Antioxidant activity in some Iranian seaweed species from Chabahar

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Abstract

The antioxidant activities of *Ulva faciata*, *Nizimuddinia zanardinii* and *Gracilaria corticata* were investigated in Chabahar, Iran. Methanol extract and n-hexane, dichloromethane and ethylacetate fractions used for antioxidative properties test by the total antioxidative activity, 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) free radical scavenging activity, reduction power, metal chelating activity and inhibition of lipid peroxidation methods. The methanolic extracts of three seaweed species showed lower antioxidative properties. The most effective antioxidant properties were observed from the EA fractions of *G. corticata* and *U. faciata*. dichloromethane (DCM), fraction of *N. zanardinii* showed significantly higher total antioxidative activity, DPPH radical scavenging and power reduction in comparison to its n-hexane, EA fractions and crude MeOH extract. There was a strong correlation between the reduction power (r^2=0.94) and the total phenolic content of the seaweeds extracts and fractions. The results indicate *U. faciata* and *G. corticata* can be potential sources of natural antioxidants and may be efficiently used as nutraceuticals.

Keywords: Antioxidative activity, Phenolic contents, *Ulva faciata*, *Nizimuddinia zanardinii*, *Gracilaria corticata*

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Introduction

Polyunsaturated fatty acids are susceptible to oxidation in free radical chain reactions (Hsieh and Kinsella, 1986). The radical chain reaction can be inhibited by various antioxidants. Antioxidants are agent for preserving food quality by prevention of oxidative deterioration of lipids and proteins. There is an increasing interest for natural antioxidants because of the safety problems of synthetic antioxidants, such as butylated hydroxyl anisol and butylated hydroxyl toluene (Amarowicz *et al*., 2000). Also natural antioxidants can protect the human body from reactive oxygen species and free radicals, and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food (Kinsella *et al*., 1993). In the body, oxidation of biomolecules such as DNA, proteins, and lipids is linked with the onset and progression of diabetes (Sheik-Ali *et al*., 2011), cancer, and heart disease (Salvatore *et al*., 2005). It has therefore been suggested that consuming foods riched by antioxidants can prevent diseases (Hart *et al*., 2014). Over the last two decades, a wide range of phytochemicals from terrestrial food plant materials have already been evaluated, but less attention has been given to the study of marine seaweeds for the antioxidative activities (Yangthong *et al*., 2009). Seaweeds bioactive substances have great chance to be used as antioxidants and antitumor drugs (Bocanegra *et al*., 2009).

Marine algae are rich source of bioactive compounds (Zubia *et al*., 2009), hence a documented antioxidant activity of these seaweeds for use in food and pharmaceutical supplements is necessary. In recent years, some marine algae extracts have been demonstrated to have strong antioxidant properties (Nagai and Yukimoto, 2003), but there are no more unfaling publication on the antioxidant activities of seaweeds extract from southern coast of Iran (e.g. Chabahar).

The Iranian seaweed species belong to 150 genera, 32 families and 15 orders. The most edible consuming of green, red and brown algae such as *Ulva faciata* (green algae), *Nizimuddinia zanardinii* (brown algae) and *Gracilaria corticata* (red algae) in the south of Iran is pickling and soup production, but the reports on the antioxidant activities of Iranian seaweeds are rare.

Therefore, the aim of the present study was to evaluate the antioxidant capacity of the extract from these seaweeds, in vitro. The alcoholic extracts of these algae were prepared and examined for antioxidant activity using DPPH scavenging, total antioxidant activity, metal chelating activity, reduction power and inhibition of lipid peroxidation assays. Their total phenolic contents measured using the Folin-Ciocalteu's method. The correlation between the total phenolic contents and antioxidant capacities of the samples were also investigated.

Materials and methods

The three seaweed species investigated in this research include *Ulva faciata, Nizimuddinia zanardinii* and *Gracilaria corticata* were collected from the coasts of Chabahar Bay in autumn 2013. A random selection of different plants was taken from the shore, packed in cool boxes and transported immediately to the laboratory. Samples were washed to remove sand and epiphytes and then stored at −18°C. Samples were freeze-dried then ground into a powder using a blender and stored in vacuum-packed bags at −80°C prior to extraction.

