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Chemical composition and functional properties of two brown seaweeds from the Qeshm Island, Iran

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Abstract

Seaweeds have been introduced as an important sources of food and pharmaceutical active agents since last century. In this study, the chemical composition and functional characteristics of (common names) *Nizamuddinia zanardinii* and *Iyengaria stellata*, were investigated. The total carbohydrate and ash contents were the two most abundant components in these seaweeds but their crude lipid and fiber contents were low. The protein content was higher in *I. stellata* followed over *N. zanardinii*. Atomic absorption spectrophotometry of the ashes showed that *I. stellata* contained higher amounts of both macrominerals (Ca, Mg) and trace elements (Fe, Zn) than *N. zanardinii*. Results of antioxidant properties showed that total phenolic (3.37 mg $100g^{-1}$), total antioxidant (27.08 mg g^{-1}) and reducing power (22.76%) were higher in *N. zanardinii* than *I. stellata*, while DPPH free radical scavenging (7.16%) was higher in *I. stellata*. The results suggest that both the seaweeds could be used as a potential source of nutrient and antioxidant compounds in food and pharmaceutical industries.

Keywords: Brown seaweeds, Chemical composition, Antioxidant activity, Mineral composition

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Introduction

Seaweeds are marine large and diverse groups of photosynthetic organisms like plants which form the primary biomass in the coastal regions (Miyashita, 2009). About 9000 species of seaweeds have been identified and broadly classified into three principal classes based on their pigmentation: green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae (Miyashita *et al*., 2013). Marine seaweeds are one of the living reproducible resources of the marine environment with potential food (Kumar *et al*., 2011) for human and animals, and medicine applications. Macroalgae are a rich source of polysaccharides, vitamins, antioxidant, dietary fiber, fatty acids, and minerals (Sinéad Lordan *et al*., 2011) and they are a potential source of secondary metabolites (Narayani *et al*., 2011). These bioactive compounds are a major resource for drug development (Kuda *et al*., 2005). In addition, marine macroalgae are used as sources of phycocolloids, thickening and gelling factors in food industries (Ruperez and Calixto, 2001). Algae also are a rich source of antioxidant compounds (Lim *et al*., 2002; Kuda *et al*., 2005; Chandini *et al*., 2008; Kokilam *et al*., 2013) and it has been reported that brown algae comparatively have higher antioxidant potential in comparison with green and red algae. (Kokilam *et al*., 2013). Polyphenols, carotenoids, tocopherols, terpenes, ascorbic acid and alkaloid are the natural compounds with antioxidant potential in seaweeds (Kokilam *et al*., 2013). These compounds react rapidly with radicals of oxygen such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous and exogenous factors, and prevent from a wide range of human diseases (Chandini *et al*., 2008). In recent years, natural antioxidant compounds have attracted major interest as replace synthetic ones in prolonging the shelf life of food and cosmetics by delaying oxidation. In addition, biologically active compounds from marine algae have been proposed for nutraceuticals and functional foods (Balboa *et al*., 2013). Marine algae are distributed in the southern coast of Iran especially around seashores of Busheher and Hormuzgan provinces. Due to the presence of various species of seaweed in the Persian Gulf, reports on the antioxidant properties and chemical composition of seaweeds from Iran are very limited. Therefore, the current study was performed to measure the antioxidant properties and chemical composition of two various species of brown seaweeds. In this research, proximate composition, physicochemical properties, mineral content, total phenolic and antioxidant activity, DPPH free radical scavenging ability and reducing power of both *Nizamuddinia zanardinii* and *Iyengaria stellata* were evaluated. *N. zanardinii* and *I. stellata* (BØrgesen) are two various species of brown seaweeds with a similar class, Phaeophyceae, *N. zanardinii* belongs to the order Laminariales and family Alariaceae and *I. stellata* belongs to the order Scytosiphonales and family Scytosiphonaceae.

Materials and methods

Samples collection

Brown seaweeds *I. stellata* and *N. zanardinii* were collected from the coast of Qeshm Island in February 2014. The samples were washed thoroughly with

seawater to remove disturbing factors such as epiphytes and dirt particles, followed by shade-drying for two to five days. After that, the samples were transferred to the laboratory and the shade dried seaweeds were powdered to less than 1 mm particles in size. The milled seaweed samples were stored in sealed plastic bags at -20°C for further experiments.

