the three locations of Tang, Chabahar, and Gowatr in pre-monsoon was significantly lower than at post-monsoon (p> 0.05). However, there were no significant differences between samples harvested from studied locations at pre- and post-monsoon, separately (p> 0.05).

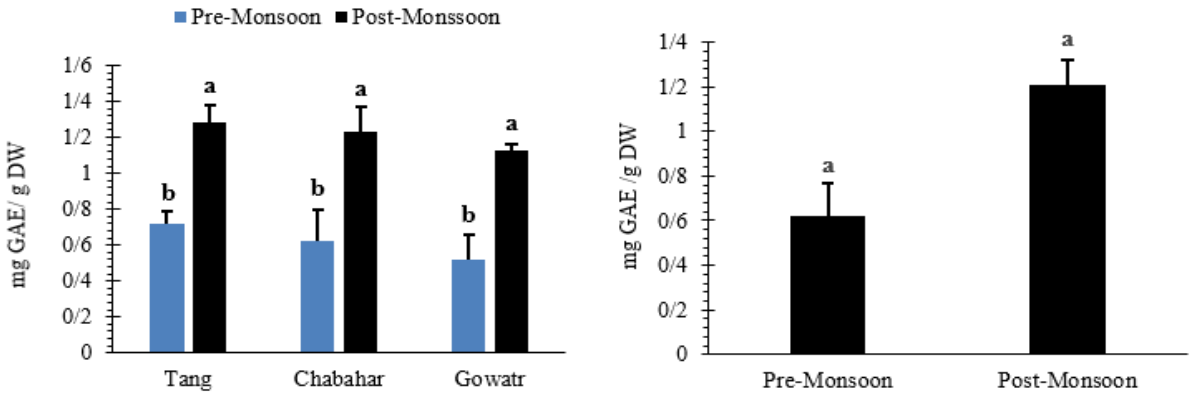


Figure 4: Comparison of the FRAP content in *Sargassum ilicifolium* extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea p>0.05).

The FRAP content in *S. illicifolium* extract at pre-monsoon ranged from 0.43 to 0.81 mg GAE/g DW, whereas at post-monsoon, it was found to be 1.11 to 1.39 mg GAE /g DW.

Total phenolic content (TPC)

Compression of The TPC content in *S. illicifolium* extract is presented in Figure 5. Based on the results for both the pre-(3.42±0.0.38 mg GEA/g DW) and post-(3.77±0.65 mg GAE/ g DW) monsoon, no significant differences were observed (sig=0.12; t=-1.41; DF=12.87). However, no significant differences were observed between the three locations of Tang, Chabahar, and Gowatr at pre- and post-monsoon, separately (p>0.05). The TPC compounds in the extract harvested in the pre-monsoon ranged from 3.12 to 4.08 mg GEA/g DW, whereas the foe sample harvested in the post-monsoon was found at 2.79 to 4.91 mg GEA/g DW.

Total flavonoid content (TFC)

As shown in Figure 6, there was no significant difference between TFC content measured in samples harvested from the pre-(12.45±2.31 mg QE/g DW) and post-(28.14±4.56 mg QE/g DW) monsoon for Methanolic *S. illicifolium* extract (Sig=0.07; T=- 9.19; DF=11.85). However, the TFC content between the three locations of Tang, Chabahar, and Gowatr at pre-

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monsoon was significantly lower than at post-monsoon (p>0.05). However, there were no significant differences between samples harvested from all studied locations at pre- and post-monsoon, separately (p>0.05). The content of total flavonoid compounds in the pre-monsoon ranges from 8.38 to 16.07 mg QE/g DW, while in the post-monsoon it is found at 21.81 to 32.17 mg QE/g DW.