One hundred gram of dried sample was suspended in methanol for 72 h. The solution was filtered through Whatman No. 1 filter paper and the pooled filtrate was concentrated in rotary vacuum evaporator (50°C), then partitioned successively with n-hexane (150 mL × 3), DCM (150 mL × 3) and EA (150 mL × 3), concentrated in *vacuo* to furnish n-hexane, DCM, and EA fractions, respectively.

The total phenolic content was estimated as gallic acid equivalents (GAE), according to Folin–Ciocalteu reagent as described by Karagözler *et al*. (2008), with slight modifications. A 30 µL aliquot of each sample (1.0 mg mL^{-1} ethanol) was added to 4.6 mL deionized water and 0.1 mL Folin–Ciocalteu reagent, and the contents were thoroughly mixed. After 3 min, 0.3 mL of 2% sodium carbonate solution was added, and the mixture was mixed thoroughly. The control contained all the reaction reagents except the sample. After 2 h incubation under medium agitation, the absorbance was measured at 760 nm using a spectrophotometer and compared to a gallic acid calibration curve. Total phenolics were determined as GAE.

Total antioxidant activity (TAC) was determined according to Prieto *et al*. (1999). Briefly, the extract (1 mg mL^{-1}) was mixed with 3.0 mL reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 min in a water bath. The absorbance was measured at 695 nm. Ascorbic acid used as standard.

The free radical scavenging activity of all extracts was evaluated by DPPH. In brief, 0.1 mM of DPPH solution in methanol was prepared, and 1 mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125, 250, 500 and 1000 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. α-Tocopherol was used as the reference. The capability of scavenging the DPPH free radical was calculated by the following formula:

DPPH scavenging effect (% inhibition) $= (A_0 - A_1) \times 100/A_0$

where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference.

Reducing power of extracts obtained from seaweeds was determined by Lim

et al. (2007) method with some modifications. Briefly, 1.0 mL of extracts/fractions (1 mg/mL in MeOH) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The reaction mixture was incubated at 50ºC for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged (6000×g) for 10 min. Two and half mL of the supernatant was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance of all sample solutions was measured at 700 nm.

The iron ion-chelating activity was determined by the method of Dinis *et al*. (1994). Briefly, an aliquot (1.0 mL) of each sample was mixed with 0.05 mL $FeCl_2$ (2.0 mmol/l), 0.2 mL ferrozine (5.0 mmol/L) and 2.75 mL distilled water. The mixture was shaken vigorously at room temperature in the dark for 10 min, and the absorbance of the iron ions–ferrozine complex at 562 nm was measured. EDTA was used as the positive control. The ability of sample to chelate iron ions was calculated using the following equation: Chelating activity (%) = $[1 – (A_{sample} - A_{blank}) / A_{control}] \times 100$

Here, $FeCl_2$ solution substituted by distilled water was used as a blank, and the sample substituted by distilled water was used as a negative control.

Lipid peroxidation inhibition was determined by acid thiobarbituric reaction using egg yolk as an oxidable substrate based on the Alves *et al*. (2012) method with some modifications. The system was generated with 0.25 mL homogenized egg yolk in 10% PBS (0.2 M; pH 7.4), 0.025 mL $FeSO_4$ 0.07 M (to start lipid peroxidation), and 0.25 mL of algal extract/ fraction (2 mg/mL). The mixture was incubated at 37°C for 30 min. After incubation, 0.75 mL 20% (v/v) trichloroacetic acid and 0.75 mL 0.8% (w/v) thiobarbituric acid were added. Then the mixture was shaken and heated at 100°C for 15 min and centrifuged at 2,000×g for 10 min and measured at 532 nm. Percentage of inhibition of lipid peroxidation was expressed at the inhibition rate (%) = $[1- (A_{sample} / A_{positive\ control})] \times 100$, where A_{sample} and $A_{positive\ control}$ refers to sample and positive control absorbance (sample absence), respectively.

One-way analysis of variance (ANOVA) was used by the Statistical Program for Social Sciences (SPSS, USA, ver. 19.0) to assess for any significant differences between the means. Differences between means at the 5 % (p<0.05) level were considered significant.

