EVALUATION OF ANTIOXIDANT CAPACITY OF METHANOL EXTRACT AND ITS SOLVENT FRACTIONS OBTAINED FROM FOUR MOROCCAN MACRO ALGAE SPECIES

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Abstract

In vitro antioxidant activities of methanolic extract and its solvent fraction (petroleum ether (PE), ethyl acetate (EA), dichloromethane (DCM), butanol (BuOH) and aqueous) were obtained from four Moroccan macroalgae species: *Ulva rigida*, *Enteromorpha intestinalis*, *Fucus spiralis* and *Bifurcaria bifurcata* using DPPH scavenging activity, metal chelating activity and total phenol content. Brown algae, *Bifucaria bifurcata* and *Fucus spiralis* contained higher amounts of polyphenols than green algae, *Enteromorpha intestinalis* and *Ulva rigida* . DCM fraction of *Fucus spiralis* and EA fraction of *Bifucaria bifurcate* showed higher phenolic content (29,79 and 24,44 mg gallic acid equivalent/g DW extract respectively) when compared to other solvent fraction and total methanol extract. DPPH radical scavenging activity of algae species extract tested showed lower activity than that of a standard compounds: ascorbic acid (EC_{50} = 0,11 mg /ml) and α tocopherol (EC_{50} = 0,215 mg /ml). Aqueous fraction of *Enteromorpha intestinalis* exhibited the most effective scavenging ability on DPPH radical (68,70%) than other fraction at the concentration of 3 mg/ml followed by aqueous and EA fraction of *Fucus spiralis* (49,50% and 50% respectively). The antioxidant activity of seaweed extracts increased in concentration-in a dependent manner. In accordance with DPPH results, potent chelation abilities were again detected in methanolic extract and its solvent fraction. All solvent and aqueous fraction of *Ulva rigida* and *Fucus spi*ralis exhibited equivalent chelating effects as compared to EDTA-Na2 standard compound (IC_{50} was lower than 0,05mg/ml). The other fractions of *Bifucaria bifurcata*

and *Enteromorpha intestinalis* showed less potent chelating effect as compared to other seaweeds (IC_{50} was high than 0,4mg/ml). However, there was no direct relationship between antioxidant activity and the phenolic content, suggesting that polyphenol play a minor role in the metal chelating ability and other compounds such as polysaccharides, protein, organic acid which can contribute to the overall antioxidant activities.

Keywords: Fucus spiralis, Ulva rigida, Enteromorpha intestinalis, Bifucaria bifurcate, antioxidant activity

Introduction

Seaweed of marine macro algae are a potential renewable resource in marine environment. About 6000 species of seaweed have been identified and have been grouped into different class which includes: green (Chlorophyceae), brown (Pheophyceae) and red (Rhodophyceae) based on their pigmentation (Dawczynski et al., 2007). Algae, as photosynthetic organisms, are exposed to a combination of light and high oxygen concentration at the origin of the formation of free radicals and other oxidative reagents. But, the awareness of the lack of structural damage in their organs has led the scientific community to consider that their protection against oxidation comes from their natural content, or production under stress in antioxidant substances. Seaweed provides an excellent bioactive compounds with potential antioxidant activity such as polyphenol, carotenoids, alkaloids, terpens, and tocopherol (Ragan Glombitza, 1986; Heo et al., 2009; Hu et al., 2008). Polyphenols derived from seaweed may be more potent that analogous polyphenols derived from terrestrial plant sources due to the presence of up to eight interconnected phenol rings (Hemat, 2007). Phlorotanins, a group of phenolic compounds which are restricted to polymers of phloroglucinol have been identified from several brown algae (Koivikko et al., 2007). These compounds have been reported to possess strong antioxidant activity. In addition, polysaccharides have also been demonstrated to possess excellent antioxidant potential (Yan et al., 1999; Zhao et al., 2008). Carotenoids, the natural pigments react rapidly also with free radical and retard or decrease the extent of oxidative deterioration (Akoh and Min, 1997). Reactive oxygen species such as hydroxyl, super oxide and peroxyl radicals which are formed in human tissues cells result in extensive oxidative damage that leads to age related degenerative conditions, cancer and a wide range of other human diseases (Reaven and Witzum, 1996, Aruoma 1999). Thus, the consumption of antioxidants and addition of antioxidant in food materials protect the body as well as foods against these events. Seaweed constitutes a commercially important renewable resource

and can be used as fertilizers, food additives and animal feed. Any studies evaluating the antioxidant proprieties of seaweed enhance their utility value.

