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Seasonal variation of fucoxanthin content in four species of brown seaweeds from Qeshm Island, Persian Gulf and evaluation of their antibacterial and antioxidant activities

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Abstract

Fucoxanthin contents of four species of brown seaweeds *(Dictyota indica, Padina tenuis, Colpomenia sinuosa* and *Iyengaria stellata)* from intertidal zone of Qeshm Island, Persian Gulf were assessed in summer and winter of 2016. In addition, some physicochemical properties (pH, temperature, salinity and conductivity) of seawater were monitored on the same time. The antibacterial activity of algal extracts was determined by disc diffusion assay, minimum inhibitory and minimum bactericidal concentration tests; and the antioxidant activity through ferric reducing antioxidant power method. The fucoxanthin contents of the studied seaweeds were higher in winter. *D. indica* showed the highest amount of fucoxanthin (approximately 462.79 and 210.72 µg/g) in both seasons, which makes it commercially applicable e.g. in food and pharmaceutical industries. Furthermore, *D. indica, I. stellata, P. tenuis* and *C. sinuosa* showed a strong ferric reduction power in both seasons. Considerable inhibition zone against gram negative (*Escherichia Coli*: PTCC1330) and gram positive bacteria (*Staphylococcus aureus*: PTCC1112) were also observed.

Keywords: Fucoxanthin, Brown seaweed, Antibacterial activity, Antioxidant activity, seasonal variation

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Introduction

Seaweeds or marine macroalgae contain a broad range of secondary metabolites like carotenoids, vitamins and minerals that are responsible for their anticancer, antioxidant, antimicrobial and many other biological activities (Rodriguez-Jasso *et al*., 2011). Since a long time ago, seaweeds were used by humans in many countries especially in Southeast Asian countries as food owing to their health benefits (Nisizawa *et al*., 1987). Carotenes (unsaturated hydrocarbons either linear or cyclic in one or both ends of the molecule) and xanthophylls (oxygenated derivatives of carotenes) are two main groups of natural carotenoids. Human body is not capable to synthesize carotenoids, hence, they should be provided from external sources (Ishida and Bartley, 2005). Fucoxanthin is one of the most important carotenoids (xanthophlls) found in the marine environment (Dembitsky and Maoka, 2007) which causes brownish color of brown seaweeds and gold color of diatoms (unicellular planktonic microalgae) (Beppu *et al*., 2009; Miyashita *et al*., 2013). Furthermore, it is responsible for the valuable biological activities including antioxidant (Fung *et al*., 2013); anticancer, anti angiogenic (Nakazawa *et al*., 2009); antiobesity and anti-inflammatory (Maeda *et al*., 2005; Maeda *et al*., 2007); due to its unique structure (Miyashita and Hosokawa, 2008; Nakazawa *et al*., 2009; Terasaki *et al*., 2009). It contains an allenic moiety and some other functional groups containing oxygen like epoxy, alcohol and ester, as shown in Fig. 1.

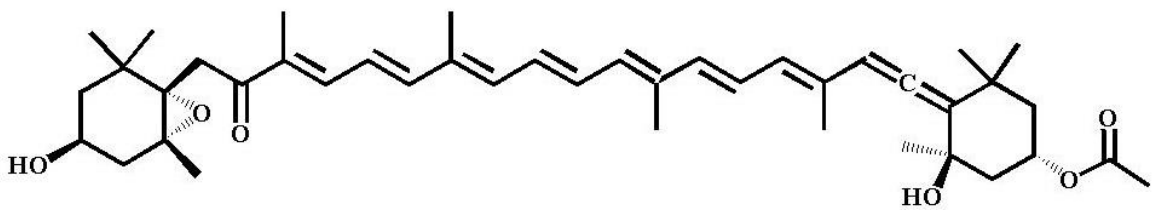


Figure 1: Chemical structure of fucoxanthin molecule.

