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Seasonal variation of total lipid, fatty acids, fucoxanthin content, and antioxidant properties of two tropical brown algae (*Nizamuddinia zanardinii* and *Cystoseira indica*) from Iran

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Abstract The present study investigates the seasonal changes in lipid components, fucoxanthin (Fx), and functional long-chain n-3 (omega-3) polyunsaturated fatty acids (PUFAs) and also evaluates for their antioxidant activity of two tropical brown algae, *Nizamuddinia zanardinii* and *Cystoseira indica*, collected from the southeast coast of Iran. Results show a significant amount of fucoxanthin that is possibly due to the specific conditions of sampling area in terms of oxidative stress and the waves of southwest monsoon and thermal stress. Total lipid in October and November was higher compare to rest of the year. The percent of PUFA in *N. zanardinii* increased coinciding with a period of relative cold weather, but PUFA in *C. indica* increased in May when the weather is warm. However, in both species, seasonal variation in DPPH radical scavenging activity, CUPRAC, and total phenolic content did not show a particular pattern. A correlation between fucoxanthin content and DPPH radical scavenging activity was evident. Both algal species had higher phenolic content over the peak of maturity and fertility when the plant was tallest (October to December for both species and April for *N. zanardinii*). In general, variation of these factors in a tropical area such as Oman Sea is different from temperate regions. These changes in many cases are not as tangible, and further investigations are required to assess the variations.

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Introduction

Seaweed can be divided into the brown (Phaeophytes), green (Chlorophytes), and red seaweeds (Rhodophytes) (Dawczynski et al. 2007; Jaswir et al. 2013a). Red and brown algae have been used as human food sources and traditional medicine since ancient times (Dawczynski et al. 2007; Rohani-Ghadikolaei et al. 2012). Polyunsaturated fatty acids (PUFA), mostly omega 3 fatty acids and the carotenoid fucoxanthin, which are found in brown seaweeds, have a significant effect on human health (Haugan and Liaaen-Jensen 1994; Dembitsky and Maoka 2007; Miyashita et al. 2011; D'Orazio et al. 2012). Based on its unique molecular structure, fucoxanthin shows high biological activity, including antioxidant (Nomura et al. 1997; Heo and Jeon 2009; Sudhakar et al. 2013), anticancer (Kotake-Nara et al. 2001, 2005; Hosokawa et al. 2004; Jaswir et al. 2013a), and antidiabetic (Maeda et al. 2006; Hosokawa et al. 2010) activities. At present, the main source of n-3 PUFA is marine fish, however, considering reduction in marine fish stock, finding a new source of PUFA is necessary (Nomura et al. 2013). Several studies have reported that the amount of lipid in seaweed varies with respect to factors such as geographic location, season, temperature, salinity, and light intensity (Sánchez-Machado et al. 2004; Terasaki et al. 2009; Gerasimenko et al. 2010, 2011; Nomura et al. 2013).

Thirty-nine species of brown algae have been reported from Iranian coastal area of Persian Gulf and Oman Sea (Gharanjik 2005), but there are few studies on the nutritional value and bioactive compounds of brown algae from Iran (Tabarsa et al. 2012; Moghadam et al. 2013). In this study, we examined fluctuation of antioxidant properties, fatty acid,

and fucoxanthin content with seasons. *Nizamuddinia zanardinii* and *Cystoseira indica* are tropical brown algae distributed in southwest Asia including India, Oman, Qatar, Pakistan, Yemen, and Iran (Silva et al. 1996; Wynne and Jupp 1998; Sahoo et al. 2001; Abid et al. 2005; Gharanjik 2005). The main reason for choosing these two species for the present study is their high density and biomass in Iran's southeast coast (Abkenar et al. 2008) and also their great potential for mariculture. Cultivated *C. indica* in the tidal zone of Chabahar Bay, Iran, shows faster growth rate and higher biomass in comparison with some other algae in the area (Farahpour et al. 2010). Chabahar Bay is located in the southeast of Iran and northern part of the Oman sea (25° 17′ 28″ N, 60° 38′ 15″ E). The coastal area is an exposed area to strong ultraviolet radiation due to the location in a tropical area. This can cause the development of reactive radical species (ROS) and other strong oxidizing agents, and marine algae growing in such warm water change their metabolism to produce antioxidants as a defense (Zubia et al. 2007).