Results

The yields of total methanolic extract and fractions of three seaweeds are given in Table 1. Extractants have an impact on the yield. Among the total methanolic extracts of three seaweeds, *U. faciata* exhibited higher yield followed by *G. corticata* and *N. zanardinii*. Among the different solvent fractions, the highest yield was observed in n-hexanic fraction, whereas

the lowest was for the DCM fraction. The higher yield of n-hexanic fractions compared to the other two fractions of the same species showed that most of the compounds in these seaweeds were low in polarity and fat-soluble.

The phenolic contents in total methanolic extract (Table 2) were significantly dfferent between species (p<0.05). Result shows that the total phenolic content of the *G. corticata* and *U. faciata* methanolic extracts were significantly higher than the *N. zanardinii*. EA fraction of *U. faciata* and *G. corticata* showed higher phenolic content of 9.12 and 7.61 mg GE/g of seaweed extract, respectively (Table 2), when compared to other fractions and MeOH extracts.

Total antioxidant activity of the total methanolic extract and fractions of three seaweeds is presented in Table 3. Higher activity was observed in EA fraction of *U. faciata* and *G. corticata*, and DCM fraction of *N. zanardinii*. Total antioxidant activity of total methanolic extracts was significantly different between these three seaweeds (p<0.05).

The scavenging activities of DPPH free radicals are shown in Table 4. Total methanolic extract from *G. corticata* showed significantly higher scavenging activity (p<0.05) in comparison with the other two species (Table 4). Among the fractions of *U. faciata*, EA fraction had higher activity (p<0.05) as compared to other fractions of the same species. Similar results were also seen in case of EA fraction of *G. corticata* and DCM fraction of *N. zanardinii*. Seaweed extract values in this study were lower than those obtained using α-Tocopherol.

The reducing power of MeOH extracts and solvent partitioned fractions from three seaweeds was determined by measuring the amount of reductones included in the samples. Reduction powers of methanolic extracts/fractions of three red seaweeds are recorded in Table 5. Significantly higher reduction power was observed for MeOH extracts/fractions of *G. corticata* compared to *U. faciata* and *N. zanardinii*. EA fraction of *U. faciata* showed maximum reducing power than the other two seaweeds (p<0.05). The reducing powers of the samples were found to be in the following order: BHT > EA> DCM> MeOH> n-hexane. All extracts demonstrated reasonable ferrous ion chelating efficacy (Table 5). Metal chelating activity (%) of MeOH extracts of *U. faciata* and *G. corticata* was higher than *N. zanardinii*. n-hexanic extract of *U. faciata* showed higher metal chelating activity than that of two other seaweeds, but metal chelating activity of DCM fraction of *G. corticata* was higher than *U. faciata* and *N. zanardinii*. EA fraction of *U. faciata* and *G. corticata* were better chelators of ferrous ion compared to *N. zanardinii* and other fractions of the same species.

Inhibitory activity of the egg yolk peroxidation at 2 mg/mL of MeOH extracts/fractions of three seaweeds was recorded in Table 5. EA and DCM fractions of all three seaweeds exhibited significantly higher inhibition ability than MeOH extract and n-hexane fractions.

Table 1: Yield of total extract (g/100 g dry seaweed) and fractions (as % of total methanolic extract) of three seaweeds.

Seaweeds	MeOH extracts	Fractions		
		n-Hexane	DCM	EA
U. faciata	9.32 ± 0.81^{a}	31.36 ± 1.2^{a}	9.72 ± 1.3^{ab}	27.54 ± 1.59^{a}
N. zanardinii	6.44 ± 0.54^{b}	28.02 ± 1.02^{b}	11.30 ± 1.65^{a}	24.38 ± 0.78^{b}
G. corticata	6.71 ± 0.62^{b}	27.42 ± 2.03^{b}	8.26 ± 0.42^{b}	18.68 ± 1.55^{c}

All the values are mean ± standard deviation (n =5); a,b Column wise values with different superscripts are significantly different (p<0.05). MeOH Methanol, DCM Dichloromethane, EA Ethyl acetate.

Table 2: Total phenolic content (mg gallic acid equivalents/g extract) of total extract and fractions obtained from three different seaweeds.