All of these characteristics make fucoxanthin very applicable in pharmaceutical and food industries (Plaza *et al*., 2008). Seasonal and spatial fluctuation in ecological conditions can lead to the different properties of the same genus and species of seaweeds growing in various latitudes all over the world. Previous studies on many species of seaweeds have shown that brown seaweeds have a higher antioxidant activity than the red and green genera due to the presence of fucoxanthin (Jiménez-Escrig *et al*., 2001; Matanjun *et al*., 2008; Prabhasankar *et al*., 2009; Taheri, 2016). The antibacterial activity of brown seaweeds has also been reported (Shanmughapriya *et al*., 2008; Kolanjinathan *et al*., 2009; El-Shafay *et al*., 2016; Perez *et al*., 2016; Kordjazi *et al*., 2019). But, there are not enough

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data about the biological activities of brown seaweeds from Persian Gulf. The aim of this study is extraction of fucoxanthin from four species of brown seaweeds (*Padina tenuis*, *Colpomenia sinuosa*, *Iyengaria stellata and Dictyota indica*) collected from intertidal zone of Qeshm Island, Persian Gulf, at two seasons (summer and winter) to compare the fucoxanthin contents and biological activities. Furthermore, the antibacterial and antioxidant activities of their extracts were investigated.

Materials and methods

Sample collection

Four species of brown seaweeds *(D. indica, P. tenuis, C. sinuosa* and *I. stellata)* were collected from the intertidal zone of the Qeshm Island, Persian Gulf, Iran (26° 56.059′ N and 56° 16.485′ E) (Fig. 2), in winter and summer of 2016. The collection dates were selected by high tide chart according to the national cartographic center of Iran (http://iranhydrography.ncc.org.ir).

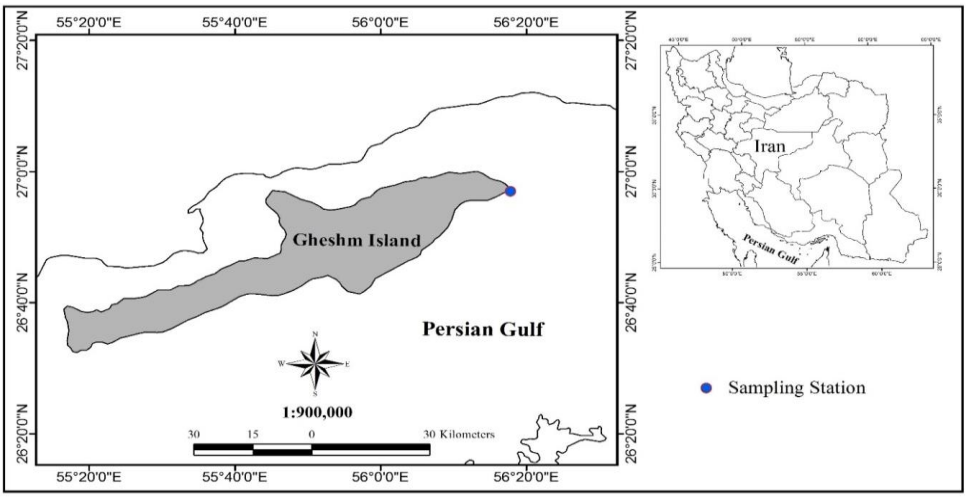


Figure 2: The location of seaweeds sampling site.

After collection, the seaweeds were washed carefully with sea water to remove external substances (epiphytes, other particles and invertebrates), transferred to the laboratory and washed again with tap water and distilled water and were air dried at shadow. The fully dried seaweeds were ground to powder and kept in glass vials in dark and cold condition to avoid degradation until analysis. During seaweeds sampling, some physico-chemical characteristics of the seawater (temperature, pH, salinity and conductivity) were also measured by multi-function water quality meter (Lutron wa2017sd).

Fucoxanthin extraction

Fucoxanthin extraction was carried out according to Haugen method (Haugen *et al*., 1992); with some modifications. All extraction solvents and fucoxanthin standard were of analytical grade and purchased from Sigma Aldrich (Steinheim, Germany). 2 grams of the

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powdered seaweed were mixed with 20 ml of methanol and stirred by a magnetic bar for 24 h at room temperature. After settlement of the insoluble part, the upper layer was further separated by centrifuging at 6000 rpm for 5 min and the supernatant was collected. This procedure was repeated three times.

