Seasonal variation of antioxidant activity and properties in Ulva lactuca Linnaeus, 1753: A compression study in pre and post monsoon to the northern coasts of the Oman Sea

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Research Article

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Abstract

Ulva lactuca (Ul) is a green macroalgae that contains commercially valuable molecules components. The purpose of this study was to evaluate and compare seasonal variations of antioxidant activity (AOA) of *U. l.* sample harvested from locations of Tang, Chabahar and Gowatr in the northern coast of the Oman Sea (IRAN). Sampling was carried out during April to May (spring) and October to November (autumn) 2020, representing pre- and post-monsoon, respectively. No significant difference ($P > 0.05$) were observed between samples harvested in pre- and post-monsoon from all three locations, except for the ferric reducing antioxidant power (FRAP) content. The FRAP on post-monsoon shows a significant difference between all studied locations ($P < 0.05$). In this season, the FRAP content was measured 69 ± 0.05; 0.60 ± 0.06 and 0.50 ± 0.06 mg GAE g^{-1} for Tang, Chabahar and Gowatr, respectively ($P < 0.05$). However, a high linear correlation (> 0.95) was calculated for parameters showing a significant contribution by β-carotene (βC) with FRAP content ($R = -0.004$) in pre-monsoon, as well as total phenolic content (TPC) with anthocyanin content (ACN) ($R = -0.013$); Half-maximal inhibitory concentration (IC_{50}) with total flavonoid content (TFC) ($R = -0.010$) and radical scavenging activity assay (DPPH) with IC_{50} content ($R = 0.022$) in post-monsoon. AOA analysis along with PCA (principal component analysis) in pre-monsoon confirm relationships and FRAP, TPC, ACN and βC determined sample distribution along PC1 (main components 1), while DPPH, IC_{50} and TFC along the PC2. However, in post monsoons all parameters determined sample distribution along PC1.

Introduction

Due to the special geographical features and ecological valuables, the Oman Sea and coasts are known as one of the rarest ecosystems in the worldwide (Al-Majed et al. 2008). Algae is one of the most important organism's lives in these Sea (Egan et al. 2013).

The findings of recent decades have been reported macroalgae are containing many potent antioxidants, antimicrobials, antivirus, anti-inflammatory, antitumor and anticancer traditional properties, which make them for suitable ingredients to possible applications such as nutraceuticals, pharmaceuticals, cosmetic industries and functional foods (dominguez and loret 2019; Arguel and Sapin 2020; Cagalj et al. 2021).

Green algae as the most diverse algae group in the world has become the focus of many studies, because they are easily accessible and extracts from them are a rich source of antioxidants, carotenoids, polyphenols and flavonoid compounds, as well as natural colorant in various natural product classes (Pérez et al. 2016; Handajani and Prabowo 2020; Prasedya et al. 2020; Fournière et al. 2021). Among the various green algae species from the genus *Ulva lactuca* L. to the Chlorophyta phylum (Ulvaceae family, order Ulvales) is one of the most common species that abound in the north coast of the Oman Sea, Iran (Sohrabipour and Rabii 1999). This species contains commercially valuable biologically active molecules components which are important in different industries.

The study of the bioactive components of algae has increased in recent years and most of the focus has been on the identification of new components (Mekinic et al. 2019; Medina-Torres et al. 2017; Sanjeewa et al. 2017). But, the high potential impact of seasonal variations on metabolite and bioactivity profiles of algae, often was ignored in analytical studies and drug discovery.

Although, the effects of various seasonal acclimation on algae bioactive compounds have been reported in various studies (Prasedya et al. 2019; Karkhaneh Yousefi et al. 2020). But, the monsoon acclimation was not well studied (Billones et al. 2016; Mahdi Abkenar et al. 2021). The monsoon has a significant impact on the environmental and ecological features of the Oman Sea and coasts (Mahdi Abkenar et al. 2021). Algal bloom is one of the most important phenomenon of the monsoon effects (Hunter 1993).

Despite the fact that antioxidant activity (AOA) and the content of phenolic compounds of algae have been widely investigated, comprehensive studies on qualitative and quantitative characteristics in various seasonal conditions using various AOA measurement methods are still lacking. In addition, information about AOA of the Ul from the northern coast of the Oman Sea is rather limited. Due to effect of environmental indices on mitigate oxidative damage of algae in this study we evaluate and compare seasonal variations of antioxidant components (total phenol, flavonoids, β-carotene and anthocyanin contents) and AOA of Ul sample harvested in three different locations of Tang, Chabahar and Gowatr in the Oman Sea coast (IRAN) at different seasonal variation of pre (spring) - and post (autumn) monsoon.

Materials And Methods

Chemicals

Sulfuric acid, Folin–Ciocalteu reagent, and aluminum chloride were from Merck (Germany); ammonium molybdate, potassium acetate, DPPH (2, 2-diphenyl-1- picrylhydrazyl), and sodium phosphate, gallic acid, rutin, ascorbic acid, were from Sigma-Aldrich (Germany).

Seaweeds collection

Seaweed *Ulva lactuca* (Ul) specimens were obtained during the low tide times (according to the tide time table obtained from www.tideforecast. com) in 2020 for two seasons during April to May (pre-monsoon) and October to November (post-Monsoon) representing the autumn and spring randomly from the northern coasts of the Gulf of Oman, Iran (Tang port: Longitude, 25˚ 35′ 79″ N; Latitude: 59˚ 89′ 02″ E, Chabahar port: Longitude, 25˚ 27′ 71″ N; Latitude: 60˚ 66′ 77″ E; and Gowatr port: Longitude, 25˚ 17′ 59″ N; Latitude: 61˚ 50′ 22″ E). Seaweed taxonomic was determined by the off-shore fisheries research center. Algae was identified by Aleem (1993), they belonged to the family: *U. lactuca* Linnaeus from Chlorophyta.

Preparation of algae methanolic extract

After the collection, algae is taken to the laboratory in a plastic bag that contains sea water to prevent evaporation. Epiphytic and foreign materials are removed by first being washed in sea water and then

distilled water to separate the potential contaminants. Dry samples were prepared by drying fresh seaweed in the air at room temperature for 7 days to dry and stored in a plastic bag for further analysis. (González del Val et al. 2001).

For the *Ul* extract preparation, first dry samples are based and powdered. Then 20 g of the Ul powder which was dried in air and added to 10 mL of 80% methanol and stored at 37 °C for 6 hours in an orbital shaker incubator (AXYOS, Australia) and centrifugation (Kubota, KN-70, HD8292, Japan) in 1500 × RPM for 15 minutes. After 72 hours of obtaining agent extracts centrifuged. Finally, the extract obtained was filtered by WHATMAN no.1 filter paper and was concentrated to dry to produce a rough extract residue. Extract was diluted with solvents (50 mg mL^{-1}) and stored at 4C used for further analysis (Chidambararajan et al. 2019).

Antioxidant activity (AOA)

The AOA of the Ul extract was determined with two different mechanisms of action, i.e., hydrogen atom transfer (HAD) (DDPH) and electron transfer (ET) (FRAB) (Granato, et al. 2018).