The aim of this study was to determine the seasonal variation of fucoxanthin, n-3, and n-6 PUFAs, total lipid, and the antioxidant potential in different seasons of the brown algae *N. zanardinii* and *C. indica* from Iran with regard to their potential for food and medicinal purposes.

Materials and methods

Two brown seaweed, *Nizamuddinia zanardinii* (Schiffner) P.C.Silva and *Cystoseira indica* (Thivy & Doshi) Mairh, samples were collected seasonally from Chabahar coast located in southeast of Iran and north part of Oman sea in 2013 and 2014. Immediately after collection, the seaweed samples were cleaned and washed with seawater to remove sand, debris, epiphytes, and other extraneous matter attached to the thalli and transported to the laboratory where they were frozen at −80 °C.

Total lipid content The methods of Nomura et al. (2013) with slight modification were used to determine total lipid. All extractions were carried out under dim light, and air in the extraction vessel was replaced with nitrogen to prevent possible degradation of carotenoids or lipids. Whole parts of the algae were sextracted with ten times (*w*/*v*) the amount of chloroform–methanol (1:2, *v*/*v*) at room temperature for 2 h. The solution was filtered, and this step was repeated two times. Ten times (*w*/*v*) the amount of chloroform–methanol–water (1:2:0.8, *v*/*v*/*v*) was added to the residue obtained by the second extraction. After extraction, all three filtrates were combined, and the solvent was removed under vacuum using a rotary evaporator. The dried extract was dissolved again with chloroform–methanol–water (8:4:3, *v*/*v*/*v*) in a separator funnel. After shaking the funnel vigorously, the lower layer was evaporated under vacuum in a rotary evaporator. The last traces of the remaining solvents and water were removed under high vacuum and a small amount of ethanol in order to obtain total lipids (TL). TL content was expressed as mg g^{-1} DW. This was calculated from fresh weight-based measurements of TL and the moisture content of the fresh samples. The moisture content was measured by oven drying the fresh sample at 105 °C (AOAC 1997) until a constant weight was obtained.

Analysis of Fucoxanthin content by HPLC

All HPLC analyses were carried out using a KNAUR Smartline system SYSS003 (Germany) equipped with a pump (Smartline Pump 1050), auto-sampler (Smartline Autosampler 3950 with sample cooling), and a photo diode-array spectrophotometric detector (Smartline UV Detector 2600 with fiber optics). Fucoxanthin content in the TL was determined by reversed phase HPLC (RP-HPLC) with methanol–acetonitrile (7:3 *v*/*v*) as the mobile phase at a flow rate of 1.0 mL min^{-1} (Terasaki et al. 2009). All RP-HPLC analyses were carried out at 28 °C using a C18 column (250×4.6 Nucl Eo DUR 100-5 C18). An aliquot of TL was dissolved in the mobile phase and filtered with a 0.22-μm membrane filter. Then, an aliquot of the filtered sample was submitted to HPLC analysis. The detection wavelength was 450 nm for detecting fucoxanthin (Jaswir et al. 2013b; Mori et al. 2004; Nomura et al. 2013; Terasaki et al. 2009). A calibration curve prepared using authentic standard was used for quantification. The standard fucoxanthin (>95 % purity) was isolated from *C. indica* lipids as outlined below.

The method described by Haugan et al. (1992) with slight modification was adopted for the extraction and purification of fucoxanthin. The ground dry algae were extracted several times with cold acetone–methanol (7:3 *v*/*v*) on ice bath and left standing at room temperature in darkness under N_2. The extract was evaporated to dryness at 30 to 35 °C on a rotary evaporator, and the residue was dissolved in methanol. The residue was partitioned in a separator funnel between *n*-hexane and 90 % aqueous methanol (*v*/*v*). The *n*-hexane phase was discarded, and the aqueous hypophase was transferred to diethyl ether. The extract is evaporated to dryness on a Rotavapor, and the pigments were dissolved in benzene and applied to a silica gel (Kiesel gel 60, Merck, Germany) column. Elution was initially with *n*-hexane (100 %), and fucoxanthin was recovered in the *n*-hexane/acetone fraction (6:4 *v*/*v*). Column chromatography was repeated two or three times. The residue from the hexane/acetone (6:4 *v*/*v*) evaporation step was re-dissolved in methanol. The concentration of fucoxanthin was checked by HPLC (1 mL min^{-1} at 450 nm, methanol, and acetonitrile were the mobile phase). Purified fucoxanthin was determined by Fourier transform infrared spectroscopy (FT-IR). The characteristic wave number of specific functional group identified in purified sample was: OH