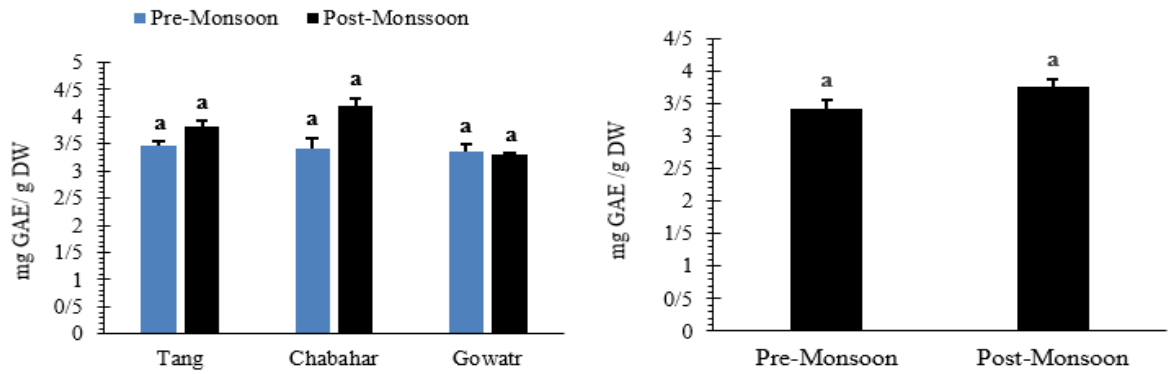


Figure 5: Comparison of the TPC content in S. i. extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea (p>0.05).

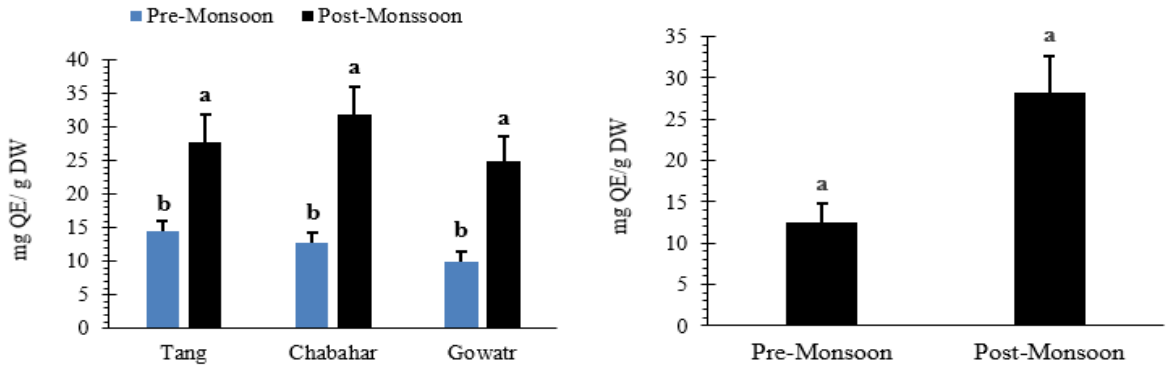


Figure 6: Comparison of the TFC content in *Sargassum illicifolium* extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea (p>0.05).

Total anthocyanin content

Figure 7 shows no significant (p>0.05) seasonal variation for the anthocyanin content of *S. illicifolium* extract harvested in pre (3.97± 0.68 mg C-3-GE/100g DW) and post (8.23± 1.04 mg C-3- GE/100g DW) monsoon (Sig=0.24; T=- 10.28; DF=13.77). However, there was no significant difference found in the three locations of samples harvested pre-monsoon (p>0.05); Meanwhile, we observed a statistically significant difference for samples harvested post-monsoon between different locations (p<0.05). Based on the results, at the post-monsoon, the anthocyanin content in samples harvested from Gowatr (7.00±0.62 mg C-3- GE/100g DW) was significantly lower than in Tang (9.10±0.43 mg C-3- GE / 100g DW) and Chabahar (8.59±0.42 mg C-3- GE/100g DW) (p<0.05). The content of anthocyanin compounds in the pre-monsoon ranged from 3.15 to 5.11 mg C-3-GE/100g DW, while in the post-monsoon it was found at 6.28 to 9.59 mg C-3-GE/100g DW.

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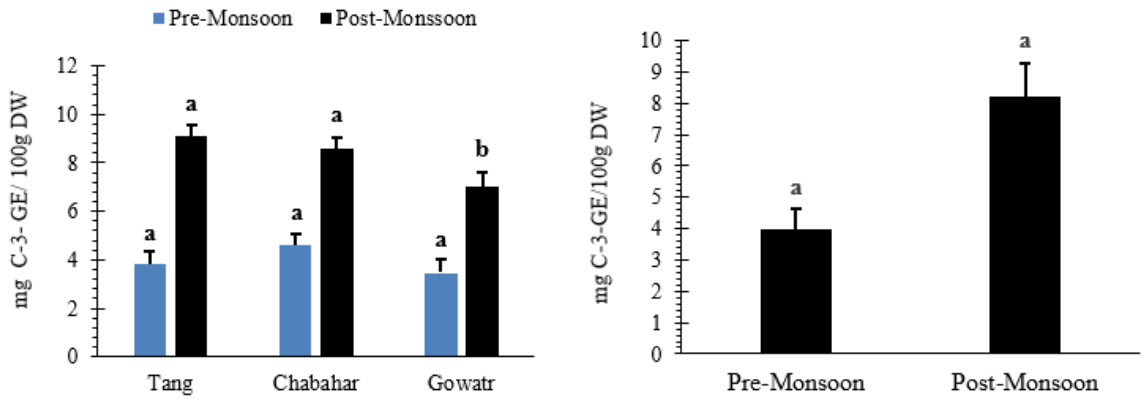