Chemicals

Folin-Ciocalteau reagent, gallic acid, ammonium molybdate, ascorbic acid, phosphate buffer, Sodium carbonate, Iron(III) chloride-6-hydrate, Trichloroacetic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and potassium ferricyanide, were purchased from sigma-Aldrich chemical co. Inc. (St. Louis, Mo, USA).

Estimation of proximate composition of the seaweed samples

Ash content

The ash content of seaweeds was quantified gravimetrically according to the method of Seedevi *et al.* (2015). The ash content was expressed as percent of dry weight.

Crude fiber content

Fiber quantified using the Fibertec System (IKA, Germany) (Robledo and Freile Pelegrín, 1997). The fiber content of the samples reported as percent of dry weight.

Extractable fat

Determining the fat percentage was done by Soxhlet method and by using petroleum ether solvent (Peinado *et al*., 2014).

Protein

Total protein was determined by the micro-Kjeldahl method. The protein was specified by using a nitrogen conversion coefficient of 6.25 (Akgül *et al*., 2015). Data reported as a percentage of dry weight.

Moisture content

The moisture content of the seaweeds was determined by drying following method of Seedevi *et al.* (2015).

Estimation of total carbohydrate content

Estimation of total carbohydrate was conducted by Phenol-sulphuric acid method (Syad *et al*., 2013). The total carbohydrate content of seaweeds was estimated based on Standard glucose curve and the results were expressed in percentage.

Evaluation of mineral contents

Mineral contents were quantified using the atomic absorption spectrophotometry (Perkin-Elmer Analyst 800 with flame furnace) (Syad *et al*., 2013). The minerals analyzed were Calcium, Iron, Magnesium, Manganese, Copper, Cadmium, Lead, and Zinc and the results were expressed as mg/100g dry weight.

Preparation of samples extract for antioxidant activities

Extraction was conducted in three steps. The extraction of seaweed sample was performed with distilled water. Briefly, 50 grams of the algal powder was mixed with 1000 ml of distilled water, were shaken in an incubator shaker (IKA® KS 4000 ic control, Germany) at room temperature for 24 h at 200 rpm and the mixture was centrifuged at 3500 rpm (5810R,

Eppendorf, Germany) for 10 min at 4°C and filtered with Whatman no 4 filter paper. Both concentrate and supernatant were freeze-dried and the concentrate was weighed. At the secondary and third steps, distilled water was added to the concentrate at the ratio of 1:20 (W/V) and the above process was repeated. The dried triplicate extracts were pooled and stored at -80°C until analyzed. Each dried extraction was then dissolved in distilled water at a concentration of 5mg/ml as a stock solution. The stock solution was applied both for the determination of TPC and antioxidant activities.

Total phenolic content (TPC)

TPC in the aqueous extracts was assessed with Folin– Ciocalteu reagent based on the method of Taga *et al*. (1984). Various concentrations of 0.001 mg ml^{-1} to 1.0 mg ml^{-1} of standard stock solution were prepared. Briefly, 200 ml of 2% Na_2CO_3 was mixed with 100 µl of test solutions. After 2 min, test solutions were mixed with 100 µl of 50% Folin-Ciocalteau reagent and were placed at temperature 25◦C for 30 min. Spectrophotometer (Biochrom, England) was used to read the absorbance value at 750 nm. Gallic acid was used as a standard. Total phenolic content was assessed by comparison with the standard calibration curve and the results were expressed as milligram Gallic acid equivalent per 100 gram of extract.

Total antioxidant activity

Total antioxidant activities of aqueous extracts were assessed based on the method of Sathya *et al.* (2013). Briefly, 0.3 mL of test sample solution at different concentrations (10-100 mg ml^{-1}) was added to 3 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was placed in a Ben Murray with temperature 95°C for 90 min. Then the absorbance was read at 695 nm. Ascorbic acid was used as standard and total antioxidant capacity reported as milligram ascorbic acid equivalent per gram of extract.