group (3400 cm^{-1}), C–H stretch (3005–2850 cm^{-1}), allene (1924 cm^{-1}), C═O acetate (1732 cm^{-1}), conjugated C═O (1645 cm^{-1}), CH_2 stretch (1605–1455 cm^{-1}), geminal methyl (1380–1360 cm^{-1}), C–O acetate (1338, 1258, and 1249 cm^{-1}), and *trans*-distributed –C═C– (1210–963 cm^{-1}) which agreed with the previous reported data (Haugan et al. 1992; Rajauria and Abu-Ghannam 2013).

Fatty acid analysis of total lipids

Fatty acid methyl esters were prepared as per the method of Prevot and Mordret (1976). Briefly, 1 mL *n*-hexane and 0.2 mL 2 N sodium hydroxide in methanol were added to an aliquot of total lipid (20 mg), vortex for 10 s, and incubated at 50 °C for 30 min. After incubation, 0.2 mL of 2 N HCl in methanol was added to the solution and vortex for 60 s to obtain the upper *n*-hexane layer containing the fatty acid methyl esters (FAMEs). The fatty acid analysis was by gas chromatography (GC) (Shimadzu) using a flame ionization detector (FID) and a capillary column (BPX-70 25 M×0.22 MM 0.25 μM). The carrier gas was nitrogen at a flow rate of 1.3 mL min^{-1}. The detector, injector, and column temperatures were 280, 250, and 25 °C, respectively.

Antioxidant extraction and analysis

Because different components in the crude extracts of seaweeds, antioxidant activities depend on different reactions and mechanisms. Therefore, in this study, three methods were employed to evaluate the total antioxidant activity. All antioxidant extractions were carried out away from direct sunlight to reduce the possibility of oxidation. Methanol (15 mL) was added to the sample (300 mg) and homogenized for 1 h at room temperature. The methanol extract was evaporated to dryness at 30 °C using a rotary evaporator, and the residue was dissolved in methanol (20 mL).

Analysis of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay The scavenging effects of methanol extract were determined using the method of (Duan et al. 2006). Briefly, 2.0 mL of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 2.0 mL of sample and incubated for 30 min in the dark at room temperature. The absorbance of samples was measured at 517 nm and using methanol as the blank. The scavenging effect (%) was calculated as:

$$\text{Scavenging}\ (\%) = \left[1-\left(A_{\text{sample}}-A_{\text{sample blank}}/A_{\text{control}}\right) \times 100\right]$$

where A_{sample} is the absorbance of the sample with treatment, $A_{\text{sample blank}}$ is the absorbance of sample only without DPPH, and A_{control} is the absorbance of methanol with the treatment.

Copper-reducing antioxidant capacity (CUPRAC) The CUPRAC reagent was prepared according to the method of Apak et al. (2004). Briefly, 1 mL sample and 0.1 mL distilled water were added to a test tube containing 1 mL of copper (II) chloride solution (10^{-2} M), neocuproine (Nc) solution (7.5×10^{-3} M), and ammonium acetate (NH_4Ac) buffer at pH 7.0 to make up a final volume of 4.1 mL. After shaking, the test tube was left in the dark for 30 min and the absorbance measured at 450 nm using distilled water as the blank.

Total phenolic content Distilled water (50 mL) was added to dried samples (2 g) and autoclaved at 121 °C for 15 min. The extract was centrifuged (2220×*g* for 10 min) and filtered. The total phenolic content of algal extracts, prepared as above, was determined according to the method of Chaovanalikit and Wrolstad (2004). To 0.5 mL aliquot of algal extract, 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of distilled water were added. After 10 min, 1.5 mL of 20 % Na_2CO_3 solution was added. The sample was thoroughly mixed and placed at room temperature for 1 h. Absorbance was measured at 755 nm, and gallic acid was used for the preparation of the standard curve.