The coastlines of Morocco are an abundant resource of seaweeds with broad species of diversity, but no study has been made to explore the antioxidant potential of seaweeds. Seaweeds are exploited mainly for the industrial production of phycocolloïds such as agar-agar, alginate and carrageenan, and not health aspects. The antioxidant activities of many types of seaweeds with Moroccan coastal area are still unexplored, although there is paucity data on the antioxidant potential of seaweeds which was harvested in Morocco. The first step in the search of a versatile antioxidant system based on seaweeds is to characterize their antioxidant system. In this study, four macro algae species were harvested from the coastal area of Morocco during spring time. These included *Ulva rigida* and *Enteromorpha intestinalis* belonging to Chlorophyceae and *Fucus spiralis* , *Bifurcaria bifurcata* belonging to the class of Phaeophyceae. The main objective of this present study is to evaluate the antioxidant capacity of the seaweeds extracts by screening total methanol extract and five different fractions (petroleum ether (PE), ethyl acetate (EA), dichloromethane (DCM), butanol (BuOH) and aqueous) using a range of chemical reaction assays: DPPH* scavengers assays and metal chelating activity (Ferrozine test) and by total phenol content.

Materials and Methods:

Collection and Plant material preparation

The seaweed extracts used in this present study were prepared from four macroalgae species *Ulva rigida* and *Enteromorpha intestinalis* belonging to Chlorophyceae and *Fucus spiralis* , and also *Bifurcaria bifurcata* belonging to the class of Phaeophyceae. They were collected from the coastal area of Sidi Bouzid near El jadida city (Morocco) in spring 2013. The algal species were hand-picked and thoroughly washed with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. Morphologically distinct thalli of algae were placed separately in new polythene bags and were kept in ice box containing slush ice and transported to the laboratory. Samples were washed thoroughly under ambient temperature using tap water to remove the surface salt and then blotted to remove excess water. Fresh material was cut into small pieces and preserved at a temperature of -20°C until it was used.

Preparation of seaweed extracts and fractions

The pulverized moisture free seaweed material (20 g) was extracted in 250 mL of methanol in a soxhlet extractor for 7 hours at 40°C. The total

extract was filtered and the obtained filtrate (crude extract) was concentrated in a rotary evaporator at 40°C to dryness. Dried extract was dissolved in 90% aqueous methanol for fraction. First fractionation was carried out with 100 ml petroleum ether (PE). PE fraction was collected and aqueous methanol phase was evaporated in a rotary evaporator to give a semisolid. A semisolid was dissolved in 200 mL of distilled water and Fractioned with 100 ml ethyl acetate (EA), dichloromethane (DCM) and n-butanol (BuOH). Resulting fractions including aqueous were evaporated to dryness. Dried fractions were dissolved in methanol and stored in coloured vials for further analysis.

Estimation of total phenolic content (TPC)

Phenolic contents of ethyl acetate extract were estimated by the method of Senevirathene et al., 2006. 100 µL aliquot of sample was mixed with 2 mL of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. After incubation, 100µL of 50% Folin-Ciocalteau's phenol reagent was added and then reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using UV-visible spectrophotometer (6305-UV-visible spectrophotometer (190-1000 nm) – JENWAY).Gallic acid was used as a standard and a calibration curve was prepared with a range of concentration from 10 to 200 mg/L. Phenolic content was expressed as gallic acid equivalent.

DPPH° (2,2 - diphenyl - 2 - picrylhydrazyl hydrate) radical scavenging activity

The free radical scavenging activity of the seaweed extracts was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) following the method of Blois 1958. This method is based on the reduction of stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical antioxidants in a methanol solution. In the presence of antioxidants, the purple colour of the DPPH radical solution changes to a bright yellow and the intensity of this can be monitored spectrophotometrically. Used as a reagent, 3,9 mL DPPH solution (0,1mM) was added to 100 µL of seaweed extracts at different concentrations. After 30 minutes, absorbance was measured at 517 nm using UV-visible spectrophotometer (6305-UV-visible spectrophotometer (190-1000 nm) – JENWAY). Thus, all the measurements were measured in triplicates and the percentage scavenging was calculated as shown by the formula:

Radical scavenging activity (%) = $[(A_0\text{-}A/\ A_0) \times 100]$, Where A_0 = Absorbance of control;

A = Absorbance of Sample

A curve of extract concentration against % DPPH was generated to estimate the concentration of extract needed to cause a 50% reduction of the

initial DPPH concentration. This value is known as EC_{50} (efficient concentration, also called oxidation index) and was expressed in terms of mg per ml. This assay was done in triplicate for each sample, and then the mean values were used to calculate the EC_{50}. Ascorbic acid, α-tocopherol were also used as positive controls.