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

The DPPH scavenging activity assay of the Ul extract was investigated following Duan et al (2006). Briefly, 1 mL of 0.15 mM DPPH were added to a different extract dilution (amounted to 1 mL). Then, the reaction mixture was incubated in the dark at room temperature for 30 minutes. 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 (IC_{50}) were calculated by the linear regression analysis and expressed as mean of three determinations. IC_{50} was used as an index to compare the AOA of individuals. Ascorbic acid was used as positive control and reducing power was expressed as mg ascorbic acid equivalents (AAE) per mL dry weight of sample (DW).

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay were determined using the Oyazu (1986) modification method. Briefly, 1 mL the Ul extract was added to 1 mL 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% of TCA was added to this reaction mixture. Aliquot 1 mL from the incubation mixture were 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 expressed as equivalent to mg gallic acid (GAE) per g dry weight sample (DW).

Total Phenolic and Flavonoid Content

Total phenolic content (TPC) of the Ul extract were determined by the Folin-Ciocalteu method (Cagalj et al., 2021) using a UV spectrophotometer (Shimatzu, UV-160A, Japan) with minor modification. Briefly, 20 µl of the Ul extract reacted with 1.50 mL of distilled water and 125 µL of 10% of folin-Ciocalteu reagent. The solution was mixed and after a minute, 375 µL of 20% sodium carbonate solution and 475 µL distilled water were added. The mixture was left in the dark for 2h at room temperature. Absorbance was read at 720 nm using a spectrophotometer. The Standard curve (0-500 mg L^{-1}) was prepared using galic acid for calculation of phenolic content (y = 0.004-x, r^2 = 0.9999). The TPC were expressed as mg Gallic acid equivalents (GAE) per g dry weight sample (DW).

Total content flavonoids (TFC) of extract the Ul assessed using the aluminum chloride colorimetric method described by Cagalj et al. (2021). Briefly, 250 µL the Ul extract was mixed with 750 µL 96% ethanol, 50 µL 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. Standard calibration curve (0-100 µg mL^{-1}) is plotted using a Quercetin solution (Y = 0.0076-0.0042, r^2 = 0.9412). TFC is expressed as mg Quercetin equivalent (QE) per g dry weight sample (DW).

Total anthocyanin content

Determination of total anthocyanin content was measured using a differential protocol spectrophotometric pH, as described by Muanda (2001) with minor modifications. Briefly, 0.5 mL of the Ul extract was 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 blank. Then, the extract combined with 3.5 mL 0.025 M sodium acetate buffer; pH=4.5. Again, the mixture was stirred and left at room temperature for 15 minutes. Absorbance of mixture was measured at the same wavelength (512 and 700 nm). Total anthocyanin content was calculated using the following equation:

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

Where A is absorbance= ($A_{\lambda 515}$–$A_{\lambda 700}$) 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; □ is the molar absorbtivity of cyanidin-3-

glucoside =24825; C is the concentration of the buffer in mg mL^{-1} =0.025. The results were expressed as milligram of cyanidin-3-glucoside equivalents (mg C-3-GE) per 100 gram of dry weight of sample (DW).

The β-Carotene content

Quantification of β-carotene were carried out following Eijckelhoff and Dekker (1995) with minor modification. Briefly, 20 g the Ul crude extract was solubilized in 10 mL 85% acetone-hexane and centrifuged at 3000 rpm for 15 minutes at 4 °C. Then, the absorbance measured at 453, 505, 645 and 663 nm using a UV spectrophotometer (Shimatzu, UV -160A, Japan). The contents of the β-carotene are calculated according to the following equations and expressed as mg per g of dry weight (DW).

$$\beta - Carotene\ (mg\ 100g^{-1}\ DW) = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

Statistical analysis

All statistical analysis was conducted using SPSS Software (New York, USA) version 16.0 in a triplicate. Shapiro-Wilk and Levenes tests were used to check the normality and homogeneity of variance, respectively. To determine whether there are differences between means, ANOVA analysis and Duncan's new multiple distance test is applied to correlate the potential of antioxidants in the samples. In addition, compression is made between monitored factors and locations in pre and post monsoon with independent sample tests. A principal component analysis (PCA) was carried out to obtain an overview of the equations and differences in antioxidant activities and properties of the Ul extract to detect patterns in the data and to reduce the dimensions of information for discrimination analysis. Data is declared a means of ± standard deviation (SD) of three replication determinations. P value <0.05.

Results

DPPH (%) scavenging activity

Table 1, indicating no significant variation of scavenging effects from *Ulva lactuca* (Ul) extract tested at 1, 10, 100 and 1000 µg mL^{-1} on the DPPH (%) of methanolic extract of the Ul harvested in pre- and post-monsoon among three locations of Tang chabahar and Gowatr (P> 0.05).

Table 1

DPPH radical scavenging activity (%DPPH) of 1, 10, 100 and 1000 µg mL^{-1} of the Ul extracts in different seasons.

Seasons	Concentration (µg mL^{-1})	Stations		
		Tang	Chabahar	Gowatr
Pre- (Spring)	1	39.46±1.37	35.72±1.74	37.9±1.91
	10	42.19±83	39.80±2.01	40.15±0.45
	100	47.28±3.34	45.20±1.15	46.79±0.74
	1000	50.43±1.19	48.96±1.23	50.90±3.01
Post- (autumn)	1	47.19±0.85	44.76±0.99	41.38±1.09
	10	54.27±3.45	52.96±1.36	49.76±2.59
	100	57.98±2.36	55.80±2.78	52.26±2.34
	1000	60.50±2.54	59.12±2.46	55.81±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).

IC_{50}

As can be seen in the Fig 3, The IC_{50} content of ascorbic acid as a standard antioxidant has not shown a significant difference (P <0.05) between both pre- (104.48± 6.96 mg AAE mL^{-1}) and post- (108.41±7.89 mg AAE mL^{-1}) monsoon (sig= 0.28; t= -111; DF = 15.75). However, no significant different were observed between three locations of Tang, Chabahar and Gowatr during pre and post monsoon (P> 0.05). IC_{50} content in the Ul extract in pre-monsoons ranged from 94.41 to 115.40 mg AAE mL^{-1}, whereas in post monsoons were found in 98.51 to 118.71 mg AAE mL^{-1}.

Ferric Reducing Power Assay (FRAP)

The Fig 4, presents the compression of the FRAP content in the Ul extract among three different locations and seasonal variations on the Northern coast of the Oman Sea. According to the results there was no significant difference found between the Ul extract which was harvested in pre- (0.58±0.08 mg GAE g^{-1} DW) and post- (0.59±0.09 mg GAE g^{-1} DW) monsoon (sig= 0.85; t= -0.18; DF= 15.59). However, there was no significant difference found in three locations of samples harvested at pre-monsoon (p> 0.05); Meanwhile, a statistically significant difference was observed in the samples harvested in post-monsoon between all locations (P <0.05). The highest (0.69 ± 0.05 mg GAE g^{-1} DW) and the lowest (0.50±0.06 mg GAE g^{-1} DW) of the FRAP content were observed at the Tang and Gowatr site, respectively. The FRAP content measurement in the Ul sample harvested from Chabahar was 0.60±0.06 mg GAE g^{-1} DW. The content of this index in the Ul extract harvested in the pre-monsoons ranges from 0.42 to 0.71 mg GEA g^{-1} DW, and the sample harvested in the post-monsoon was 0.44 to 0.75 mg GEA g^{-1} DW.