Seaweeds	MeOH extracts	Fractions		
		n-Hexane	DCM	EA
U. faciata	3.36 ± 0.77^{a}	4.12 ± 0.1^{b}	4.81 ± 0.26^{a}	9.12 ± 0.94^{a}
N. zanardinii	2.14 ± 0.32^{b}	1.03 ± 0.12^{c}	0.98 ± 0.14^{c}	0.75 ± 0.11^{c}
G. corticata	4.2 ± 0.21^{a}	2.44 ± 0.53^{a}	6.49 ± 0.10^{b}	7.61 ± 0.67^{b}

All the values are mean ± standard deviation (n =5); a,b Column wise values with different superscripts are significantly different (p<0.05). MeOH Methanol, DCM Dichloromethane, EA Ethyl acetate.

Table 3: Total antioxidant activity (mg ascorbic acid equivalents/g extract) of total extract and fractions obtained from of three seaweeds.

Seaweeds	MeOH extracts	Fractions		
		n-Hexane	DCM	EA
U. faciata	0.54 ± 0.07^{b}	1.12 ± 0.1^{a}	2.23 ± 0.12^{c}	31.22 ± 1.22^{a}
N. zanardinii	0.24 ± 0.01^{c}	0.33 ± 0.02^{b}	9.92 ± 0.03^{a}	1.91 ± 0.04^{c}
G. corticata	2.2 ± 0.11^{a}	0.64 ± 0.02^{b}	4.26 ± 0.23^{b}	16.46 ± 0.11^{b}

All the values are mean ± standard deviation (n =5); a,b Column wise values with different superscripts are significantly different (p<0.05). MeOH Methanol, DCM Dichloromethane, EA Ethyl acetate

Table 4: DPPH free radical scavenging activity (%) of total extract and fractions obtained from of three seaweeds (Concentration of extracts used = 1000 µg).

Seaweeds	MeOH extracts	Fractions		
		n-Hexane	DCM	EA
U. faciata	18.36 ± 0.17^{c}	29.17 ± 0.49^{a}	30.34 ± 0.19^{b}	79.32 ± 2.14^{a}
N. zanardinii	15.22 ± 0.44^{d}	7.44 ± 0.77^{c}	44.34 ± 1.23^{a}	11.21 ± 0.89^{c}
G. corticata	19.66 ± 0.51^{b}	22.33 ± 1.12^{b}	8.29 ± 0.92^{c}	72.26 ± 1.21^{b}
α-Tocopherol	94.26 ± 0.1^{a}			

All the values are mean ± standard deviation (n =5); a,b Column wise values with different superscripts are significantly different (p<0.05). MeOH Methanol, DCM Dichloromethane, EA Ethyl acetate.

Table 5: Reduction power, Methal chelating activity and Inhibition of Egg Yolk Lipid Peroxidation of the crude methanolic extracts and solvent fractions (MeOH, n-hexane, dichloromethane and ethylacetate) of the three seaweeds.

Seaweeds	MeOH extracts	Fractions		
		n-Hexane	DCM	EA
Reduction power				
U. faciata	0.58 ± 0.01[b]	0.47 ±0.01[a]	0.68 ± 0.01[b]	0.92 ± 0.02[a]
N. zanardinii	0.32 ± 0.04[c]	0.28 ±0.01[c]	0.30 ± 0.02[c]	0.27 ± 0.01[c]
G. corticata	0.64 ± 0.01[a]	0.39 ±0.02[b]	0.79 ± 0.01[a]	0.85 ± 0.01[b]
BHT	0.97 ± 0.01			
Methal chelating activity				
U. faciata	42.18 ± 1.61[a]	21.31 ±0.48[a]	54.55 ± 1.21[b]	65.45 ± 1.24[a]
N. zanardinii	29.16 ± 0.93[b]	11.66 ±1.54[c]	14.44 ± 0.52[c]	15.23 ± 0.61[b]
G. corticata	43.61 ± 1.22[a]	19.34 ±0.72[b]	57.75 ± 1.72[a]	64.66 ± 0.85[a]
EDTA	98.27 ± 0.31			
Inhibition of Egg Yolk Lipid Peroxidation				
U. faciata	37.22 ± 0.41[a]	22.05 ±0.84[a]	63.44 ± 1.01[a]	69.24 ± 1.77[a]
N. zanardinii	15.22 ± 0.45[b]	9.16 ±0.94[c]	25.66 ± 0.88[b]	35.12 ± 1.44[c]
G. corticata	36.88 ± 1.14[a]	17.55 ±0.37[b]	63.76 ± 1.33[a]	64.33 ± 1.09[b]

All the values are mean ± standard deviation (n =5); a,b Column wise values with different superscripts are significantly different (p<0.05). MeOH Methanol, DCM Dichloromethane, EA Ethyl acetate.