Figure 7: Comparison of the anthocyanin content in *Sargassum ilicifolium* extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea (p>0.05).

β-carotene content

Figure 8 shows no significant (p>0.05) seasonal variation in the contents of β-carotene in *S. illicifolium* extract in the pre (0.076±0.015 mg/g DW) and the post (0.079±0.010 mg/g DW) monsoon (Sig=0.42; T= -0.57; DF=13.81). The β-carotene content between all studied locations showed a significant difference during pre- and post-monsoon (p<0.05). The highest and lowest levels of β-carotene at pre-monsoon were observed in Chabahar Bay (0.088±0.006 mg/g DW) and Tang (0.060±0.016 mg/g DW) locations, respectively (p<0.05). The β-carotene content measurement in the sample harvested from Gowatr at pre-monsoon was 0.079±0.006 mg/g DW. For post-monsoon, the β-carotene content in the sample harvested from Gowatr (0.068±0.003 mg/g DW) was significantly lower than the Tang (0.085±0.006 mg/g DW) and Chabahar Bay (0.086±0.008 mg/g DW) samples (p<0.05). The β-carotene content in the methanolic extract of *S. illicifolium* harvested in pre-monsoon ranges from 0.042 to 0.093 mg/g DW, while in post-monsoon it is found at 0.066 to 0.095 mg/g DW.

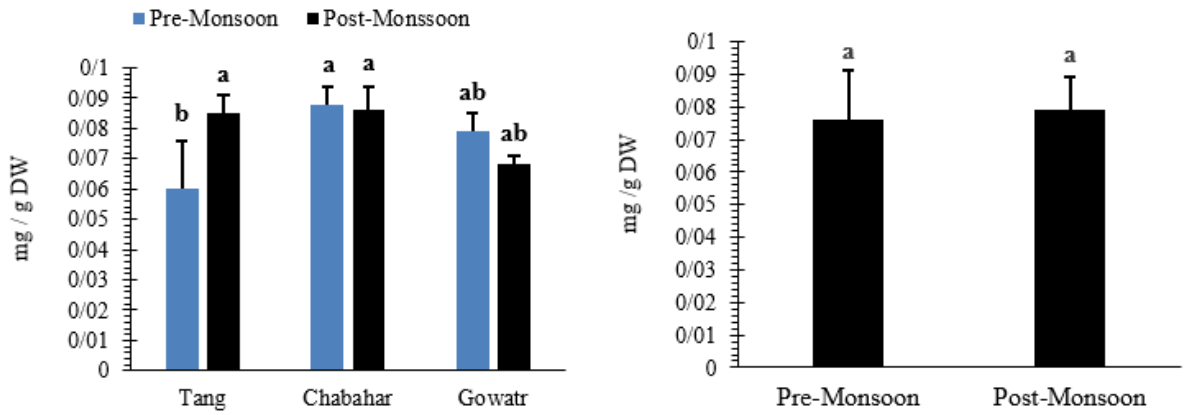


Figure 8: Comparison of the β-carotene content in *Sargassum illicifolium* extract between three different locations (Left) and monsoon conditions (Right) on the northern coast of the Oman Sea (p>0.05).

Correlation analysis

The Pearson correlation analysis was carried out to determine the relationship between each antioxidant activity index and the properties of *S. illicifolium* extract in different seasonal samplings. As shown in

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Table 2, there was a significant strong positive correlation between IC_{50} with FAR content (r=0.83; p<0.001) in pre-monsoon.

However, at post-monsoon (Table 3), there was a significant negative correlation between IC_{50} and DPPH content (r=-0.67; p<0.05). However, we observed a significantly positive correlation between TPC (r=0.68; p<0.05) and anthocyanin (r=0.77; p<0.05) with β-carotene content.