Total Phenolic content (TPC)

Compression of The TPC content in the Ul. extract presented in the Fig 5. Based on the results for both pre- (3.32±0.77 mg GAE g^{-1} DW) and post- (3.38±1.00 mg GAE g^{-1} DW) monsoon, no significant variations were observed (sig= 0.27; t= -1.13; DF= 15.97). However, no significant differences were observed between three different locations of Tang, Chabahar and Gowatr during pre and post monsoon (P> 0.05). The TPC compounds in extract harvested in the pre-monsoons range from 2.14 to 4.13 mg GAE g^{-1} DW, whereas foe samples harvested in the post monsoon were found at 2.15 to 4.95 mg GAE g^{-1} DW.

Total Flavonoid Content (TFC)

As shown in the Fig 6, there were no significant differences between the pre- (0.38±0.11mg QE g^{-1} DW) and the post- (0.35±0.07 mg QE g^{-1} DW) monsoon for methanolic the Ul extract (Sig = 0.54; T=0.62; DF=14.04), as well as between three different locations of Tang, Chabahar and Gowatr during pre and post monsoon (p> 0.05). The content of total flavonoid compounds in the pre-monsoons ranges from 0.22 to 0.55 QE g^{-1} DW, while the post monsoon was found at 0.25 to 0.47 mg QE g^{-1} DW.

Anthocyanin Content (ACN)

Fig 7, showing no significant (P> 0.05) seasonal variation for the anthocyanin content from the Ul extracts harvested in pre (3.41±0.44 mg C-3-GE 100g^{-1} DW) and post (3.70±0.37 mg C-3- GE 100g^{-1} DW) monsoon (Sig= 0.15; t= -1.49; DF = 15.57). However, no significant were observed for the samples harvested in three different locations of Tang, Chabahar and the Gowatr during pre and post monsoon (P> 0.05). The content of anthocyanin compounds in the pre-monsoons ranges from 2.87 to 3.71 mg C-3-GE 100g^{-1} DW, while in the post monsoon was found at 2.98 to 4.03 mg C-3-GE 100g^{-1} DW.

The β-Carotene content (βC)

Fig 8, showing no significant seasonal variations (P> 0.05) in the contents of βC the Ul extract in the pre (0.76±0.10 mg g^{-1} DW) and the post (0.80±0.14 mg g^{-1} DW) monsoon (Sig= 0.53; t= -0.63; DF= 14.62), and among the three differential Tang locations, chabahar and Gowatr during pre and post monsoon (p> 0.05). The β-carotene content in methanolic extract of the Ul harvested in pre-monsoon ranges from 0.57 to 0.88 mg g^{-1} DW, while on the post monsoon found in 0.49 to 0.97 mg g^{-1} DW.

Correlation analysis

Pearson correlation analysis was carried out to determine the relationship between each antioxidant activity index and properties in different seasonal sampling. As shown in Table 2, there is a significant negative correlation between FAR with β-carotene content (r= -0.004; p <0.05) in pre-monsoon.

Table 2

Pearson’s correlation coefficients between the variables to pre-monsoon

Indices	DDPH	IC_{50}	FRAP	TPC	TFC	ACN	βC
DPPH	1	-0.219	0.467	-0.378	-0.105	-0.028	-0.203
IC_{50}	-0.219	1	-0.112	-0.159	0.373	-0.115	-0.258
FRAB	0.467	-0.112	1	0.544	0.058	0.297	-0.004
TPC	-0.378	-0.159	0.544	1	-0.106	0.230	0.441
TFC	-0.105	0.373	0.058	-0.106	1	0.269	-0.246
Anthocyanin	-0.028	-0.115	0.297	0.230	0.269	1	0.181
β-carotene	-0.203	-0.258	**-0.004***	0.441	-0.246	0.181	1

***=P<0.05**

However, on post monsoon there were a significant negative correlation between TPC with Anthocyanin content (r= -0.013; P <0.05), as well as between IC_{50} with TFC content (r = -0.010; P <0.05). There were also a significantly positive correlation between DPPH with IC_{50} content (r = 0.022; P <0.05).

Table 3

Pearson's correlation coefficients between the variables to post-monsoon

Variables	DPPH	IC_{50}	FRAP	TPC	TFC	ACN	βC
DPPH	1	**0.022***	0.427	-0.088	0.327	0.053	0.061
IC_{50}	0.022	1	0.544	0.340	**-0.010***	0.643	0.578
FRAP	0.427	0.544	1	0.247	0.486	0.373	0.160
TPC	-0.088	0.340	0.247	1	-0.203	**-0.013***	-0.057
TFC	0.327	**-0.010***	0.486	-0.203	1	0.419	-0.155
Anthocyanin.	0.053	0.643	0.373	**-0.013***	0.419	1	0.687
β-carotene	0.061	0.578	0.160	-0.057	-0.155	0.687	1

***=P<0.05**

Based on the PCA results in pre monsoon, the first three PCs (principal components) were extracted with the value of eigenvalue greater than 1 and together they explain 72.58% of the total variance (Table 4). PC1 explained 28.76% of data set variations and PC2 explained 22.27% (Fig 9). According to the value loaded, which expresses the correlation between PCs and variables, it can be stated that FRAP, TPC, anthocyanin and β-carotene were determined by the sample distribution along PC1, while DPPH, IC_{50} and TFC along PC2 (Fig 9a). This was proven evidenced by high r Pearson's correlation coefficients between

these variables and suggests that the AOA from the Ul extract was mainly associated with phenolic compounds. PCA analysis confirmed that the Ul harvested in Tang, Chabahar and Gowatr possess high AOA and phenolic compounds, including DPPH, TPC and FRAP contents (Fig 9b).

Table 4

eigenvectors and eigenvalues of the first three principal components (PCs) for 7 qualitative pod characters in pre-monsoon

Variable	Principal components		
	PC1	PC2	PC3
Eigenvalue	2.013	1.559	1.508
Variability (%)	28.763	22.271	21.547
Cumulative (%)	28.763	51.034	72.581
	Eigenvectors		
DPPH	-0.026	0.743	-0.176
IC_{50}	-0.355	-0.172	0.446
FRAP	0.426	0.501	0.262
TPC	0.586	-0.208	0.177
TFC	-0.187	0.027	0.671
Anthocyanin	0.330	0.080	0.435
β-carotene	0.453	-0.343	-0.175

On post monsoon, the first three PCA of the Ul were extracted with eigenvalue value greater than 1 and together they explained 79.49% of the total variance (Table 4). PC1 explained 38.48% of variations of data sets and PC2 explained 23.36% (Fig 10). According to the value loaded, which expresses the correlation between PCs and variables, can be stated that all studied parameters (DPPH, IC_{50}, FRAP, TPC, TFC, Anthocyanin and β-carotene) determine the distribution of the samples along PC1 (Fig. 10a). This is proven by the high R Pearson correlation coefficient between these variables and suggests that the AOA from the Ul extract were mainly associated with phenolic compounds. PCA analysis confirmed that samples harvested for Tang showed high AOA and phenolic compounds, including total anthocyanin, IC_{50}, FRAP, β-carotene and TFC contents (Fig. 10b).