DPPH scavenging activity

The scavenging effects of samples for DPPH radical were monitored based on the method of Rodríguez-Meizosoet *et al.* (2006). Briefly, 23.5 mg of DPPH powder was dissolved in 200 ml of methanol. This solution was diluted with methanol at a ratio of 1:20; then 0.1 ml of different concentrations of algal extracts (20-200 µg ml^{-1}) added to 3.9 ml of a methanolic solution of DPPH. The test solutions were vortexed for 1 min and kept in the dark at 25ºC for 30 min. The absorbance of all the sample solutions was measured at 517 nm by using the methanol as blank. Synthetic antioxidants Gallic acids and Ascorbic acid were used as positive controls. The DPPH free radical scavenging ability was estimated using the following formula:

Scavenging effect (%)=$[(A_0-A_1)/A_0]\times100$

A_0=Absorbance of control;

A_1=Absorbance of sample

A_0=DPPH solution without sample;

A_1=DPPH solution contain test sample

Reducing power

The reducing power of aqueous extracts was assessed based on the method of Kumar *et al.* (2011). Briefly, 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium ferricyanide (1%) were mixed with 0.5 ml of extract samples

solution at concentrations (10-320 µg ml^{-1}) and the mixture was incubated at 50ºC for 20 min. Afterward 1.25 ml of trichloroacetic acid (10%), 1.25 of ml distilled water and 0.25ml of Fecl3.6H2O (0.1%) were added to the mixture. The mixture was kept at 25 ºC for 10 min and the absorbance was measured at 700 nm. Synthetic antioxidant Ascorbic acid was used as positive control. Reducing power increases by increasing the absorbance value of the reaction mixture.

Statistical analysis

All analyses conducted in triplicates, the data reported as means±standard deviations. Statistical analysis was performed by software SPSS (Version 20). Independent samples t-test was implemented to assess for any significant differences between the means. p-value<0.05, p<0.01 regarded as significant. Microsoft Office Excel 2010 used to diagramming.

Results

The proximate composition of *N. zanardinii* and *I. stellata* were investigated and the results were tabulated in Table 1. The amounts of ash and protein in *I. stellata*, moisture and fiber were significantly higher in *N. zanardinii* (p<0.05). But the fat and carbohydrate contents of algae *N. zanardinii* and *I. stellata* were not observed a statistically significant difference (p>0.05).

Table1. Proximate composition of the *Nizamuddinia zanardinii* and *Iyengaria stellata*

Composition	***N. zanardinii***	***I. stellata***
Ash content	15.84 ± 1.26^{b} % DW	33.68 ± 3.25^{a} % DW
Crude fiber content	5.18 ± 0.07^{a} % DW	4.68 ± 0.08^{b} % DW
Crude protein content	3.18 ± 0.05^{b} % DW	8.16 ± 0.14^{a} % DW
Crude lipid content	0.41 ± 0.12^{a} % DW	0.27 ± 0.09^{a} % DW
Moisture content	8.99 ± 0.17^{a} % DW	3.77 ± 0.67^{b} % DW
Total carbohydrate content	29.10 ± 5.60^{a} % DW	34.80 ± 5.41^{a} % DW

Results expressed as Mean ±SD (n= 3). Different superscript letters in the same row indicate significant differences (p<0.05). DW: dry weight.

Mineral composition

The mineral composition of two different species of brown seaweeds was shown in Table 2. There were significant (p<0.05) differences in the concentrations of all the mineral and trace elements except Magnesium and Copper between two species of seaweeds. Among all the 8 minerals analyzed Calcium were found to be highest (2580-9586 mg $100g^{-1}$ DW) in *N. zanardinii* and *I. stellata*, respectively. The Lead was a toxic element with the lowest concentration in the analyzed seaweeds, the range of <0.5mg $100g^{-1}$ - <0.5mg $100g^{-1}$ for the *N. zanardinii* and *I. stellata*, respectively.

Table 2: Mineral composition (mg $100g^{-1}$ dry weight) determined by atomic absorption spectrophotometry in *Nizamuddinia zanardinii* and *Iyengaria stellate.*

Mineral	Seaweeds	
	N. zanardinii	***I. stellata***
Ca	2580 ± 278.74^{b}	9586.66 ± 476.069^{a}
Mg	1316.66 ± 32.14^{a}	1316.66 ± 25.16^{a}
Fe	90 ± 10^{b}	326.66 ± 15.27^{a}
Mn	2.06 ± 0.49^{b}	14.43 ± 0.30^{a}
Zn	1 ± 0.1^{b}	1.53 ± 0.05^{a}
Cu	0.73 ± 0.57^{a}	0.53 ± 0.05^{a}
Pb	<0.5	<0.5
Cd	<0.2	0.2 ± 0

Results expressed as Mean ± SD (n= 3). Different superscript letters in the identic row indicate significant differences (p<0.05).