Results

Figure 1 shows photographs of *N. zanardinii* and *C. indica*. Observations of the algae at different seasons showed that *N. zanardinii* has two peaks of productivity over the period late October to late November and late March to late April when the plants were tallest. On the other hand, *C. indica* only has one peak during late October to late November. In the late September, juvenile algae are dominant while in late October and November, adult spore-bearing plants are present and with sporulation begin to reproduce. With the warm weather in July, *C. indica* was not found and the density of *N. zanardinii* was low.

Table 1 shows the changes in total lipid (TL) and fucoxanthin (Fx) contents (mg g^{-1} DW) of *C. indica* and *N. zanardinii*. TL content of *N. zanardinii* in February is significantly ($p<0.05$) higher than in June ($p<0.05$), and in *C. indica*, the highest TL content was in December (86.1±6.50 mg g^{-1} DW) and the lowest in February (69.12±5.20 mg g^{-1} DW) respectively. The content of Fx in *C. indica* in all seasons was higher than in *N. zanardinii* ($p<0.05$). The highest value of Fx in *N. zanardinii* was recorded in June (1.65±0.17 mg g^{-1} DW), and in *C. indica*, it was recorded in December (3.56± 0.20 mg g^{-1} DW).

In the TL of *C. indica* and *N. zanardinii*, the major fatty acids were myristic (14:0), palmitic (16:0), oleic (18:1n-9), SDA (18:4n-3), AA (20:4n-6), and EPA (20:5n-3) (Tables 2 and 3). Among them, 16:0 was found in all the samples at highest concentration, followed by oleic acid, AA, SDA, myristic acid, and EPA. Variations of total saturated fatty acids

Fig. 1 Wild *N. zanardinii* (*left*) and *C. indica* (*right*) collected from Chabahar coast in Iran in October 2013

(SFAs), total monounsaturated fatty acids (MUFA), total n-3 PUFAs, and total n-6 PUFAs in two studied species in 4 months are shown in Tables 2 and 3. The percentage of total PUFA in *N. zanardinii* (25.95 %) in February and in *C. indica* (37.91 %) in April was significantly higher than other seasons ($p<0.05$). The higher level of PUFAs in April was more in the TL of *C. indica* (Tables 2 and 3).

The results of DPPH radical scavenging activities are shown in Table 4. The highest and lowest scavenging activity for *N. zanardinii* was in June (96.93±1.41 %) and the lowest (85.7±1.63 %) in October; for *C. indica*, the highest was in December and April (89.23±1.81 %) and the lowest (73.96± 3.02 %) in April.

C. indica had a higher CUPRAC than *N. zanardinii* in the methanolic extracts (Table 4). The CUPRAC assay showed that *N. zanardinii* has high antioxidant activity in June (1.08± 0.11) and *C. indica* in December (1.26±0.06).

Phenolic content in *N. zanardinii* was high in April (Table 4) ($p<0.05$), with a value of 1.52±0.06 mg g^{-1} DW), but in *C. indica*, it was lower than in other months (0.86± 0.03 mg g^{-1} DW). In both algae, phenolic content was low in February. In general, phenolic content of *N. zanardinii* was higher than in *C. indica* in all months (Table 4).

In the 5 months of data from *N. zanardinii* and *C. indica* in Tables 3 and 4, fucoxanthin showed a closer relation with DPPH scavenging activity than with the CUPRAC assay results and total phenolic content; correlation coefficients were 0.81 and 0.918 respectively for the two studies (Table 5).

Discussion

The lipid contents of seaweed ranged from 1 to 5 % of dry matter and varies strongly according to the species (Pise and Sabale 2010; Terasaki et al. 2009; van Ginneken et al. 2011), geographical location (Nomura et al. 2013), and season (Gerasimenko et al. 2010; Nomura et al. 2013; Terasaki et al. 2009). Tropical seaweed species have significantly lower total lipid than those from cold regions (Narayan et al. 2004). Lipid analysis of the Sargassaceae, from the subarctic zone (Terasaki et al. 2009), showed higher total lipid in contrast with tropical zone (Narayan et al. 2004; Rohani-Ghadikolaei et al. 2012). However, many researchers have reported high level of total lipids in brown algae from tropical areas (Gosch et al. 2012; McDermid and Stuercke 2003; Thinakaran et al. 2012). High levels of total lipid in the present study with seasonal change also were observed (3.1 to 7.9 % of dry weight for *N. zanardinii* and 7.2 to 8.6 % of dry weight in *C .indica*). This variation in *C. indica* showed less change because of the disappearance of the algae and the lack of sample for analysis.