Table 5

eigenvectors and eigenvalues of the first three principal components (PCs) for 7 qualitative pod characters in post-monsoon

Variable	Principal components		
	PC1	PC2	PC3
Eigenvalue	2.693	1.635	1.236
Variability (%)	38.466	23.364	17.662
Cumulative (%)	38.466	61.830	79.492
	Eigenvectors		
DPPH	0.192	0.494	0.088
IC_{50}	0.504	-0.301	0.155
FRAP	0.449	0.291	0.364
TPC	0.110	-0.276	0.762
TFC	0.241	0.605	-0.094
Anthocyanin	0.521	-0.080	-0.313
β-carotene	0.407	-0.362	-0.385

Discussion

Generally, in this study antioxidant activity of the Ul extract that harvested during pre- (spring) and post- (autumn) monsoon do not shows significant differences between the variables tested, except for FRAP content. Our results indicate that the FRAP content has a significant difference between seaweed harvested on post-monsoon between all three locations. The highest and lowest activity of the FRAP content were in Tang (0.69 ± 0.05 mg GAE g^{-1}) and Gowatr (0.50 ± 0.06 mg GAE g^{-1}), respectively. However, the FRAP content measured in Chabahar was 0.60 ± 0.06 mg GAE g^{-1}. These results indicate that AOA does not change with an increase or decrease of temperature during the pre and post monsoon, except for the FRAP content. This observation was supported by previously studies in other seaweed which also reported a different AOA test (Fellah et al. 2017; Jelandar et al. 2017; Farasat et al. 2013, 2014; Liu et al. 2011; Horincar et al. 2011). The FRAP concentration reported in this manuscript is similar to the reported for the Ul (0.60 ± 0.12 mg GAE g^{-1}) harvested in the autumn on the intertidal coast of Chabahar (Iran), using methanolic extract (Sarani Yaztapeh 2020). Moulazadeh et al (2021) reported that the Persian Gulf patched the Ul extract collected in the autumn had 64.89 ± 11.33 μmol Fe^{2+}/g. Sivaramakrishnan et al (2017) measure AOA and phenolic compounds of four green macroalgae (*Halimeda Tuna*, *Halimeda Macroloba*, *enteromorpha* sp. and *Acetabularia Acetabulum*) were collected from the coast of South Andaman (India) in three different locations. According to the author reporting the FRAP content was around from 152.89 ± 2.53 to 164.3 ± 1.04 μg g^{-1}. Likewise, seasonal variations of the FRAP content followed the patterns reported for *Halopetrical Scoparia* in the same study as significant differences found in the spring and fall (Fellah et al. 2017). Karkhaneh Yousefi et al (2020) by

measured the FRAP content in four different brown algae (*Dictyota indica, Padina tenuis, Colpomenia sinuosa and Iyengaria stellata*) harvested from Qeshm Island (Persian Gulf) reported that Antioxidant activities of all summer collected seaweeds were lower than the winter ones which were in accordance with their fucoxanthin contents. Contrary to our results, Trigui et al (2013) observed the highest free radical scavenging activity in *U. rigida* extract collected at the end of winter (February) and the beginning of spring (March) compared to the end of summer (August) and the beginning of fall (September-October). Based on the results, the authors suggested AOA increase with a decrease of temperature (Trigui et al. 2013).

However, variations of the FRAP concentration obtained from the Ul and other seaweed species can be associated with environmental factors such as various species compositions, seasonal variations, salinity, irradiation and inorganic and phosphorus nitrogen and the type of extraction solvent used (Oucif et al. 2017; Aruelles et al. 2018; Aruelles et al. 2021). Increased solar radiation and UV exposure during summer have also been associated with an increase in the production of antioxidant compounds in intertidal macroalgae at the monsoon (Garcia-Vaquero et al. 2021). In addition, inorganic nutrition released from shrimp farms in Gowatr is considered to be ignored compared to nitrogen background concentrations that occur naturally (Marinho et al. 2015c).

To investigate whether AOA from the Ul extract studied, a significant correlation was observed in the pre-monsoon between the FRAP with β-carotene contents and other antioxidant values except FRAP. It shows carotenoids having antioxidant activities in reducing Fe^{3+} radicals on the Ul. Here, there was a significant correlation between IC_{50} with free radical of DPPH, IC_{50} with TPC, and TPC with anthocyanin content in the post monsoon confirm the literature report and may indicate that this compounds are responsible for AOA or at least involved in the reaction of the effect at the post monsoon, which is appropriate with many previous studies aruelles (2021); Arguelles and Sapin 2020; Jesumani et al (2020); Sanger et al (2019); Sivaramakrishnan et al (2017); Farasat et al (2013; 2014); Chew et al. (2008).

Likewise, a lower correlation between β-carotene with DPPH content, IC_{50}, TPC, TFC and Anthocyanin which is only a phenolic compound that is not involved in AOA through this path but there may be several effects involving other active compounds.

Conclusion

In the current study, the assessment of the Ul antioxidant activities and properties extract which was harvested from three different locations during per (spring) and post (autumn) monsoon was evaluated. The FRAP content in post monsoons shows a significant difference between all three locations. The highest and lowest FARP content were found in the Tang and Gowatr, respectively. Perhaps, the location of Tang sampling does not yet have extreme environmental pollution compared to Chabahar and Gowatr such as lower tidal ranges that contribute to the sunlight exposure, and disposal of industrial human activities such as fisheries and waste making it into the sea. Based on the obtained results from this

study we could claim that macroalgae (*Ulva lactuca*) provides promising antioxidant properties that can be used in various food and pharmaceutical industries.

Abbreviations

Ul = *Ulva lactuca*

AOA = Antioxidant activity

FRAP= Ferric reducing antioxidant power

TFC= Total flavonoid content

TPC= Total phenolic content

ACN = Total anthocyanin content

βC = β-carotene

Declarations

Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions Statement

Ali Mahdi Abkenar conceived the original idea. Developed the theory and performed the computations. Verified the analytical methods. Carried out the experiment. Discussed the results and wrote the final manuscript.