Phenolic content

The total phenolic content (TPC) of *N. zanardinii* and *I. stellata* shown in Figure1. The phenolic content in extracts was significantly different between species (p<0.05). The aqueous extract of *N. zanardinii* showed higher total phenolic content (TPC) of 3.37±0.13 mg GAE $100g^{-1}$ compared to water extract of *I. stellata* 1.22±0.09 mg GAE 100g.

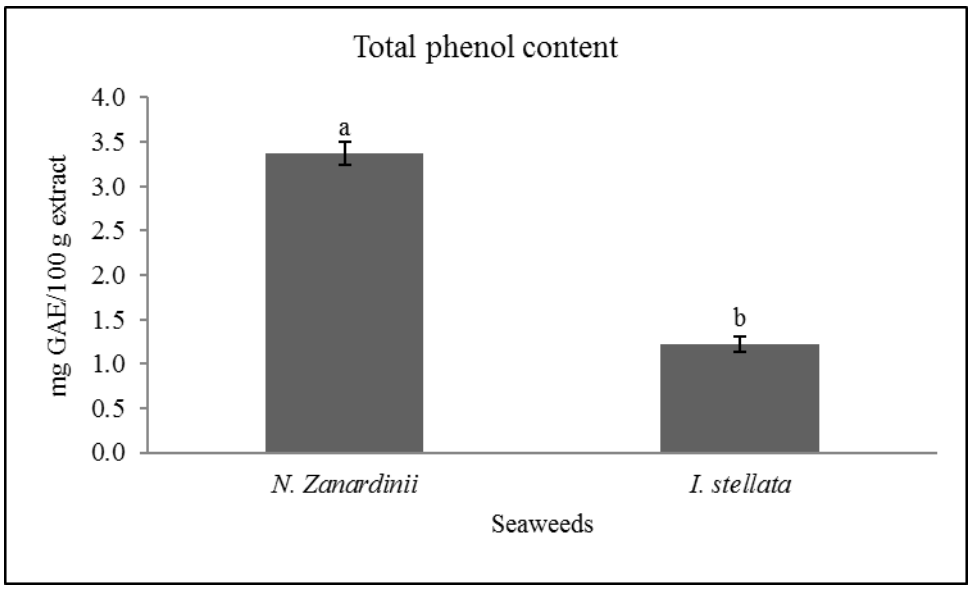

Figure 1: Total phenolic content of seaweeds extract. Different superscripts letters indicate significant differences (p<0.05). Data expressed as Mean±SD (n= 3).

Total antioxidant activity

Total antioxidant activity (TAE) is a method for determining the antioxidant potential of marine algae. In the present study, *N. zanardinii* and *I. stellata* showed the total antioxidant activity in the range of 0-27.08 mg g^{-1} and 0-5.03 mg g^{-1}, respectively at different concentrations (10-100 mg ml^{-1}). The maximum of 27.08 mg g^{-1} and 5.03 mg g^{-1} inhibition were observed at the concentration of 100 mg ml^{-1}, respectively, it can be concluded, By increasing the concentration of the extract, antioxidant activity increases. At concentrations of 10, none of the seaweeds showed antioxidant activity, but in other

concentrations the antioxidant activity of *N. zanardinii* was significantly higher than *I. stellata* ($p<0.05$).