Ferrous ion-chelating ability assay

The chelation of ferrous ions by extracts was estimated by the method of Dinis et al., 1994. 50 µl of $FeCl_2$ solution (2mM) was added to 100 µL of different concentrations of the extract. The reaction was initiated by the addition of 200 µL of Ferrozine solution 5 mM [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulphonic acid monosodium salt]. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Thereafter, the absorbance of the mixture was measured at 562 nm using UV-visible spectrophotometer (6305 - UV-visible spectrophotometer (190-1000 nm) – JENWAY). Thus, the percentage inhibition of Ferrozine–Fe^{2+} complex formation was calculated by using the following formula:

Inhibition of Ferrozine–Fe^{2+} complex (%) = $[(A_0\text{-}A/\ A_0) \times 100]$ Where A_0 = Absorbance of control; A = Absorbance of Sample.

IC_{50} was determined as the concentration of extract needed to cause a 50% inhibition of ferrosine Fe^{2+} complex. EDTA- Na_2 was used as reference standard and ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased and the measurement of the chelating activity of the coexisting chelator.

Statistical analysis

All data were analyzed using the SPSS statistical package (version20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA, followed by the Student Newman keuils post hoc test, was used to compare differences in the data ($P < 0,05$). Values are expressed as the mean ± SD.

RESULTS:

Extract and fraction yield

Significant variations in extraction yield were found among different seaweeds (**Table 1**). The highest extraction yield was recorded for the methanol extract of *Fucus spiralis* which had higher yield of 12,25% followed by *Bifucaria bifurcata* and *Ulva rigida* (10,85% and 10,75% respectively*). Enteromorpha intestinalis* had the lowest yield (8,52%). Among the fraction, aqueous fraction had a higher yield in all the seaweeds.

Table1. Yield of total extract (as % W/W of seaweed on dry weight basis) and fractions (as % of total methanol extract) of four seaweed (n = 3)

	Total ME			Fraction		
Seaweeds		**PE**	**EA**	**DCM**	**BuOH**	**Aqueous**
Ulva rigida	**10,75 ± 0,95[b]**	**21,48 ± 0,26**	**18,77 ± 0,72**	**1,84 ± 0,13**	**10,47 ±0,55**	**47,10 ± 0,14**
Fucus spiralis	**12,25 ± 0,94[a]**	**20,96 ± 0,66**	**23,55 ± 0,67**	**1,40 ±0,35**	**14,57 ±0,37**	**38,69 ± 0,32**
Bifucaria bifucarta	**10,85 ± 0,70[b]**	**22,23 ±1,49**	**7,57 ± 0,06**	**1,01 ±0,07**	**21,72 ±0,79**	**45,95 ± 0,43**
Enteromorpha intestinalis	**8,52 ± 0,68[c]**	**20,73 ±0,22**	**19,63± 0,67**	**5,10 ±0,20**	**16,72 ± 0,41**	**37,45 ± 0,19**

All the values are mean ± SD; SD: standard deviation

ME: methanol; PE: petroleum ether; EA: ethyl acetate; DCM: dichloromethane; BuOH: butanol

a-c column wise values with same superscripts of this type, indicate no significant difference (P ‹ 0,05)

Total phenolic content

Table 2. Total phenol content en mg gallic acid equivalent/g extract of methanolic extract and fraction obtained from four seaweed (n = 3)

	Total ME			Fraction		
Seaweeds		**PE**	**EA**	**DCM**	**BuOH**	**Aqueous**
Ulva rigida	**1,6 [d] ± 0,08**	**0,16 [y] ± 0,01**	**2,51 [v] ± 0,05**	**6,29 [s] ± 0, 26**	**4,82 [t] ± 0,02**	**1,46 [w] ± 0,01**
Fucus spiralis	**6,6 [b] ± 0,2**	**1 [w] ± 0,03**	**6,70 [s] ± 0,03**	**29,79 [p] ± 0,42**	**2,96 [v] ± 0,02**	**3,26 [u] ±0,15**
Bifucaria bifucarta	**8,6 [a] ± 0,3**	**1,25 [w] ± 0,04**	**24,44 [q] ± 0,51**	**14,61 [r] ± 0,32**	**6,92 [s] ± 0,26**	**3,06 [u] ±0,12**
Enteromorpha intestinalis	**3,2 [c] ± 0,02**	**0,86 [x] ± 0,03**	**3,62 [u] ± 0,03**	**4,07 [t] ± 0,06**	**6,87 [s] ± 0,04**	**3,03 [u] ±0,01**