Conflict of interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

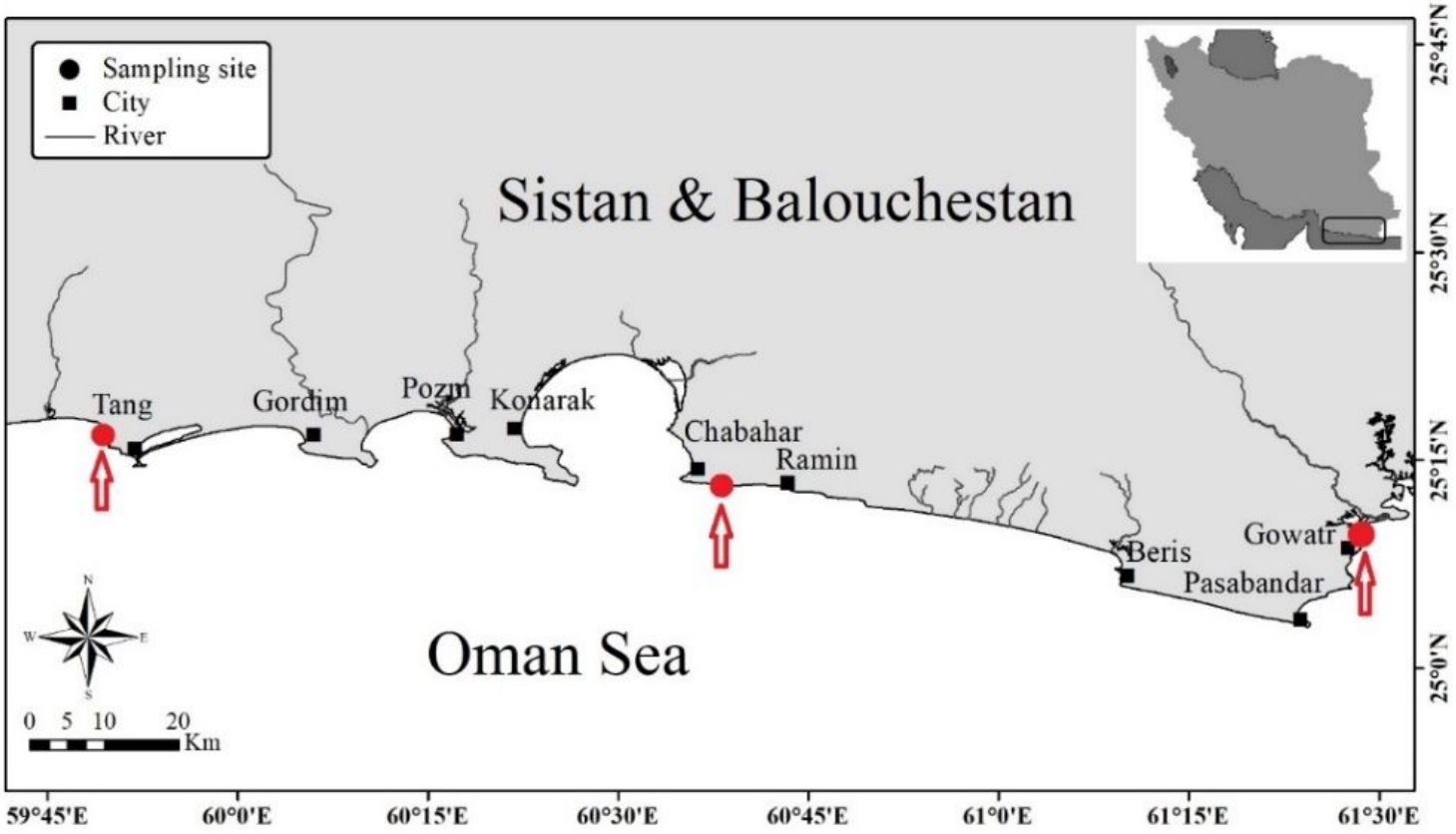

Figure 1

a sampling points on the Oman Sea, Iran.

Figure 2

a green macroalgae (*Ul*)

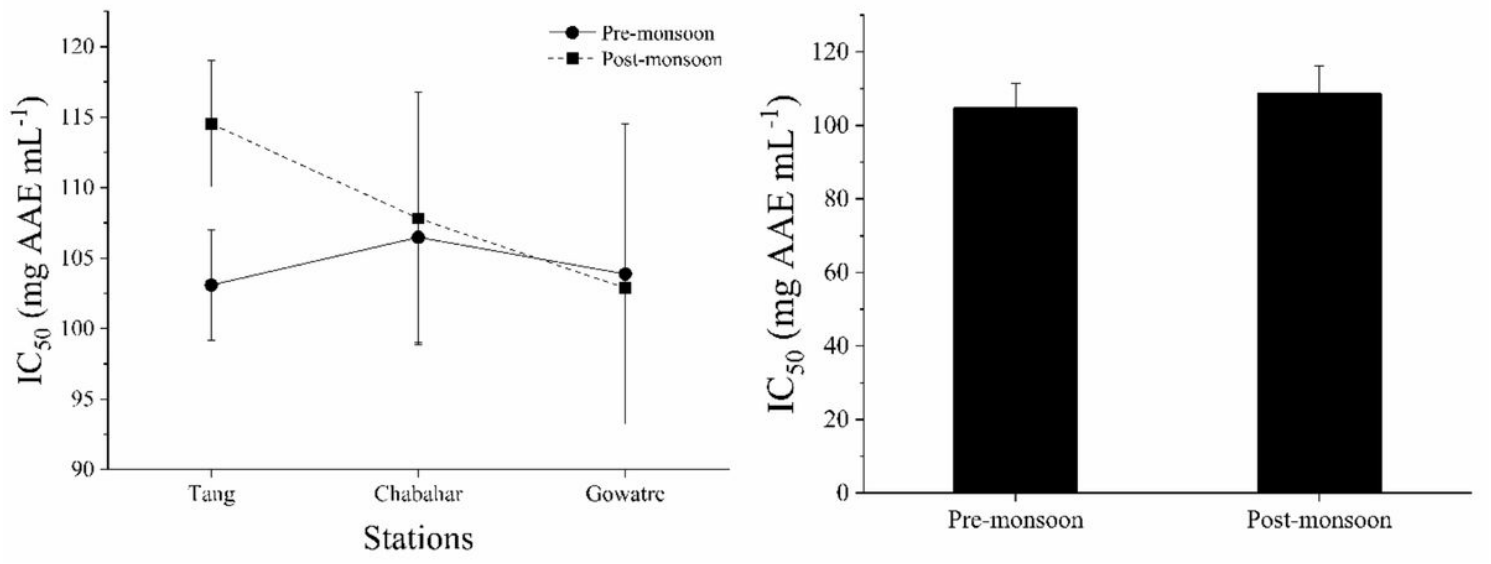

Figure 3

comparison of the IC_{50} content in the Ul extract between different locations (left) and monsoon conditions (right) in the northern coats of the Oman Sea (P> 0.05). Mean ± standard deviation.