Regarding to the correlations between phenolic contents and different antioxidant activity assays as shown in Fig. 1, low correlation was observed between DPPH (r^2=0.45; Fig.1B) and total antioxidant activity (r^2=0.51; Fig.1A), but higher correlation was observed with reduction power (r^2=0.94; Fig.1C), metal chelating activity (r^2=0.84; Fig.1D) and lipid peroxidation inhibition (r^2=0.71; Fig.1E).

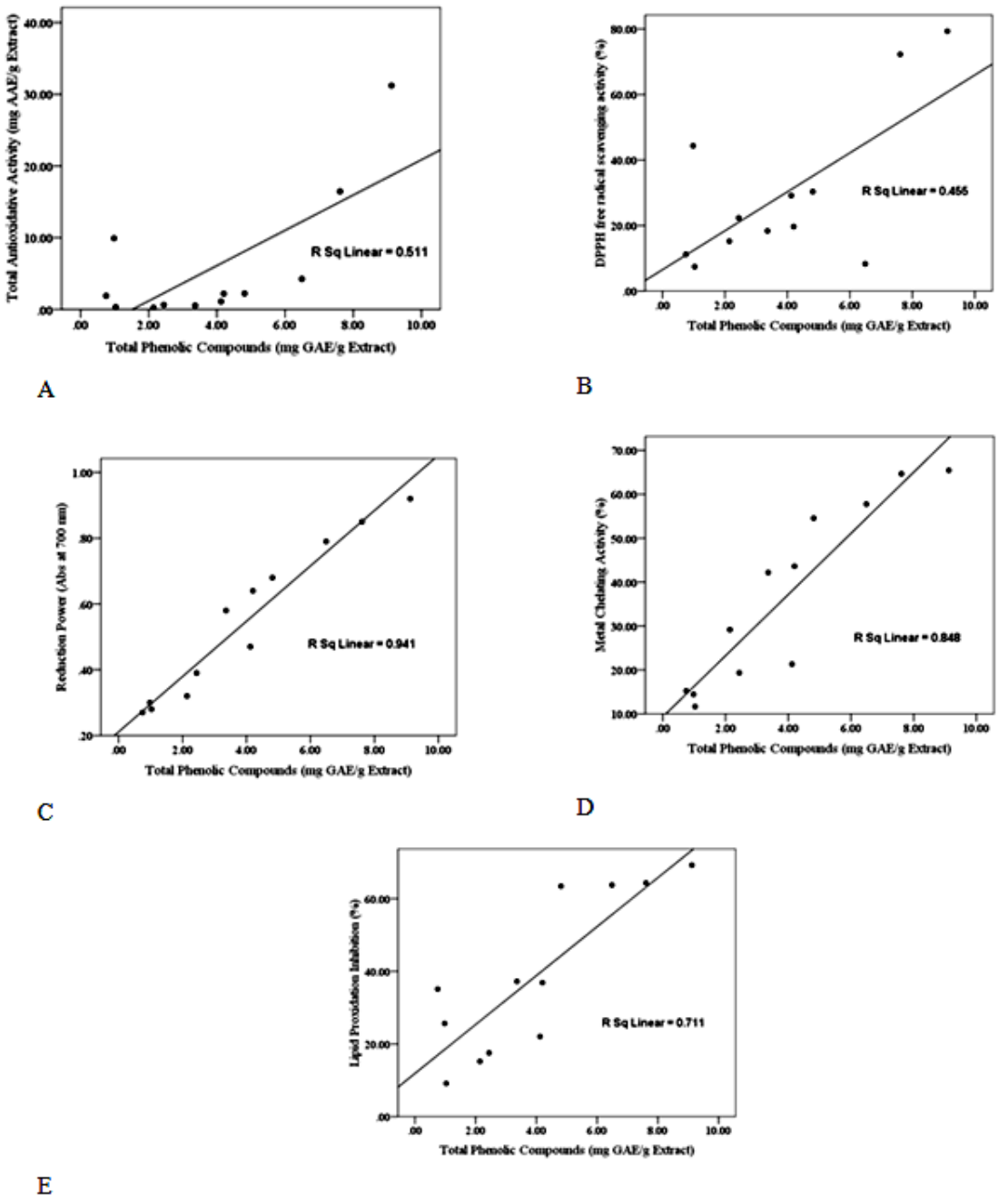

Figure 1: Relationship between antioxidant activities and phenolic contents of EtOH extracts and fractions from three seaweeds: (A) Total antioxidant activity, (B) DPPH free radical scavenging activity, (C) reducing power, (D) Metal chelating activity and (E) Lipid peroxidation inhibition, expressed as mg of gallic acid equivalents/g extract. Solid lines represent linear regression curves.

Discussion

In the present study *U. faciata* exhibited higher yield followed by *G. corticata* and *N. zanardinii*. The yield of methanolic extract in the present study was higher as compared to the earlier study by Chakraborty *et al*. (2013), who obtained 4.83, 6.54 and 5.32 g/100 g dry sample of total MeOH extract in the *Hypnea musciformis*, *Hypnea valentiae* and *Jania rubens*, respectively. However, considerably higher yields are reported from the methanol extracts of green seaweeds (Matanjun *et al.*, 2008). In different types of seaweeds, such as red and brown seaweeds, the yields of the extracts vary ranging from 2.9% to 12.7% (Ganesan *et al*., 2008, Ye *et al*., 2009).

In the present study EA fraction of *U. faciata* and *G. corticata* showed higher phenolic content compared to other fractions and MeOH extracts. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including free radical scavenging and antioxidant properties (Devi *et al.*, 2011). Earlier reports revealed that marine seaweed extracts, especially their polyphenols, have antioxidant activity (Kuda *et al*., 2005). Duan *et al.