Then, 60 ml distilled water and 60 ml hexane was added to the extract and the aqueous layer was separated by a separatory funnel and the organic phase was discarded. 40 ml chloroform was added to the aqueous phase and the organic phase was separated by the separatory funnel and the solvent was evaporated under reduced pressure at 30˚C by a rotary evaporator. The residue was kept at -20°C until further analysis.

Preparation of standard solution

0.5 mg fucoxanthin standard (Sigma-aldrich, Steinheim, Germany) was accurately weighed and dissolved in 1 ml of methanol and stored in -20°C.

High Performance Liquid Chromatography (HPLC) analysis

HPLC grade methanol was purchased from Samchun Chemicals (Seoul, Korea) and ultrapure water was obtained from a Direct Q UV-3 Millipore system. Quantitative analysis of fucoxanthin in the extracts was performed by HPLC (Agilent 1100 equipped with a quaternary pump and a G1314A variable wavelength detector (VWD)). The accompanying Agilent 1100 Chem station was employed for instrument control, data acquisition and processing. HPLC analysis was performed with an isocratic elution by 90:10 methanol-water at a flow rate of 1 mL/min on an Extend- C18 column (250 * 4.6 mm, 5 µm, Agilent, USA). The temperature was maintained at 20˚C and UV detection was performed at 450 nm. Both the extracts and standards were injected (injection volume: 20 µl) into the reverse phase column and identification was carried out by comparing the retention times of fucoxanthin standard and spiking the standards to the sample. Each experiment was repeated three times and run in triplicate.

The Recovery was calculated by adding a known amount of standard to the accurately weighed dry algae and extracting the spiked algae with the same procedure as previously described. The calibration curve was constructed by plotting the measured peak areas versus five concentrations (25–500 mg/L) of the fucoxanthin standard. Linearity of the standard curves was assessed by the coefficient of determination (R^2). Accuracy was determined using a high purity standard and precision expressed as standard deviation (SD) for each of the replicate concentrations (n = 3).

Estimation of antioxidant activity by the FRAP method

Antioxidant capacity of eight different algal extracts containing fucoxanthin was measured by Ferric reducing antioxidant power (FRAP) assay (Benzie *et al*., 1999). The FRAP

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reagent solution was prepared with 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tri(2-pyridyl) -1,3,5-triazine (TPTZ), 40 mmol/L HCl and 20 mmol/L standard solution of FeCl3.6H2O in the ratio of 10:1:1 (V/V/V). The reagent solution was heated to 37˚C and used freshly. 200µl of the reagent solution was dispensed into the 96-well plates followed by adding 20 µl of algal extracts to initiate the reaction. The absorbance was read at 593 nm after 10 minutes by ELISA reader (ELX800, BioTeK, USA). Ascorbic acid with concentration of 2 mg ml^{-1} was used as the positive control. The result of ferric reduction power was implied as mg ascorbic acid equivalent per g of dried weight of algal extract.

Estimation of antibacterial activity

Antibacterial activity of the algal extracts from four species of brown seaweeds at two seasons was investigated by paper disk diffusion assay (Bauer *et al*., 1966). One-gram positive bacteria (*Staphylococcus aureus* (ATCC 6538)) and one-gram negative bacteria (*Escherichia coli* (ATCC 25922)) were purchased from Pasteur Institute of Iran.

Briefly, a suspension of each strain was spread on Muller Hinton Agar (MHA) medium. The paper discs were placed on the agar plates, then soaking with 20 µl of the algal extract (concentration 200 mg ml^{-1} in dimethyl sulfoxide (DMSO)). The plates were incubated at 37˚C for 24 hrs. After incubation, the growth inhibition zone was quantified by measuring the diameter of the inhibition zone in mm. The solvent alone was used with the same method as a negative control.

Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration of the fucoxanthin extract was investigated by the tube dilution method. Certain concentrations of the algal extracts were introduced into the test tubes. Standard inoculum of overnight culture of each organism in nutrient broth (Merck) (10^6 cell ml^{-1}) was added to each tube. The tubes were incubated for 24 h at 37°C. The lowest concentration of the extracts that inhibits the growth of the organisms were measured which corresponds to MIC (Natarajan *et al*., 2010; Ananthan *et al*., 2011). After culturing the test organisms separately, the broth was inoculated on to the freshly prepared agar plates to assess the bactericidal effects, agar plates were inoculated with samples from each of the test tubes that showed no visible growth from the MIC test (incubated for 24 h at 37°C). The minimum concentration of extract that caused the bacteria elimination (99.9%) regarded as the MBC (Chellaram *et al*., 2009). Each experiment was repeated three times and run in triplicate.

Results

Fucoxanthin content of four different species of marine brown seaweeds (*P. tenuis*, *C. sinuosa*, *I. stellata and*

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D.indica) collected from Qeshm Island, Persian Gulf, was determined for winter and summer. In addition, the antioxidant and antibacterial activities of the seaweeds extract were investigated. The HPLC chromatograms of fucoxanthin standard and one of the algal extracts (*D. indica*) are shown in Figs. 3 and 4, respectively.

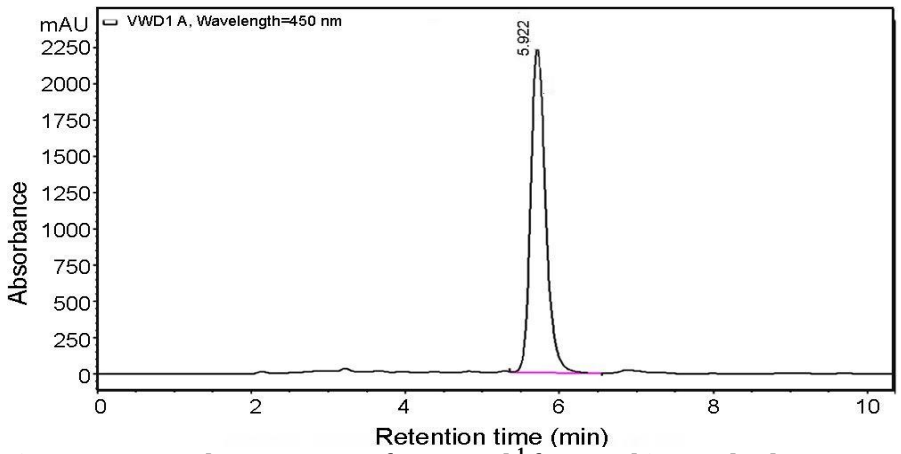


Figure 3: HPLC chromatogram of 500 ng ml^{-1} fucoxanthin standard at 450 nm.

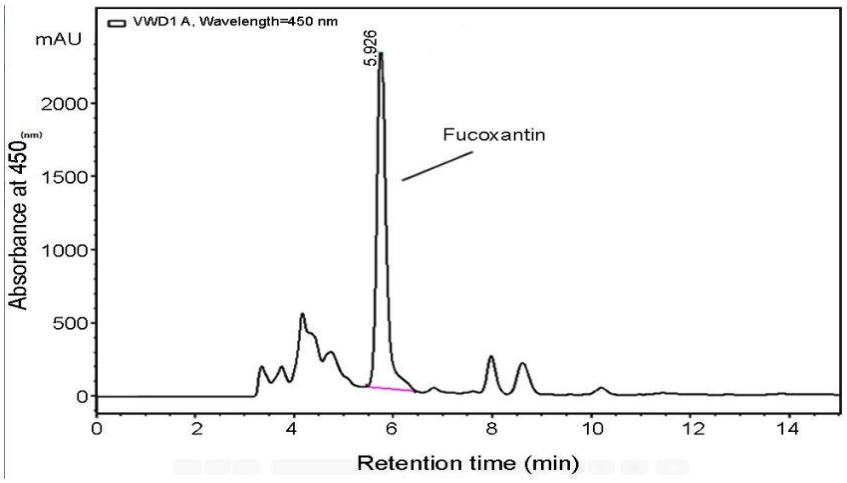


Figure 4: HPLC chromatogram of *D. indica* at winter collection.