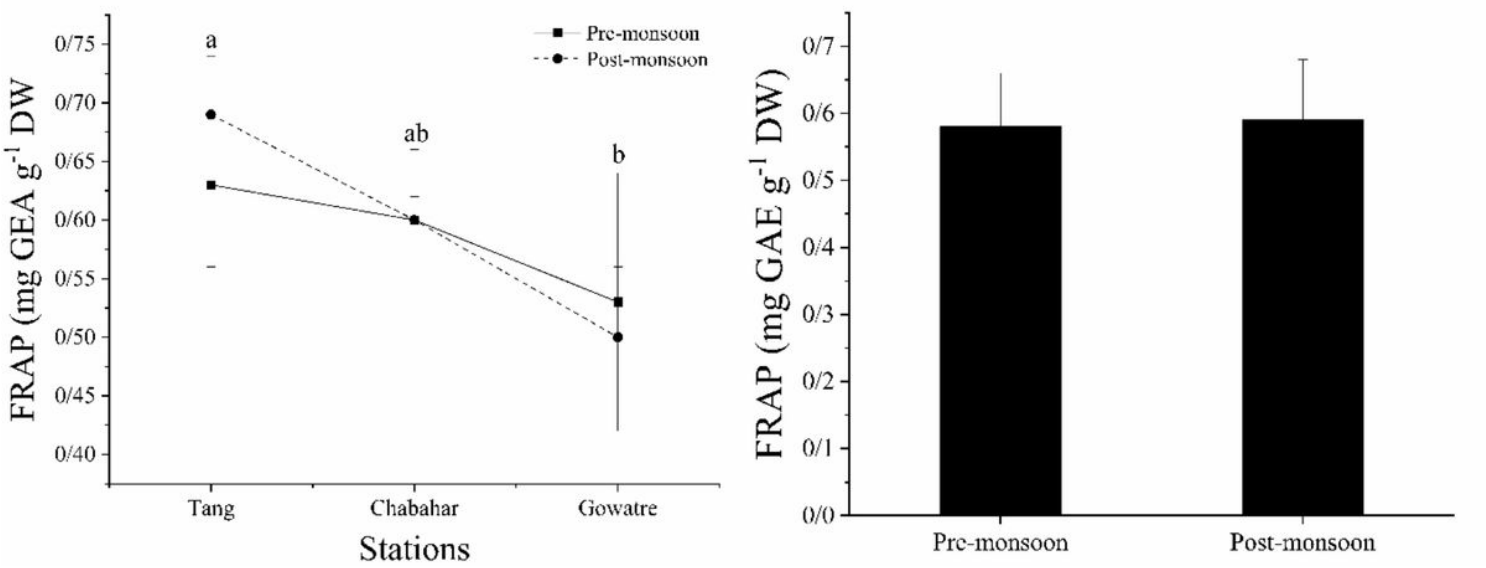

Figure 4

comparison of the FRAP content in the Ul extract between different locations (left) and monsoon conditions (right) of the northern coats of the Oman Sea (P> 0.05). Mean ± standard deviation.

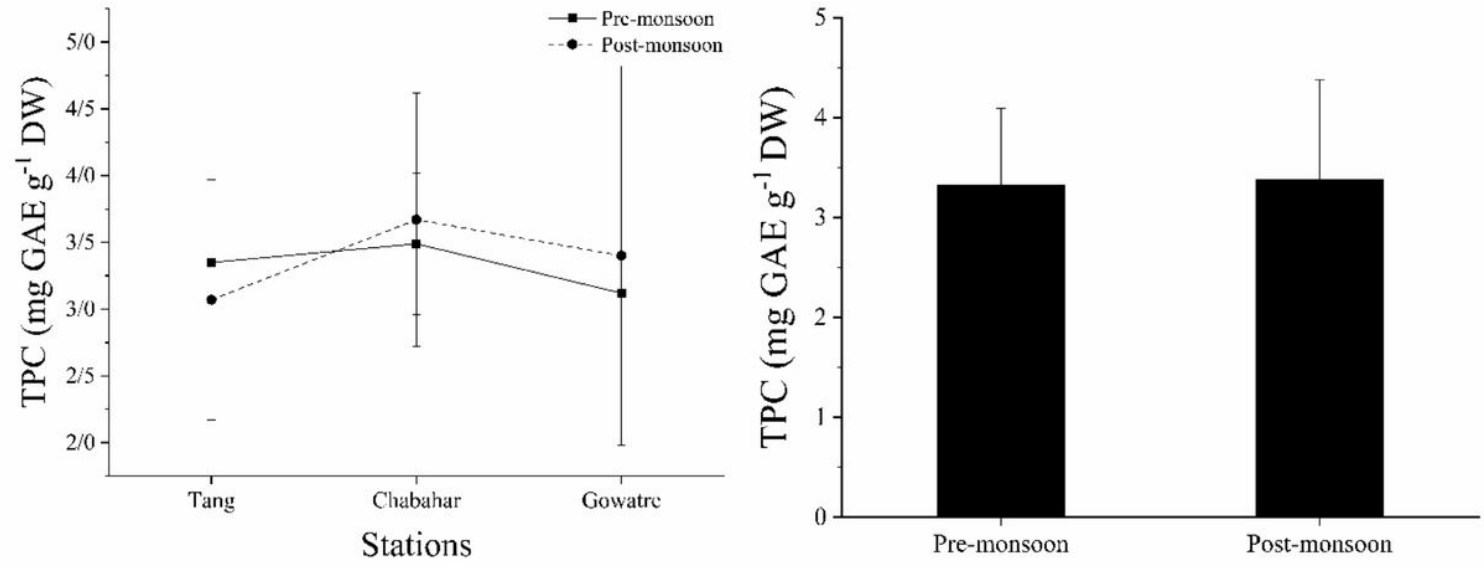

Figure 5

comparison of the TPC content in the Ul extract between different locations (left) and monsoon conditions (right) of the northern coats of the Oman Sea (P> 0.05). Mean ± standard deviation.