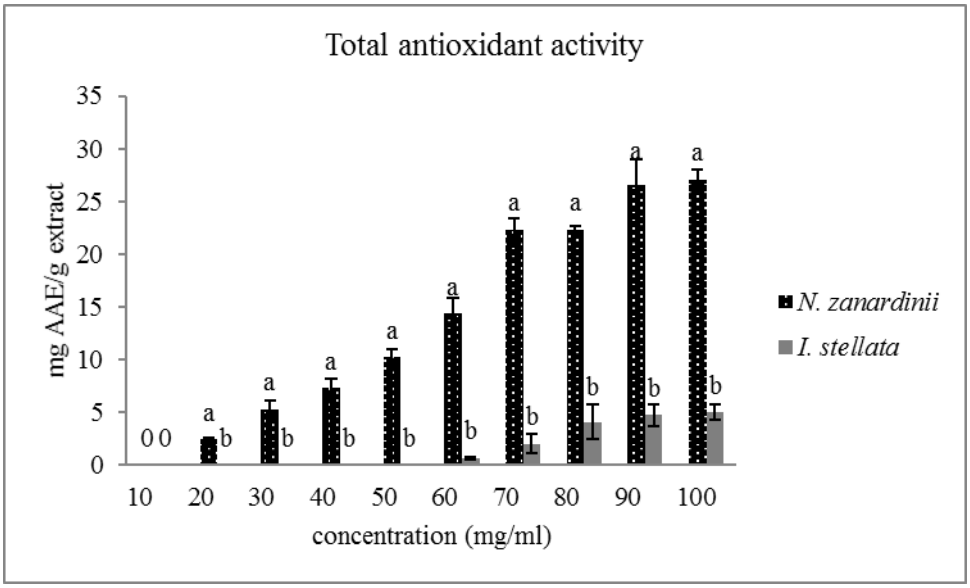

Figure 2: Total antioxidant activity of seaweeds extract. Different superscript letters in the identic concentration indicate significant differences ($p<0.05$). Data expressed as Mean±SD (n=3).

The DPPH free radical scavenging

Antioxidant activities of the aqueous extracts of two species of brown seaweed were measured by using a radical scavenging method. In the present study inhibitory effect of extracts of *N. zanardinii* and *I. stellata* on DPPH radical are determined and depends on the concentration. The 20-200 µg ml^{-1} concentration showed 1.61-2.73% and 6.04-7.16% of inhibition respectively. The highest value of 2.73% and 7.16% observed at the concentration of 200 µg ml^{-1} in *N. zanardinii* and *I. stellata*, respectively. The results showed that by increasing the concentration of seaweed extracts the percentage of DPPH free radical scavenging increased. At the highest concentration, the standards of Gallic acid and ascorbic acid showed the antioxidant activity of 95.52-87.36% respectively. Inhibitory effect of positive controls in all concentrations compared to algae extracts were significantly higher ($p<0.01$). DPPH free radical scavenging ability of an aqueous extract of brown algae *I. stellata* in all concentrations was significantly higher than *N. zanardinii* ($p<0.05$).

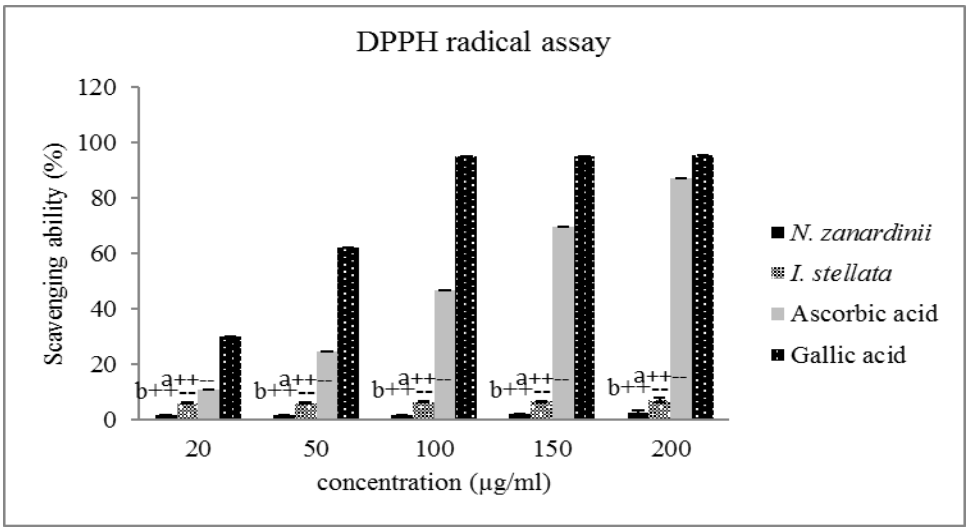

Figure 3: DPPH antioxidant activity of seaweeds extract. Different superscript letters in the identic concentration indicate significant differences (p<0.05). Different superscript emblems (++,--) in the same concentration indicate important significant differences with ascorbic acid and Gallic acid, respectively (p<0.01). Data expressed as Mean±SD (n= 3).

Reducing power

Fig. 4 shows the reducing power results of seaweeds *N. zanardinii* and *I. stellata* exhibited concentration dependent. The reducing power of *N. zanardinii* and *I. stellata* was 0.56-22.76% and 0.2-6.4% at the concentration of 10-320 µg ml^{-1} respectively, which showed a constant increase in its reducing power with increased concentration. Between the extracts in concentrations of 20 and 40 were not observed statistically significant difference (p>0.05). But in other concentrations reducing power of macroalgae *N. zanardinii* was significantly higher (p<0.05). Reducing power of ascorbic acid at all concentrations was significantly higher than the algae extracts (p<0.01).