Table 2: Pearson's correlation coefficients between the variables of pre-monsoon.

Indices	DDPH	IC50	FRAP	TPC	TFC	Anthocyanin	β-Carotene
DPPH	1	-0.24	-0.27	-0.17	-0.59	-0.58	0.35
IC50	-0.24	1	**0.83****	0.47	0.26	-0.21	-0.19
FRAB	-0.27	**0.83****	1	0.20	0.36	0.13	-0.19
TPC	-0.17	0.47	0.20	1	-0.23	0.02	0.15
TFC	-0.59	0.26	0.36	-0.23	1	0.40	-0.46
Anthocyanin	-0.58	-0.21	0.13	0.02	0.40	1	0.18
β-carotene	0.35	-0.19	-0.19	0.15	-0.46	0.18	1

Table 3: Pearson's correlation coefficients between the variables to post-monsoon.

Variables	DPPH	IC50	FRAP	TPC	TFC	Anthocyanin	β-carotene
DPPH	1	-0.67*	-0.63	-0.20	-0.22	-0.47	-0.21
IC50	**-0.67***	1	0.38	0.23	0.62	0.45	0.55
FRAP	-0.63	0.38	1	-0.13	0.16	0.54	0.05
TPC	-0.20	0.23	-0.13	1	0.52	0.42	**0.68***
TFC	-0.22	0.62	0.16	0.52	1	0.40	0.61
Anthocyanin.	-0.47	0.45	0.54	0.42	0.40	1	**0.77***
β-carotene	-0.21	0.55	0.05	**0.68***	0.61	**0.77***	1

Discussion

S. illicifolium is a good source of bioactive constituents that cope with various applications in different industries (Yende *et al.*, 2021). To our knowledge, this is the first report on the antioxidant content and activities of *S. illicifolium* in different seasons of the Iranian coast of the Oman Sea. The results indicated the difference in antioxidant activity, FRAP, TPC, TFC, anthocyanin, and β-carotene from seaweed based on different geographical locations. Based on the results, seasonal changes in DPPH radical scavenging activity showed no statistical significance between geographical locations. Nevertheless, the lowest IC50 values, corresponding to the highest radical scavenging activity, were found from October to November (post-monsoon), matching the period in which the maximum concentrations of FRAP, TFC, and anthocyanin were found, respectively. This data suggests a possible correlation between DPPH scavenging activity and TFC and TPC. This assumption was further supported by the partial least square coefficient plot revealing that both FRAP were positively correlated with IC50 at pre-monsoon and DPPH with IC50 at post-monsoon, suggesting that these variables had a positive effect on the radical scavenging activity. These results follow previous studies, which directly correlated DPPH scavenging activity with the IC 50, FRAP, and TFC found in the extracts of both seaweeds (Palanisamy *et al.*, 2017; Sharifian *et al.*, 2019; Nosrati *et al.*, 2021; Sahragard *et al.*, 2021). Nosrati *et al.*

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(2021) DPPH activity of brown algae of *Sargassum glaucescens* was reported to be between 16.1-31.9%, which was lower than our results. Palanisamy *et al*. (2017) studied the antioxidant effect of *Sargassum polycystum* and reported that DPPH and FRAP contents were 61.2% and 67.59%, respectively. The DPPH radical scavenging activity (12.08±0.28 and 7.24±0.18 mg GAE/100g) was high in *Zonaria tournefortii* and *Sphaerococcus coronopifolius* during summer. In comparison, for *Halopteris scoparia* (11.12±0.19 mg GAE/ 100g), the highest DPPH activity was observed in autumn. Likewise, the ferric reducing power for *H. scoparia* (41.93±1.08 mg GAE/100g), while the highest level of FRAP was observed at autumn in *S. coronopifolius* and *Z. tournefortii* (54.69±0.75 and 31.02±1.45 mg GAE/100g, respectively) (Fellah *et al*., 2017).

On the other hand, the analysis revealed a positive correlation between TPC and anthocyanin with β-carotene, suggesting a negative impact of β-carotene on the radical scavenging activity. However, in several studies, pigments have been reported to have vigorous radical scavenging activity (Jensen, 1966; Stengel and Dring, 1998; Petrushkina *et al*., 2017; Marinho *et al*., 2019).