Quantification of the fucoxanthin in the extracts was performed using a 5 point calibration curve which was constructed using 5 different concentrations of fucoxanthin standard. A liner correlation with R value of 0.999 for the calibration equation was found between peak areas and concentrations of fucoxanthin. The recovery was 90%. The results are shown in Table 1.

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Table 1: Fucoxanthin content of algae species at winter and summer.

Algae species	Fucoxantin Concentration (µg g^{-1}) DW[a] ± SD	
	Winter	Summer
Dictyota indica	462.79± 3.08	210.72± 1.20
Iyengaria Stellata	55.39± 1.15	26.10± 0.85
Padina tenuis	42.77± 0.95	17.61± 0.43
Colpomenia sinuosa	19.23±0.0.65	13.53± 0.35

[a] DW: Dry Weight

The fucoxanthin peak appears at 5.9 min. As shown in Table 1, *D. indica* from winter season has the highest amount of fucoxanthin (462.79 µg g^{-1}). HPLC results confirmed that all summer collected seaweeds had lower concentration of fucoxanthin compared to winter collection. As shown in Table 2, the physico-chemical properties of water were different in two sampling seasons.

Table 2: The measured physico-chemical parameters of seawater in the sampling sites at summer and winter.

Season	pH	Salinity (ppt)	Conductivity (ms cm^{-1})	Temperature (°C)
Summer	7.52	37.84	55	37.6
Winter	8.27	30.62	46	23.8

Antioxidant activity

According to FRAP method for investigation of antioxidant activity, the results are based on reduction power of Fe^{3+} complex to Fe^{2+} form, thus the reduction activities of samples were shown by µg Ascorbic acid equivalent/g (µg ASA g^{-1}) of extract dry weight (Ascorbic acid, a standard antioxidant was considered as the control). As shown in Fig. 5, the antioxidant activity of extract from *D.indica* (at winter) was higher than the other species and *I.stellata* showed the least activity. Antioxidant activities of all summer collected seaweeds were lower than the winter ones which were in accordance with their fucoxanthin contents. The decrease in reduction powers of the extracts at both seasons are as follows: *D.indica*, *I.stellata*, *P.tenuis* and *C. sinuosa*.

Antibacterial activity

The results of inhibition zone diameter of bacterial growth for extracts from four different species of brown seaweeds (*P. tenuis, C. sinuosa, I. stellata and D. indica*) collected at two seasons are shown in Table 3. According to this table, the highest inhibition zone diameter for both gram positive (*s. aureus*) and gram negative bacteria (*E. coli*) were recorded for *D.indica* extracts, 18.26 and 17.50 mm (winter) and 17.83 and 17.16 mm (summer).

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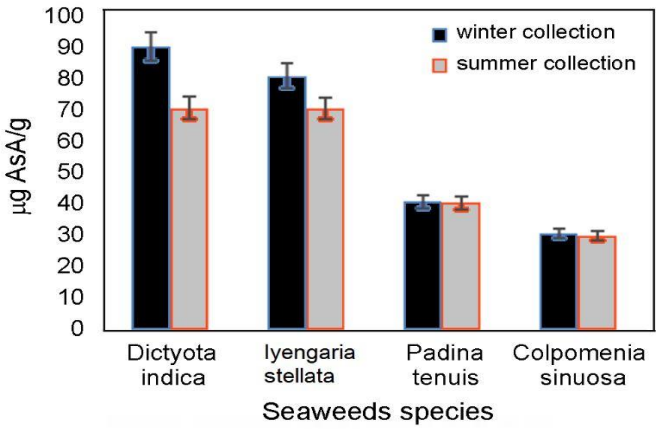


Figure 5: FRAP reduction activity (µg ASA g^{-1}) of extracts (concentration of 0.5 mg ml^{-1}) of four brown seaweeds species are expressed as mean ± standard deviation (n=3).

Table 3: Bacterial growth inhibition zone diameters (mm) of the studied algal extracts at winter and summer (means±SD) of 3 replicates.