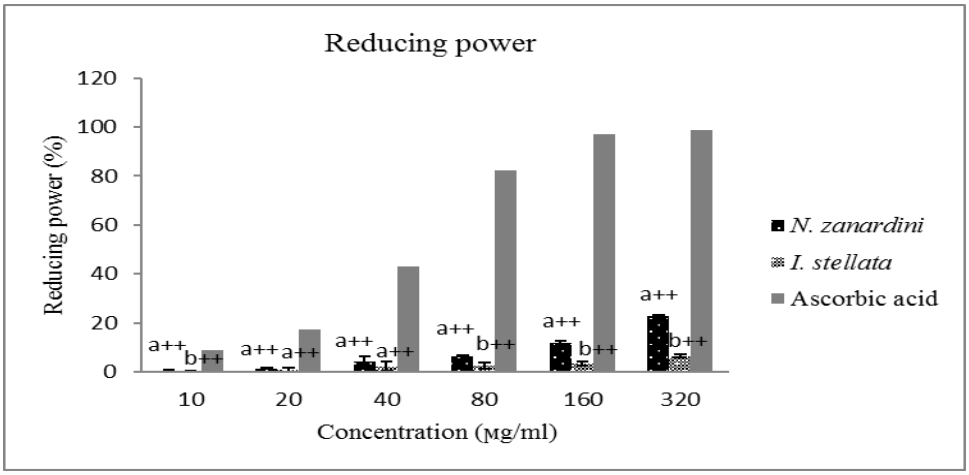

Figure 4: Reducing power of *Nizamuddinia zanardinii* and *Iyengaria stellate*. Different superscript letters in the identic concentration indicate significant differences (p<0.05). Different superscript emblem (++) in the same concentration indicate important significant differences with ascorbic acid (p<0.01). Results are expressed as Mean±SD (n= 3).

Discussion

The Proximate composition of the *N. zanardinii* and *I. stellata* was shown in table 1. In general, the amount of ash is high in the seaweeds (ranges of 8 to 40% dry weight) (Rupérez, 2002) that is the indicative of high levels of various minerals in the seaweeds (Kumar *et al*., 2011). The ash content determined for *I. stellata* was higher than reported by Rohani-Ghadikolaei *et al*. (2012) and Syad *et al.* (2013). The total fiber content of seaweeds indicated a significant correlation (p<0.05). *N. zanardinii* and *I. stellata* have a higher fiber than *Ascophyllum nodosum*, *Caulerpa racemosa*, *Codium isthmocladum* and *Eucheuma isiforme*, but the fiber was lower in these seaweeds when compared to *Sargassum filipendula* and *Padina gymnospora* (Robledo and Freile Pelegrín, 1997). In general, total fiber content was low in both seaweeds. The protein values are significantly different between the two seaweeds (p<0.05). The amount of protein in brown seaweeds is within the ranges of 3 to 15% of dry weight of seaweed (Burtin, 2003; Chakraborty and Santra, 2008; Manivannan *et al*., 2008, 2009; Rohani-Ghadikolaei *et al.*, 2011). Variations in the protein content of seaweeds can occur due to species, season and their environmental conditions (Dawczynski *et al*., 2007). Lipids are one of the components of seaweeds. The lipid level of seaweeds is generally low (1–5% of dry weight) (Burtin, 2003; Polat and Ozogul, 2008). The levels of lipids detected in the present study were more than previously reported for other brown seaweed species, namely: *Sargassum wighti* (0.21±0.001%) and *Hormophysa triquetra* (0.11±0.001%), (Kokilam *et al*., 2013). Carbohydrates are needed for important energy processes such as respiration (Kokilam *et al*., 2013). Fucoidan, laminaran, cellulose, alginate and mannitol are the common carbohydrates in brown seaweeds (Dawczynski *et al*., 2007). The carbohydrate values are not significantly different between the two seaweeds (p>0.05). These results are higher than those reported by MacArtain *et al* (2007) for *Ascophyllum nodosum* (13.1%), *Laminaria digitata* (9.9%), *Himanthalia elongata* (15%), *Undaria pinnatifida* (4.6%), Mohammadi *et al* (2012) for *Colpomenia sinuosa* (11.3%), and Syad *et al* (2013) for *S. wightii* (0.0095%). Environmental factors such as temperature, salinity and sunlight intensity can change the amount of carbohydrates in different species (Sakthivel and Devi, 2015). Moisture is an important factor in determination of the shelf-life and quality of food materials (Syed *et al*., 2013). There was a significant correlation between moisture in the seaweeds (p<0.05).