* (2006) observed higher phenolic content (73.7 GE/ g) in the ethyl acetate soluble fraction of red algae, *P. urceolata*. Also Chakraborty *et al*. (2013) reported highest phenolic content for ethyl acetate soluble fraction of *H. musciformis*, *H. valentiae* and *J. rubens* (37–205 mg GAE/g). The least total phenol content was observed in *N. zanardinii*. In the present study, for all three seaweeds, higher contents of the phenolic compounds were observed in the solvent fractions than the crude MeOH extract. Similar finding was also reported by Ganesan *et al.* (2008). This could be due to more interfering substances present in the crude extract as compared to those fractions. Moreover, it has been reported that total phenolic content increased in the fractions with increasing solvent polarity (e.g. EA and DCM) (Chakraborty *et al*., 2013).

EA fraction of *U. faciata* and *G. corticata*, and DCM fraction of *N. zanardinii* showed the total antioxidant activity. Total antioxidant activity of *Halimeda tuna* and *Turbinaria conoides* diethyl ether fractions was higher than methanolic fractions but this activity was wise versa about *Gracilaria foliifera* diethyl ether and methanolic fractions (Devi *et al*., 2011). Moreover, Ye *et al*. (2009) showed total antioxidant activity of the EA fraction of *Sargassum pallidum* was higher than *n*-BuOH, methanolic and aqueous fractions. Kumar *et al*. (2011) reported total antioxidant activity of *Gracilaria corticata* and *Ulva fasciata* methanolic extract of 0.44 ± 0.04 and 0.62 ± 0.05 mg ascorbic acid E/g extract, respectively. Total antioxidant activity of *Gracilaria corticata* metanolic extract in the current study was higher than that report. Higher activity in fractions may be due to the interferences of other compounds

present in crude (methanolic) extracts (Chandini *et al.*, 2008) and, it has also been reported that solvents used for extraction have dramatic effect on the chemical species (Yuan and Walsh, 2006).