Season	Bacterial strains	Seaweeds species			
		Dictyota Indica	***Iyengaria stellata***	***Padina tenuis***	***Colpomenia sinuosa***
Winter	*Staphylococcus aureus*	18.26 ± 0.40	16.83 ± 0.76	12.00 ± 0.50	13.30 ± 0.76
	Escherichia coli	17.50 ± 0.50	15.30 ± 1.04	11.50 ± 0.50	11.30 ± 1.02
Summer	*Staphylococcus aureus*	17.83±0.76	15.83±0.28	12.16±0.76	11.66±0.76
	Escherichia coli	17.16±0.76	15.33±0.28	11.83±0.76	11.5±0.57

The lowest inhibition zone diameters were seen for *C. sinuosa* for both strains and seasons, except to *S.aureus* in winter which was related to *P. tenuis.* Table 4 shows MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of extract from *P. tenuis*, *C. sinuosa*, *I. stellata and D. indica* species at winter and summer collection. The tested extract, especially extract from *D. indica* showed good MIC and MBC values in both seasons. No significant differences were observed between MIC, MBC values and different seasons.

Table 4 MIC and MBC values (mg ml^{-1}) of the studied algal extracts at winter and summer.

Season	Bacterial strains	Seaweeds species								
		Dictyota Indica		***Iyengaria stellata***		***Padina tenuis***		***Colpomenia sinuosa***		***Cefixim* (Control Antibiotic)**
		MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC (µg ml^{-1})**
Winter	*Staphylococcus aureus*	7.5	9.0	11.0	12.0	25.0	28.0	28.0	32.0	0.5
	Escherichia coli	8.0	9.0	12.0	12.0	30.0	30.0	32.0	32.0	4
Summer	*Staphylococcus aureus*	8.0	10.0	11.0	12.0	28.0	30.0	30.0	34.0	0.5
	Escherichia coli	8.0	11.0	12.0	12.0	30.0	30.0	32.0	32.0	4

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Discussion

Our results showed an efficient extraction procedure which released almost 90% of fucoxanthin and the HPLC analysis enabled the identification of fucoxanthin in a short time of 6 min. Fucoxanthin content in the studied seaweeds varied from 19.23 to 462.79 µg/g in winter and 13.53 to 210.72 µg/g in summer. It could clearly be seen that the amount of fucoxanthin in winter is higher than summer in all the studied species. It seems that the light exposure period and temperature alternation are two ecological factors that affected the amount of fucoxanthin in summer and winter (Nomura *et al*., 2013). It could be also concluded from previous research works that the growth rate of marine seaweeds and their bioactive contents depend on ecological aspects (sunlight, temperature, nutrients amount, etc) of their growth location (Campbell *et al*., 1999; Dean and Hurd, 2007; Terasaki *et al*., 2009). Fucoxanthin is a light harvester and acts as photo-protectant (Fung *et al.,* 2013). Attaran Fariman *et al*. (2015) studied the fucoxanthin content of two species of brown seaweeds of Oman Sea. They reported the fucoxanthin content of 0.56- 1.61 mg g for the brown algae, *Nizamuddinia zanardinii* and 2.33-3.35 mg g^{-1} for *Cystoseira indica.* The higher amount of fucoxanthin in winter (3.74 mg g^{-1}) and lower amount in summer (1.26 mg g^{-1}) was also reported for the brown algae, *Cystoseira hakodatensis* from the northern seashore of Japan (Nomura *et al*., 2013); and some other seaweeds at winter (Campbell *et al*., 1999; Dean and Hurd, 2007). These are in accordance with our findings that the fucoxanthin contents of all four species were lower in summer due to the high temperature of the seawater and long period of exposure to sunlight (approximately 16 h). Besides the environmental aspects (location and the season of harvesting) (Airanthi *et al*., 2011); many other factors like drying (Mise, 2011) and pigment extractions methods (Nisizawa *et al*., 1987); have been reported as the effective factors in the different observed amount of fucoxanthin from different seaweeds species. The reported fucoxanthin content in brown seaweeds, considering the sampling location, season of sampling and the extraction method (Jaswir *et al*., 2012; Kim *et al*., 2012; Xiao *et al*., 2012); varies significantly.