It is documented that, in temperate regions, lipid formation directly changes with season and increases in the winter. Total lipid content of brown algae increases from winter to spring in temperate and subarctic zones of the North Pacific coinciding with seedling growth (Honya et al. 1994; Nelson et al. 2002; Gerasimenko et al. 2011). In the present study, the total lipid increased especially in October and December when seedlings and juvenile algae are predominant (Table 1).

Table 1 Moisture content (%), total lipid (mg g^{-1} DW), fucoxanthin content (mg g^{-1} DW), FX_{TL} (as percentage of total lipids) of *N. zanardinii* and *C. indica*, and temperature (°C) of sampling area

	Nizamuddinia zanardinii				*Cystoseira indica*				
	MC	TL	FX	FX_{TL}	MC	TL	FX	FX_{TL}	Temp
29 June 2013	70.01	39.3±3.95a	1.65±0.17a	4.19	–	–	–	–	34.1
12 Oct 2013	77.38	79.88±15.53b	0.81±0.10cde	1.01	81.52	78.85±7.99	3.31±0.26ab	4.19	27.77
7 Dec 2013	71.36	60.3±7.21c	0.87±0.12c	1.44	83.88	86.1±6.50	3.56±0.20a	4.13	23.3
4 Feb 2014	75.66	71.02±10.16bc	0.56±0.13e	0.78	80.38	69.12±5.20	2.63±0.60bc	3.80	21.1
21 April 2014	74.05	67.07±10.53bc	1.37±0.10b	2.04	79.23	72.97±5.65	2.33±0.45c	3.19	25.83

All the values are mean±standard deviation of three samples. Significant differences ($p<0.05$) with months for *N. zanardinii* and *C. indica* are indicated by different lowercase letters

Table 2 Fatty acid composition (weight % of total fatty acids) of *N. zanardinii* in different months

21 April 2014	4 Feb 2014	7 Dec 2013	12 Oct 2013	29 June 2013	Fatty acid	Peak
0.82±0.03	0.41±0.02	0.62±0.05	1.30±0.02	0.38±0.01	C12:0	1
4.82±0.04	4.59±0.12	4.69±0.13	4.33±0.32	5.08±0.16	C14:0	2
0.61±0.02	0.68±0.02	0.70±0.10	0.48±0.04	0.58±0.08	C15:0	3
44.76±0.50	39.94±0.10	41.29±0.41	43.41±0.11	41.08±0.01	C16:0	4
4.24±0.05	3.00±0.14	3.16±0.19	3.30±0.27	2.97±0.16	C16:1n-7	5
0.93±0.02	1.34±0.07	1.21±0.02	1.14±0.06	0.14±0.05	C18:0	6
21.44±0.08	20.40±0.12	22.32±0.22	18.91±0.12	25.08±0.16	C18:1n-9	7
2.39±0.15	2.77±0.21	2.57±0.03	4.31±0.25	2.83±0.30	C18:2n-6	8
1.10±0.07	2.56±0.09	3.06±0.18	0.68±0.11	1.00±0.03	C18:3n-3	9
3.49±0.11	4.13±0.30	4.38±0.10	4.22±0.32	6.71±0.26	C18:4n-3	10
0.46±0.02	0.66±0.03	0.66±0.05	0.55±0.06	0.52±0.04	C20:0	11
0.79±0.02	1.83±0.07	1.19±0.17	1.85±0.06	1.08±0.04	C20:1n-9	12
10.43±0.10	11.78±0.27	9.99±0.18	10.10±0.02	8.08±0.17	C20:4n-6	13
0.76±0.02	0.75±0.01	0.72±0.03	0.62±0.03	0.51±0.08	C22:0	14
0.41±0.01	0.55±0.02	0.34±0.08	0.69±0.07	0.37±0.05	C22:1n-9	15
2.18±0.05	3.97±0.04	2.60±0.12	3.71±0.12	2.85±0.05	C20:5n-3	16
0.81±0.01	1.06±0.08	0.66±0.09	0.44±0.06	1.03±0.03	C22:4n-6	17
52.89 a	48.34 e	50.27 c	52.06 b	48.29 de	Total SFA	
26.95 b	25.72 d	26.67bc	24.39 e	29.44 a	Total MUFA	
20.16 e	25.95 a	23.05 c	23.55bc	22.75 d	Total PUFA	
6.69 e	10.34 b	9.92 c	8.84 d	10.38abc	n-3 PUFA	
13.47 c	15.61 a	13.13 d	14.71 b	11.89 e	n-6 PUFA	
2.01	1.50	1.32	1.66	1.14	n6/n3 PUFA	