U. faciata, EA fraction had higher DPPH scavenging activity as compared to other fractions of the same species. DPPH is a stable free radical and in the presence of a hydrogen donator, is reduced to diphenyl-pricryl-hydrazine, which has a pale yellow color. Thus, the ability of seaweeds to scavenge DPPH free radicals was determined by the decrease in its absorbance at 517 nm. Therefore, DPPH is often used as a substrate to evaluate antioxidant activity of an agent (Je *et al*., 2009). Earlier studies showed high DPPH radical scavenging activities in the EA fractions of red seaweeds, *R.confervoides*, *Polysiphonia urceolata* and *Ecklonia cava* (Duan *et al*., 2006, Wang *et al*., 2009). Chakraborty *et al*. (2013) reportedthat *H. musciformis* had significantly higher DPPH scavenging activity (82.9 %) in the EA fraction and *H. valentiae* in the DCM fraction (66.36%). They suggest that these fractions may contain compounds having polyphenolic groups with multiple -OH groups and/or center of unsaturation in their structural moieties to enable them to donate a proton to DPPH radical. Ye *et al*. (2009) found that the DPPH free-radical scavenging activities of EA fraction and n-BuOH fraction of *Sargassum pallidum* were much higher than those of the other fractions and crude extract. Also Kumar *et al*. (2011) reported DPPH free radical scavenging activity of 44.32 ± 4.3% and 51.36 ± 8.79% for *Gracilaria corticata* and *Ulva fasciata* methanolic extracts, respectively. Based on the results of present study, solvent fractions showed higher radical scavenging activity and may contain a structural enable them to donate a proton to free radicals and neutralizing them.

EA fraction of *U. faciata* showed maximum reducing power than the other two seaweeds. The reducing ability of a compound greatly depends on the presence of reductones, which have exhibit antioxidant activities by donating a hydrogen atom and breaking the free radical chains (Qi *et al.*, 2005). This study is in accordance with the earlier reports which reported that reducing power of EA extracts (Abs700 nm 1.46 ± 0.02) of *H. musciformis* were higher than n-hexanic and DCM extracts (Chakraborty 2013). Also, Kumar *et al.* (2008) reported that reducing power of MeOH (Abs 700 nm 0.07–0.74) and EA extracts (Abs 700 nm 0.013–0.467) of red seaweed *Kappaphycus alvarezii* extracts were higher than n-hexanic extract (Abs 700 nm 0.017–0.16 at 0.5–5 mg/mL). Furthermore, Wang *et al*. (2009) showed EA fractions of red seaweed *Rhodomela confervoides* exhibited potentially high reducing power (426 mg/g ascorbic acid equivalents).

Regarding to metal chelating activity, n-hexanic extract of *U. faciata*

showed higher activity than two other different seaweeds. Ferrous ions are considered the most effective pro-oxidants. Therefore, the chelating ability of the extract from seaweeds was examined in the present study. A representative chelator, EDTA, was used as the control. Fe^{2+} can form complexes with ferrozine and shifts to its characteristic purple color. In the presence of chelating agents, formation of ferrozine–Fe^{2+} is disrupted, resulting in a decrease of color development (Cho *et al*., 2007). Ye *et al*. (2009) reported EA fraction and n-BuOH fraction of *Sargassum pallidum* exhibited higher chelating abilities compared to the other fractions. Also, Chakraborty *et al*. (2013) reported EA extracts of *H. musciformis* and *J. rubens* were better chelators compared to *H. valentiae.* Iron is known to generate free radicals through the Fenton & Haber-Weiss reaction. Metal ion-chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion-chelating capacity plays a significant role in the antioxidant mechanism (Kumar *et al*., 2008). Based on the report of Lindsay (1996), compounds with structures containing two or more the following functional groups: –OH, –SH, –COOH, $–PO_3H_2$, >C=O, $–NR_2$, –S– and –O– in a favorable structure-function configuration will have chelation activity and are effective as secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.