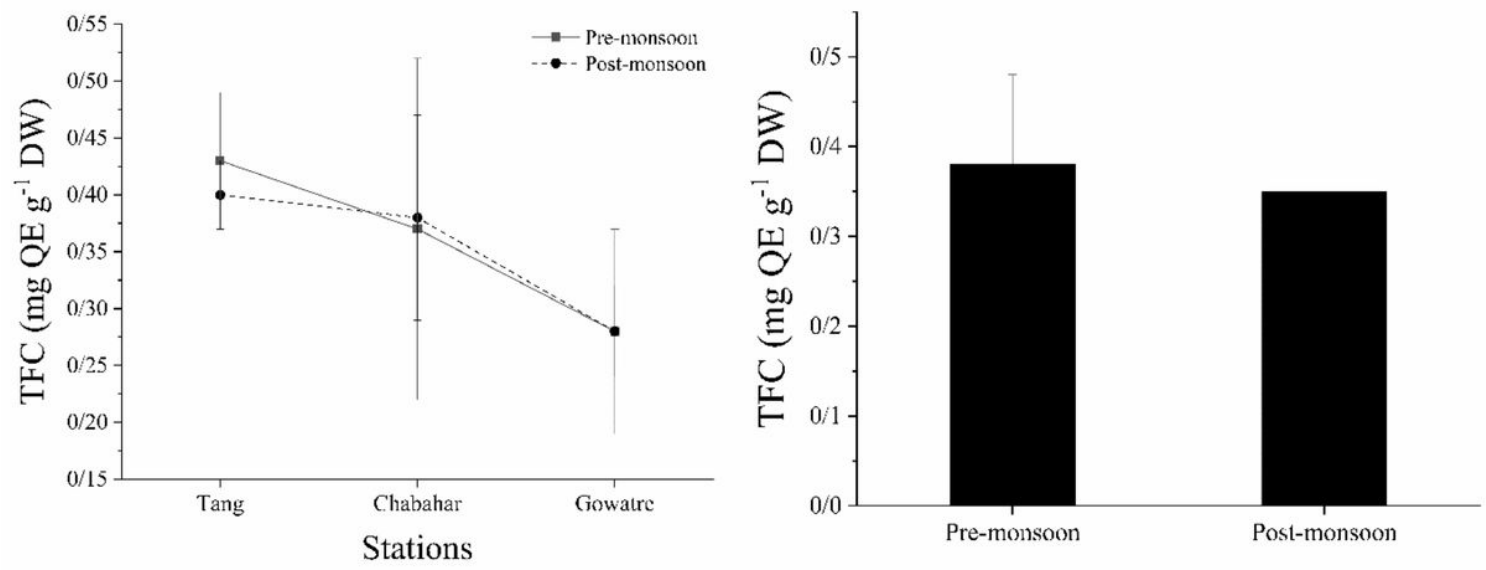

Figure 6

comparison of the TFC content in the Ul extract between different locations (left) and monsoon conditions (right) of the northern coats of the Oman Sea (P> 0.05). Mean ± standard deviation.

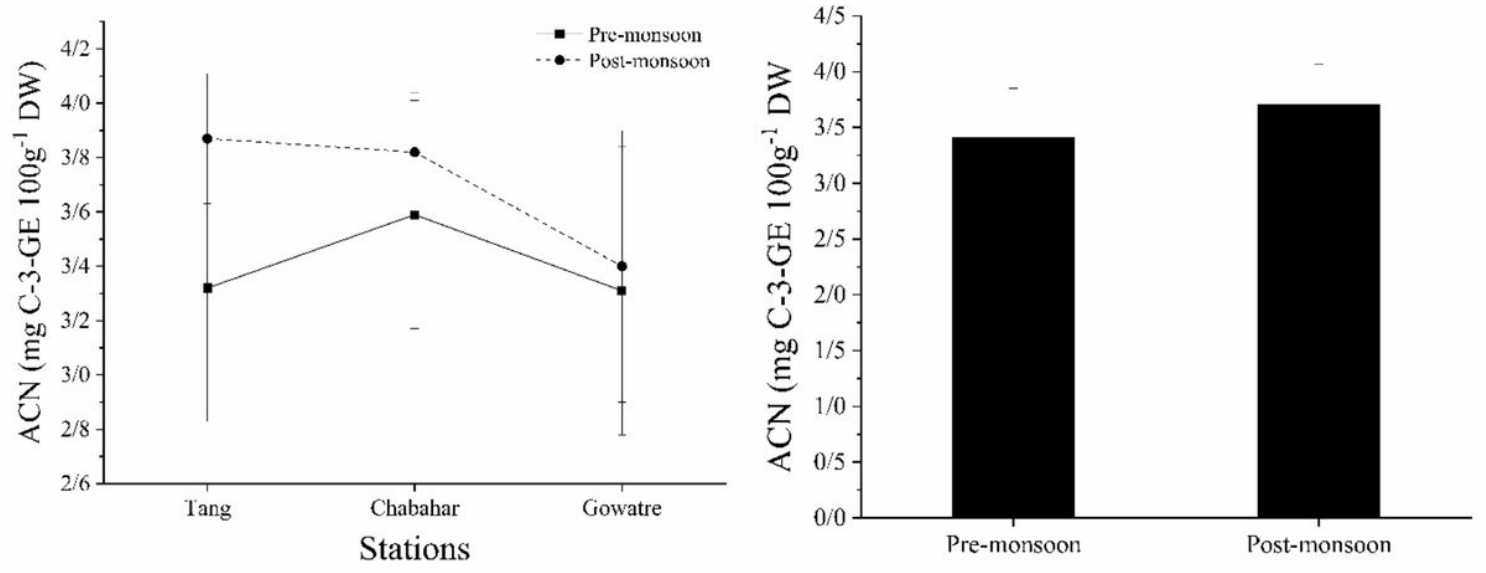

Figure 7

comparison of the ACN in the Ul extract between different locations (left) and monsoon conditions (right) of the northern coats of the Oman Sea (P> 0.05). Mean ± standard deviation.

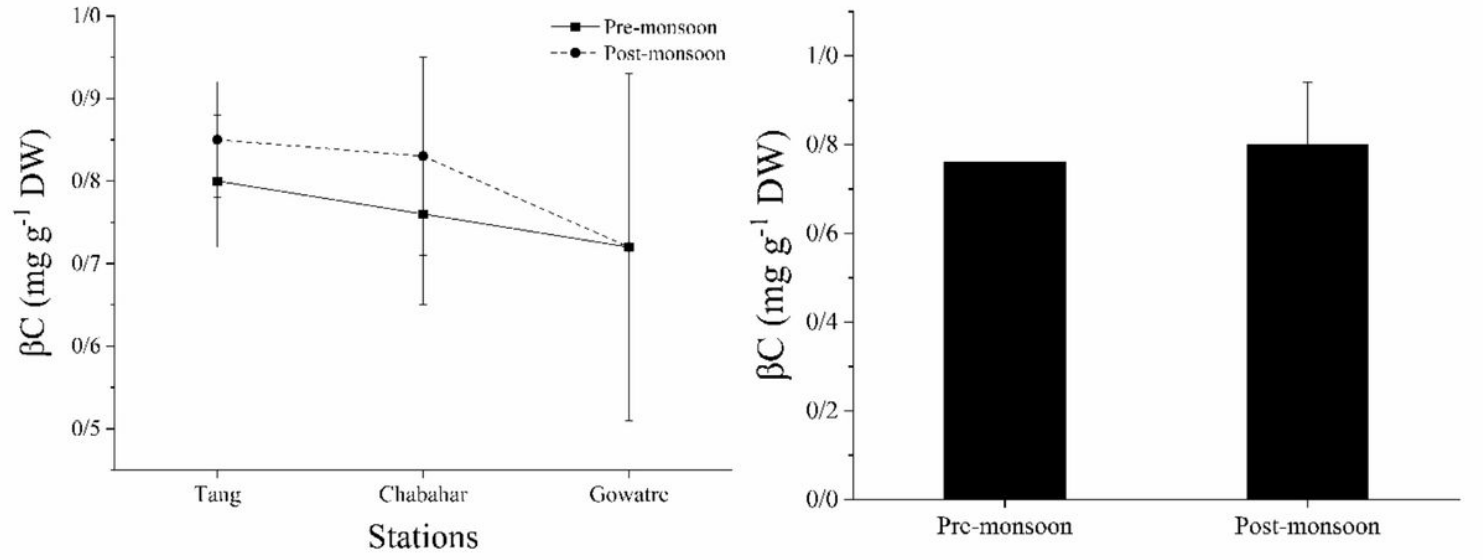

Figure 8

comparison of the βC content in the Ul extract between different locations (left) and monsoon conditions (right) the northern coats of the Oman Sea (P >0.05). Mean ± standard deviation