Data are mean values of triplicate samples±SD. Different lowercase letters in the same column indicate significant differences in mean ($p<0.05$)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Fucoxanthin is a major carotenoid present in brown seaweeds and, along with chlorophylls and β-carotene, plays an important role in light harvesting, photoprotection during excess light, and upregulation of photosynthesis (D'Orazio et al. 2012; McKew et al. 2013). The different fucoxanthin contents in different seasons are mainly due to light exposure and temperature fluctuations (Nomura et al. 2013). According to the results in Table 1, fucoxanthin content *in N. zanardinii* and *C. indica* is significantly higher than other species from tropical areas (Nomura et al. 2013; Lann et al. 2012; Jaswir et al. 2013a; Fung et al. 2013; Terasaki et al. 2009). It is likely that fucoxanthin in the studied species not only has a light harvesting role but also acts as a strong photoprotectant. In these algae, high light exposure with high oxygen concentration probably leads to formation of free radicals and other strong oxidative stress. Therefore, these conditions lead to the algae producing essential antioxidant compounds, especially in this tropical area, to protect them against oxidative stress such as UV radiation. There are many studies on the antioxidant properties of fucoxanthin against oxidative stress caused by UV-B radiation, scavenging of free radicals, and also quenching effect of singlet oxygen (Heo and Jeon 2009; Sudhakar et al. 2013; Nomura et al. 1997; Sachindra et al. 2007).

Sampling areas in the present study were rocky shores and tidal pools with high waves where organisms are exposed to many factors including sediment scouring, strong wave action, nutrient loading, herbivores, heat and desiccation, and high radiation exposure in some seasons. Organisms living in the mid- and high-shore areas of tropical rocky shores experience a greater degree of heat and desiccation in comparison with temperate shores (Macusi and Deepananda 2013). The higher content of fucoxanthin in June for *N. zanardinii* (1.65±0.17 mg g^{-1} DW) can be due to the presence of strong wave action of the southwest monsoon of the Indian Ocean (SWM) and, consequently, creation of reactive oxygen species and thermal and light stress.

The major fatty acids of the total lipids of *N. zanardinii* and *C. indica* were myristic acid, palmitic acid, oleic acid, C18, and C20 PUFA as in other brown seaweeds (Dawczynski et al. 2007; Hofmann and Eichenberger 1997; Khotimchenko 1998; Kumari et al. 2010; Li et al. 2002; Sánchez-Machado et al. 2004) (Tables 3 and 4). The fatty acid composition of

Table 3 Fatty acid composition (weight % of total fatty acids) of *C. indica* in different months

21 April 2014	4 Feb 2014	7 Dec 2013	12 Oct 2013	Fatty acid	Peak
0.39±0.03	0.28±0.04	0.92±0.08	1.32±0.09	C12:0	1
4.23±0.07	4.90±0.81	4.09±0.18	3.78±0.15	C14:0	2
0.28±0.07	0.59±0.07	0.41±0.05	0.35±0.04	C15:0	3
30.53±0.76	38.54±0.13	38.84±0.09	36.09±0.06	C16:0	4
3.79±0.10	4.23±0.07	4.53±0.12	4.92±0.07	C16:1n-7	5
0.67±0.05	1.51±0.09	0.77±0.06	0.59±0.02	C18:0	6
11.10±0.06	12.23±0.05	16.16±0.13	13.90±0.11	C18:1n-9	7
4.58±0.14	4.84±0.17	5.50±0.12	6.46±0.08	C18:2n-6	8
1.77±0.04	3.22±0.12	3.46±0.06	1.58±0.13	C18:3n-3	9
11.09±0.07	5.21±0.08	6.91±0.12	8.12±0.19	C18:4n-3	10
0.43±0.07	0.73±0.11	0.47±0.07	0.45±0.01	C20:0	11
8.20±0.07	3.29±0.02	3.57±0.17	5.43±0.16	C20:1n-9	12
13.81±0.14	12.50±0.09	10.53±0.10	12.60±0.15	C20:4n-6	13
0.34±0.04	0.49±0.09	0.60±0.03	0.66±0.03	C22:0	14
1.38±0.05	1.24±0.07	1.07±0.06	1.27±0.07	C22:1n-9	15
6.76±0.12	2.58±0.07	2.24±0.04	2.75±0.19	C20:5n-3	16
0.05±0.01	0.02±0.01	0.07±0.01	–	C22:4n-6	17
38.57 d	47.59 c	46.01bc	43.24 a	SFA Total	
c24.52	b24.01	25.33 a	25.52 a	MUFA Total	
37.91 d	28.39 c	28.71bc	31.51 a	Total PUFA	
19.62 a	11.01 d	12.61 b	bc12.45	n-3 PUFA	
18.44 b	17.36 c	16.1 d	19.09 a	n-6 PUFA	
0.93	1.57	1.27	1.53	n6/n3 PUFA	