Lipid peroxidation is a consequence of the chain reaction caused by a reactive oxygen species leading to the generation of products such as lipid hydroperoxide, which has unpaired electrons or shows the ability to attract electrons from other molecules causing direct or indirect DNA damage (Zhu *et al*., 2004). Lipid peroxidation is a major cause of pathological effects such as cardiovascular disease, cancer, and brain dysfunctions; it also leads to the development of food rancidity and off-flavours. Therefore, determining the degree of lipid peroxidation and antioxidant activity is significantly important in order to screen the antioxidant from natural product. The present study correlates well with earlier study of Zubia *et al*. (2009), reporting EA and DCM fractions are the major seaweed fractions harboring the principle antioxidative component that inhibits lipid peroxidation. Also in Chakraborty *et al.* (2013) study, EA fraction of *H. musciformis* registered significantly higher TBARS inhibition ability (2.71 MDAEC/kg) than all the other extracts/fractions.

In the present study, inhibition of egg yolk lipid peroxidation in the EA and DCM fractions of *U. faciata* and *G. corticata* may be due to the presence of polyphenolic compounds which were reported to disrupt free-radical chain reaction by donating a proton to fatty acid radicals to terminate chain reactions (Karawita *et al*., 2005).

Although methanolic and n-hexanic fractions of *N. zanardinii* had relatively higher content of polyphenols, it exhibited much lower inhibition activity. The antioxidant activities of phenolic compounds were different due to their different structures (Ye *et al*., 2009). Therefore, there is a wide degree of variation between different phenolic compounds in their effectiveness as an antioxidant. Although the methanolic and n-hexanic fractions had significantly higher content of phenolic compounds, these phenolic compounds showed lower inhibition of egg yolk lipid peroxidation than those with different structures. This is maybe one of the reasons that these fractions exhibited lower inhibition activity.

Phenolic compounds are very important plant constituents because they exhibit an antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Norra, 2011). It has been reported that the antioxidant activity of the extracts from various types of seaweeds might be correlated with total phenolic content (Ganesan *et al*., 2008; Ye *et al*., 2009). Therefore, in the present study, total phenolic contents of methanolic extract and fractions were determined and correlated with antioxidant activities. Correlation result in this study is in agreement with Matanjun *et al*. (2008) that reported phenolic content in the seaweed extracts and showed much higher correlation with reducing power (R^2=0.96) than the radical-scavenging activity (R^2=0.56). Some authors claim that there is low correlation between total phenolic content and antioxidative properties (Cho *et al*., 2010). Contrary, some authors reported high correlation between phenolic compounds and different antioxidant activity of seaweeds (Lu and Foo, 2000, Siriwardhana *et al*., 2003). For example, Devi *et al.* (2008) reported that the correlation between total polyphenolic content and DPPH, is the highest (R^2 = 0.9514) and with reducing power is the lowest (R^2 = 0.6357). Therefore, they conclude that the best method to determine the antioxidant capacity of seaweed is the DPPH method. This finding is not in accordance with the present study that reducing power showed the highest correlation and DPPH showed lowest correlation by total phenolic content of different seaweeds.

Other research about antioxidative effects of Iranian seaweeds is available on *Nizamuddinia zanardinii* and *Cystoseira indica* (Attarn Fariman *et al*., 2015), but their findings were not supported by our results. They have reported more than 85.7% DPPH radical scavenging activity for *N. zanardinii* methanolic extract for all 5 months that is very indefinite and insubstantial.

Based on our results, the antioxidant properties of phenolics are a result of their ability to act as reducing agents, metal chelator, and some extent free radical quenchers, but the lower

correlation between DPPH values and the phenolic contents in the seaweed extracts indicated that there might be some effects involving the other active compounds.

The present study reveals the potent antioxidant properties of three seaweeds species, *U. faciata, N. zanardinii* and *G. corticata,* available along with the southeast coast of Chabahar, Iran.

Total antioxidant activities, DPPH free radical scavenging activities, reducing powers, capacities of metal chelating and inhibitions of lipid peroxidation of methanolic extracts and its fractions from three seaweeds were evaluated *in vitro*. EA fractions of *U. faciata* and *G. corticata,* exhibited higher antioxidant activities, while DCM fraction of *N. zanardinii* showed the highest antioxidant capacity, except metal chelating activity and lipid peroxidation inhibition. There was a strong correlation between the reduction power (r^2=0.94) and total phenolic content of the seaweed extracts/fractions. The present findings indicate that these seaweeds can be potential sources of natural antioxidants. Further study is needed in order to identification of specific compounds responsible for the relatively antioxidant activities in these seaweeds.

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