Our results demonstrate that the total phenolic content was not significantly different between autumn and spring in *S. illicifolium* samples. The TPC found in this study was previously reported for water (209 µg GAE/g DM) and ethanol extracts (354 µg GAE/g DM) of *Saccharina latissima* (Farvin and Jacobsen, 2015). This difference may likely be related to the solvents' polarity (Marinho *et al*., 2019). On the other hand, the concentrations of TPC reported here for *S. illicifoilum* (2.79-4.91mg GAE/g DM) are similar to those reported for *L. digitata* harvested monthly on a sheltered rocky shore in Brittany (France), ranging seasonally from 0.09 to 0.19% DM (using phloroglucinol as standard; Connan *et al.*, 2004). Likewise, the seasonal variation in the TPC followed the pattern reported for *S. latissima* in the same study, with the highest values found in winter and the lowest in summer/autumn (Marinho *et al*., 2019). However, Fellah *et al*. (2017) reported that TPC was higher in *S. coronopifolius* (144.33±1.76 mg GAE/100g) in autumn. The TPC content in *Zonaria tournefortii* in the summer (12.08 ± 0.28 mg GAE/100g) was significantly higher than in the spring (6.61 ± 0.14 mg GAE/100g) and autumn (3.73 ± 0.28 mg GAE/100g). It could be that the annual variation in phenolic content is dependent on diverse species composition and the different environmental conditions obtained in each season. The phenolic compound content of macroalgae varies with the reproductive stage of algae and with physical factors such as light density and quality, photoperiod, and temperature (Fellah *et al*., 2017). Gobbo-Neto and Lopes (2007) report that these factors have correlations and do not act in isolation; they may jointly influence the secondary metabolism.

To our knowledge, the amount of TFC did not show significant differences between the samples harvested in spring (pre-monsoon) and autumn (post-monsoon). In comparison, samples harvested in spring showed a higher level of

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TFC compared to samples harvested in autumn. TFC varied seasonally, reaching a maximum in summer and a minimum in winter. Flavonoids are UV-absorbing compounds that function as UV screens in terrestrial plants, among many other vital functions. For instance, UV-B can induce quercetin, which is primarily found in epidermal cells. This role is assumed to be played by simpler UV-absorbing compounds, such as mycosporine-like amino acids, in the aquatic environment (Rozema *et al.,* 2002). Nevertheless, structural identification of flavonoids in red and brown seaweeds, and microalgae, has recently been reported, contradicting the general assumption that these compounds could be exclusively present in terrestrial plants (Klejdus *et al*., 2010; Rodríguez-Bernaldo de Quirós *et al*., 2010; Goiris *et al*., 2014). The TFC found in the present study (8.38-32.17 mg QE/g DM over all seasons) is much higher than in several brown and green seaweed species originating from the Black Sea (6.5–229 μg RE/g DM).

The qualitative pigment composition did not change seasonally, whereas the number of specific pigments changed seasonally and with β-carotene concentrations that could be of commercial interest (Marinho *et al*., 2019).

β-carotene is the first and most crucial carotenoid (Butler *et al*., 2018). Our results reveal a seasonal pattern in the concentration of β-carotene, which ranged from summer and autumn. However, the samples harvested in the Tang location post-monsoon were significantly higher than those harvested pre-monsoon. Furthermore, the increased β-carotene content observed in this location in autumn is supported by studies showing that this increase can be a response to, i.e., shade and depth adaptation to increase light-harvesting efficiency (Ramus *et al*., 1977; Fortes and Lüning, 1980). These results can be correlated with the lower irradiance and short day length observed during autumn and winter in Iran. On the other hand, several specific pigments changed seasonally, and with β-carotene concentrations that could be of commercial interest, varying seasonally from 5.29 to 23.46 μg/g DM of *S. latissima* in September, November, and January by Hjarnø Havbrug A/S in the vicinity of Horsens Fjord, in the inner Danish waters (Denmark) (Marinho *et al*., 2019).

Conclusions

The algal biomass harvested from April to May (spring) and October to November (autumn) showed seasonal differences in S. illicifolium biomass, except for IC50. However, it exhibited a blend of characteristics from the Tang, Chabahar, and Gowatr sites in terms of FRAP content, total flavonoids, total anthocyanin, and β-carotene, highlighting the importance of considering these factors when evaluating its antioxidant potential. The FRAP content positively affected the radical scavenging activity, especially IC 50 in spring (pre-monsoon). In autumn (post-monsoon), β-carotene had a negative effect on the radical scavenging activity, especially TFC and anthocyanin content.

Conflicts of interest

The author does not have any conflicts of interest to declare.

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