Data are mean values of triplicate samples±SD. Different lowercase letters in the same column indicate significant differences in mean ($p<0.05$)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

seaweeds is sensitive to temperature (Khotimchenko 1998; Nomura et al. 2013; Sewón et al. 1997). A decrease in temperature usually results in an increase of polyunsaturated fatty acid (PUFA) and a decrease of saturated fatty acid (SFA) (Nomura et al. 2013). A sma increase in PUFA in the cold season in *N. zanardinii* was observed (Table 3), but a remarkable point in this study is the higher amount of PUFA in *C. indica* than in *N. zanardinii*, especially in April (37.91 %). However, Kumari et al. (2010) reported that the amount of PUFA in *C. indica* is 47.5±2.71 % which is comparable with temperate area species. This difference may be due to temperature differences at the different locations.

In addition, nutritionists and nutrition standards suggest a ratio of omega-6 to omega-3 essential fatty acids of 1:1.15 to 1:2 (Hamazaki and Okuyama 2003), whereas in the western diet this ratio is between 15:1 and 16.7:1 (Simopoulos 2002). In this study, the n-6:n-3 ratio for *N. zanardinii* is between 2.01:1 and 1.14:1, which indicates an acceptable level for

Table 4 DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) radical scavenging assay (%), CUPRAC assay (absorbance), and total phenolic content (mg g^{-1} DW) of *N. zanardinii* and *C. indica* in different months

	Nizamuddinia zanardinii			*Cystoseira indica*		
	DPPH	CUPRAC	TPC	DPPH	CUPRAC	TPC
29 June 2013	96.93±1.41a	1.08±0.11a	1.2±0.04a	–	–	–
12 Oct 2013	85.7±1.63b	0.86±0.04bc	1.3±0.08ac	88.66±1.45a	1.04±0.56a	1.3±0.06a
7 Dec 2013	87.83±2.07bc	0.92±0.03ab	1.1±0.05ab	89.23±1.81a	1.26±0.06a	1.0±0.05bc
4 Feb 2014	96.16±1.04a	0.9±0.04b	1.0±0.07b	82.90±2.66b	1.01±0.15ab	0.9±0.03c
21 April 2014	93.1±4.03ac	0.88±0.05c	1.5±0.06c	73.96±3.02c	0.72±0.14b	0.8±0.03c

All the values are mean±standard deviation of three samples. Significant differences ($p<0.05$) with months for *N. zanardinii* and *C. indica* indicated by different lowercase letters

Table 5 Correlation coefficients of the fucoxanthin contents with the antioxidant activity

	Nizamuddinia zanardinii			*Cystoseira indica*		
	DPPH	CUPRAC	TPC	DPPH	CUPRAC	TPC
FX	0.81	0.47	0.3	0.918	0.8	0.46
DPPH		0.35	0.03		0.85	0.95
CUPRAC			0.04			0.21

use as an additive in the human diet for balancing the ratio of omega 3 and omega 6 fatty acids.