Atomic absorption spectrophotometry determination of the ashes showed that these seaweeds contained high amounts of the macrominerals and trace elements are required in human diet. *I. stellata* contains more minerals due to having more ash content. Differences in the amount and composition of polysaccharides in cell walls of seaweeds could be explained due to differences in the biosorption of minerals and trace elements (Davis *et al*., 2003; Bocanegra *et al*., 2009). The Iron, Magnesium and Copper content determined for seaweeds in this study were

higher than brown seaweeds reported by Krishnaiah *et al*. (2008); Rohani-Ghadikolaei, *et al.* (2012) and Astorga-España *et al.* (2015). The Cadmium, Lead, Copper and Zinc consumption would be 3.77, 0.91, 10.79 and 25.35 µg day^{-1}, respectively which FAO/WHO expert committee has allowed these values of elements for daily consumption. Copper and Zinc content in *N. zanardinii* and *I. stellata* were lower than reported limits by FAO/WHO (Subba Rao *et al*., 2007).

Phenolic compounds are commonly found in plants but earlier reports revealed that marine seaweed extracts possess antioxidant properties (Lim *et al*., 2002). Antioxidants phenolic are the most effective compounds in brown seaweeds (Kuda *et al*., 2005). Polyphenols, phlorotannins, and fucoxanthin are the major active compounds in different seaweed extracts that contain several biological activities including antioxidant properties (Yan *et al*., 1996). Total phenol content in *N. zanardinii* studied in this work was lower than *N. zanardinii* reported from Chabahar coast (Fariman *et al*., 2015). Further, phenol content in *I. stellata* tested in this research was lower than *I. stellata* reported from Bandar Moalam (Moein *et al*., 2015).

Natural antioxidants are not limited to terrestrial sources and reports show that seaweeds are rich sources of natural antioxidant compounds (Cox *et al*., 2010). Total antioxidant activity in *N. zanardinii* is higher than those reported by Kokilam *et al.* (2013) for *Hormophysa triquetra* (24.0±3.05 mg g^{-1}) and *Sargassum wightii* (20.0±2 mg g^{-1}).

DPPH is a useful reagent for evaluation of free radical-scavenging activities of compounds and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When DPPH is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is formed, with the simultaneous change in the color of the solution from violet to pale yellow (Sathya *et al*., 2013). DPPH radical scavenging activity of the positive controls, Gallic acid, and ascorbic acid, was higher than water extract of the *N. zanardinii* and *I. stellata* in all of five tested concentrations. DPPH radical scavenging activity in *I. stellata* was higher compared to *Nizamuddinia zanardinii* and *Cystoseira indica* (Fariman *et al*., 2015). But DPPH radical scavenging in *N. zanardinii* tested in this research was lower than *Nizamuddinia zanardinii* reported from Chabahar coast (Fariman *et al*., 2015).

In the reaction system, antioxidant substances in the samples cause reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form by breaking the free radical chain by donating a hydrogen atom. The reaction of reductions with special precursors of peroxide leads to prevention of the formation of peroxide (Seedevia *et al*., 2015). Thus, the antioxidant activity of an aqueous extract related to the reductive activity. The increased absorbance value of samples indicates increased reducing power. Higher absorbance value means stronger reducing the power of sample. Reducing power in *N. zanardinii* and *I. stellata* were higher than *Colpomenia sinousa*, *Cystoseria myrica* and *Iyengaria stellata* (Moein *et al*., 2015).

Seaweeds have been introduced as important sources of food and pharmaceutical active agents since last

century. The two seaweeds analyzed for their chemical composition were found to be interesting potential sources of carbohydrate and minerals owing to their high levels. Protein content was high in *I. stellata*. Results of antioxidant properties showed that total phenolic content, total antioxidant activity and reducing power were high in *N. zanardinii* than the *I. stellata*, while DPPH free radical scavenging was higher in *I. stellata*. So, these seaweeds can be considered as potential sources of natural antioxidants. Persian Gulf seaweeds studied can be useful as a substitute for synthetic antioxidants, medical applications or as additives. The findings of this work can be helpful to future research to identify bioactive antioxidant compounds.

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