Biological properties

There is an increasing demand on using natural product as food (Senthil *et al*., 2013); and health products. Thanks to the proper environmental conditions of Qeshm Island in Persian Gulf, the study of antioxidant and antibacterial activities of some abundant brown seaweed for finding rich sources of fucoxanthin seemed to be of great importance. There are some reports concerning that the main reasons of antioxidant activity of seaweeds are total phenolic compounds and fucoxanthin content (Jiménez-Escrig *et al*., 2001; Airanthi *et al*., 2011; Novoa *et al*., 2011; Thomas and Kim, 2011; Wijesingher and Jeon, 2012). Similar to

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current study, there is a strong correlation of antioxidant activities with fucoxanthin content. Yan *et al*. (1999) investigated the antioxidant activity of several species of marine seaweeds and introduced fucoxanthin as the major component of the seaweeds causing the antioxidant activity (Yan *et al*., 1999). Studying the antioxidant activity and fucoxanthin content of diatoms (Xia *et al*., 2013); showed the same results. Furthermore, the antioxidant activity of brown seaweeds and fucoxanthin and its metabolite, fucoxanthinol were reported as the main reason of antioxidant activity in some brown seaweeds (Sachindra *et al*., 2007; Airanthi *et al*., 2011). Pirian *et al*. (2017) reported the antioxidant activity of some brown seaweed from Persian Gulf (*Sargassum boveanum, Sargassum vulgare, Sargassum angustifolium, Polycladia myrica* and *Sirophysalis trinodis*) which were approximately: 20, 70, 50, 60, and 58 µg ASA/g, lower than the measured antioxidant activities of the current studied seaweeds. In our study, the algal fucoxanthin containing extracts were tested against one gram positive and one gram negative bacteria, i.e *Staphylococcus aureus* and *Escherichia coli* and the results were expressed as inhibition zone diameter in millimeters. The inhibition zone was about 11-18 mm for *S.aureus* and 11-17 for *E. coli.* The inhibition zone for gram positive bacteria (*S. aureus*) of *D.indica* extract was higher than the other species, approximately 18 mm and the lowest inhibition zone diameter was approximately 13 mm for *C.sinuosa* extract. As it could be seen from Table 3, there is no significant difference in the antibacterial activity in different seasons. However, it seems that the difference in the antibacterial activities between different species of the studied brown seaweeds correlates with the fucoxanthin content of the algal extracts (Table 1). Of course, there are other bioactive metabolites in the extract rather than fucoxanthin that could be responsible for the observed antibacterial results. However, due to the employed extraction method, our extract contains higher amounts of fucoxanthin. Gonzalez Val *et al*. (2001) screened the antimicrobial properties of some red, green and brown seaweed extracts and found that the brown seaweeds (*Dictyota* sp.1 and sp. 2, *Padina pavonica* and …) had high antimicrobial activity (Inhibition zone: higher than 15 mm) (Gonzalez Val *et al*., 2001). The reported research works also show that bioactivity of seaweeds depend on different factors like seasonal variation, the extraction methods (Sreenivasa Rao and Parekh, 1981) and the extraction solvent (methanol, ethyl acetate, n-hexane and benzene or chloroform and etc.) (Rosell and Srivastava, 1987; Sastry and Rao, 1994; Febles *et al*., 1995). Of course, the antibacterial activity of seaweeds could be also related to the content of other bioactive metabolites like alkaloids, terpenoids, steroids, (Güven *et al*., 2010; Ornano *et al*., 2014), polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and

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carotenoids (Rodriguez *et al*., 2010; Priyadharshini *et al*., 2011).

In conclusion, the present study on four species of brown seaweeds i.e *Dictyota indica, Padina tenuis, Colpomenia sinuosa* and *Iyengaria stellata* from Queshm Island showed that their fucoxanthin content varies in different seasons and it is higher in winter which could be related to temperature variation and light exposure period. Besides, the algal extracts containing fucoxanthin showed rather good antioxidant and antibacterial activities especially in *Dictyota indica* which also contains the highest content of fucoxanthin. So, it could be considered as a potential candidate for further studies for introducing a rich source of fucoxanthin to the food and pharmaceutical industry. Furthermore, our study promotes the future research on the other abundant species of seaweeds and their bioactive metabolites in the region of Persian Gulf and Oman Sea.

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