In both species, we observed a slight monthly variation of total phenolic content but with a different pattern. Seasonal phenolic contents measured in this study are in good agreement with previous studies, with maximum levels accruing in different seasons (Kamiya et al. 2010; Mannino et al. 2013; Plouguerné et al. 2006; Steinberg 1995). Total phenolic contents are generally affected by different abiotic factors such as temperature, irradiance levels, nutrient availability and salinity, or biotic factors such as grazing pressure and the reproductive state of the algae (Airanthi et al. 2011; Kamiya et al. 2010; Mannino et al. 2013; Steinberg 1995; Stiger et al. 2004). Algae past their peak of maturity and fertility show higher phenolic content (Steinberg 1989; Stiger et al. 2004). According to our results, both algal species displayed higher phenolic content over these peaks when the plant was tallest (October to December for both species and April for *N. zanardinii*). In this study, *N. zanardinii* had two peaks of maturity and fertility which is agreement with a previous study for *N. zanardinii* at the Socotra Archipelago off Yemen (Kemp 1998). Pavia et al (1997) reported that increased UV-B radiation led to an increase in phlorotannins in *Ascophyllum nodosum*. Furthermore, in plant tissue, phenolic compound synthesis increases with increase of water temperature (Aquino-Bolaños and Mercado-Silva 2004). Ragan and Jensen (1978) reported that in *Fucus vesiculosus*, there is a high correlation between surface water temperature and phenolic content. Moreover, phenolic content in nutrient-rich waters is increased (Arnold et al. 1995; Stiger et al. 2004). In the present study, the higher amount of phenolic content is in June, possibly due to increased water temperature and increased nutrients caused by the summer monsoon and the existence of high waves in this area.

The major active compounds in brown algae extract detected with DPPH radical scavenging assay is polyphenol and fucoxanthin (Airanthi et al. 2011; Ganesan et al. 2008). There is a synergic relationship between antioxidant activity of brown seaweeds and the combination of sucoxanthin and phenolic content (Airanthi et al. 2011; Fung et al. 2013). In this study, a relationship between fucoxanthin content and DPPH radical scavenging activity was evident with correlation coefficient of (r^2=0.81) for *N. zanardinii* and (r^2=0.91) for *C. indica*. In CUPRAC assay, the correlation with fucoxanthin was reasonable in *C. indica* (r^2=0.8). Similarly, the correlation of fucoxanthin content of *Undaria pinnatifida* with CUPRAC assay gave a correlation coefficient of 0.752 (Fung et al. 2013), indicating that our results are comparable with previous reports for brown algae.

Phlorotannins, the largest group of phloroglucinol-based polyphenols that are accumulated only in marine brown algae, suggested that they were the most likely active component responsible for the main antioxidant activity in brown seaweeds (Li et al. 2011). They exist in various types of different molecular size and degree of polymerization, and this may influence the antioxidant activity (Wang et al. 2012). In this study, one of the reasons for different antioxidant levels in different months between DPPH radical scavenging, CUPRAC, and Folin assays could be due to the structural variations in the phenolic compounds and the selectivity of each assay. The Folin assay measures only hydrophilic antioxidants, and DPPH radical scavenging is a solvent dependent assay, whereas the CUPRAC assay is more selective and able to simultaneously assay lipophilic and hydrophilic antioxidants (Apak et al. 2008).

CUPRAC assay values are comparable to ABTS/TEAC and Folin assay values for polyphenols, but simple sugars and citric acid are not oxidized in CUPRAC (Badarinath et al. 2010; Prior et al. 2005). Çelik et al (2008) reported that the correlation between total phenolic content and CUPRAC assay in herbal tea gave a correlation coefficient of 0.966, showing that total phenolic content and CUPRAC correlated well in herbal plant extracts. The negative correlation between TPC and CUPRAC requires further investigation to better understand this variation apart from the fucoxanthin and polyphenol contents in brown algae.

Our present study is the first report on the isolation, purification, identification, and seasonal change of bioactive compounds, fucoxanthin, fatty acids, and also the antioxidant potential of the tropical marine brown algae, *N. zanardinii* and *C. indica*, with significant pharmaceutical, medicinal, cosmetic, nutraceutical, food, and agricultural importance from the Chabahar coast of Iran.

In a general, brown algae have important bioactive compounds such as fucoxanthin, SDA, omega 3 EPA, and omega 6 ARA. Among these compounds, fucoxanthin is the key for better functional understanding of brown algal lipid features. This study has also demonstrated that fluctuation in the fatty acid and fucoxanthin contents cannot be attributed to specific environmental changes.

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