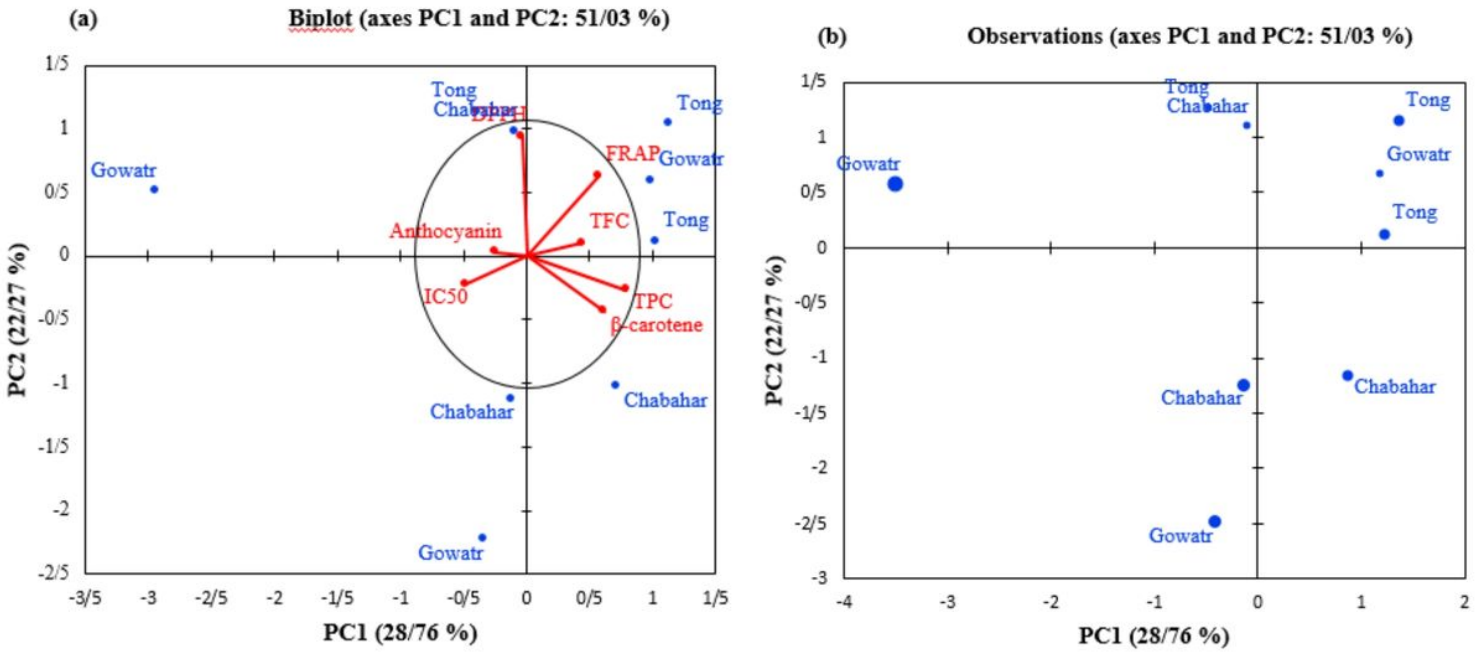

Figure 9

projections of the variables (a) on to the factor plane defined by principal components (PC1 and PC2); Projection of the scores (b) onto the factor pane in different location.

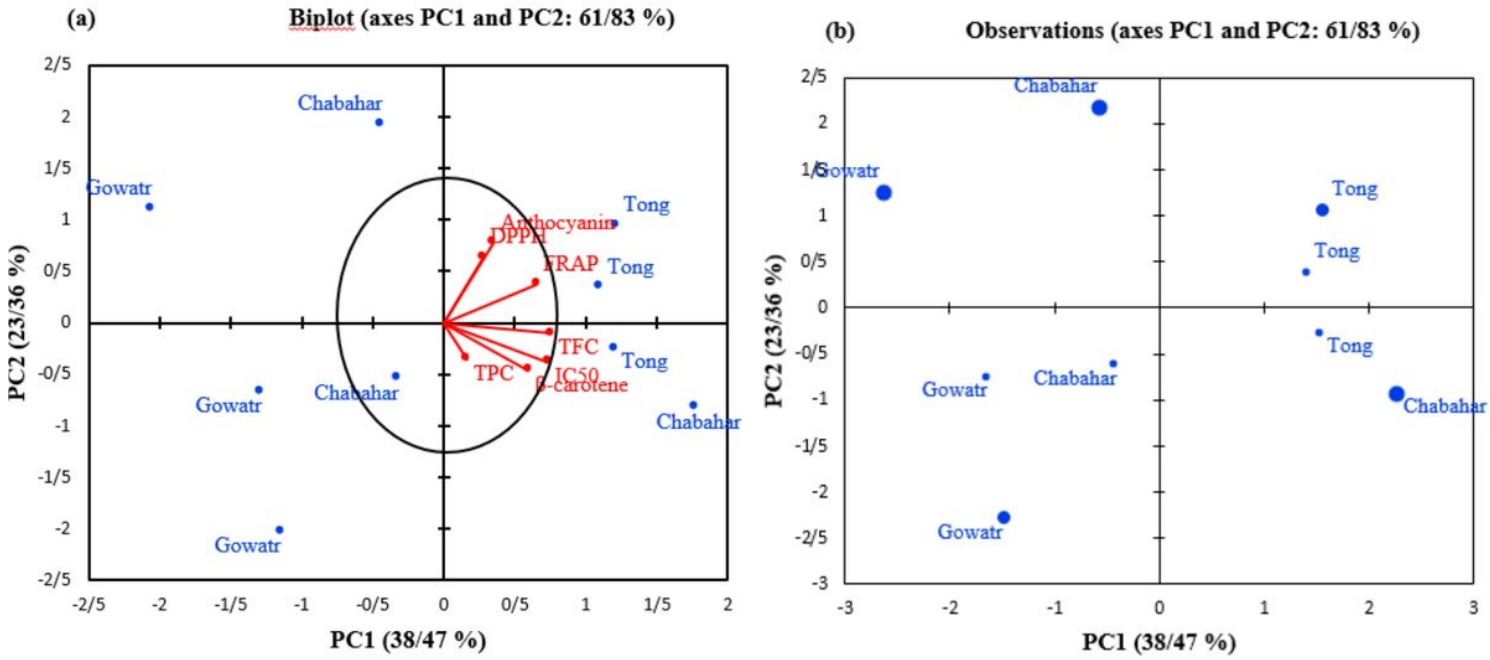

Figure 10

projections of the variables (a) onto the factor plane defined by principal components (PC1 and PC2); b Projection of the scores (b) onto the factor plane in different location