All the values are mean ± SD; SD: standard deviation

ME: methanol; PE: petroleum ether; EA: ethyl acetate; DCM: dichloromethane; BuOH: butanol

a-d column wise values with same superscripts of this type indicate no significant difference (P ‹ 0,05)

p-y Row wise values with different superscript of this, indicate significant difference (P ‹ 0,05)

The seaweed species showed significant differences in total phenolic content of crude methanol extract and solvent fraction ranging from 0,16 to 29,79 mg gallic acid equivalent/g DW extract (**Table 2**). Brown algae, *Bifucaria bifurcata* and *Fucus spiralis* contained higher amounts of polyphenols than green algae, *Enteromorpha intestinalis* and *Ulva rigida* . DCM fraction of *Fucus spiralis* and EA fraction of *Bifucaria bifurcate showed* higher phenolic content (29,79 and 24,44 mg gallic acid equivalent/g DW extract respectively) when compared to other solvent fraction and total methanol extract. In the case of *Ulva rigida* and *Enteromorpha intestinalis*, the high values of phenolic content were found in DCM and BuOH fraction respectively (6,29 and 6,87 mg gallic acid equivalent/g DW extract respectively).

DPPH radical scavenging activity

DPPH radical scavenging activity differed between the algae species tested (Figures 1 and 3). All the activity were however relatively lower than that of a standard compounds. The reference compounds, ascorbic acid (EC_{50} = 0,11 mg /ml) and α tocopherol (EC_{50} = 0,215 mg /ml) exhibited higher radical scavenging effect when compared with all the seaweed extracts tested. The DPPH scavenging activity of ascorbic acid was more than 99% at concentration 0,3 mg /ml when the DPPH scavenging activity of α tocopherol was 97% at concentration 0,6 mg /ml (Figure 2). Concentration dependency of antioxidant activity was investigated as a function of DPPH radical scavenging activity. The antioxidant activity increased with increasing concentration in all the extract. At concentration of 3mg /ml, no considerable differences was found in DPPH radical scavenging activity of total methanolic extract from four macroalgae tested varying of 29,9% in *Ulva rigida* and *Bifucaria bifurcata* to 32% in *Enteromorpha intestinalis* and *Fucus spiralis*. The comparison of DPPH radical scavenging activity of solvent fraction from seaweeds are shown in Figure 3. Among all seaweed extract, aqueous fraction of *Enteromorpha intestinalis* exhibited the most effective scavenging ability on DPPH radical (68,70%) than other fraction at concentration 3 mg/ml followed by aqueous and EA fraction of *Fucus spiralis* (49,50% and 50% respectively). The aqueous, EA and BuOH fraction of *Ulva rigida and others fraction of Fucus spiralis* (PE, DCM and BuOH fraction) showed relatively less scavenging potentials (less than 33%). The lowest values of DPPH radical scavenging activity was observed in solvent fraction of *Bifucari bifurcata* which was lower than other seaweed extract solvent fraction of *Bifucari bifurcata* which was lower than other seaweed extract.

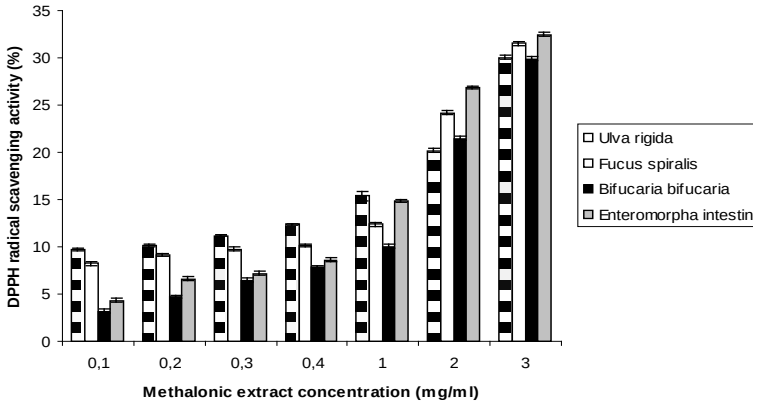

Figure 1: DPPH radical scavenging activity (%) of total methanolic extract obtained for four seaweeds (n = 3)

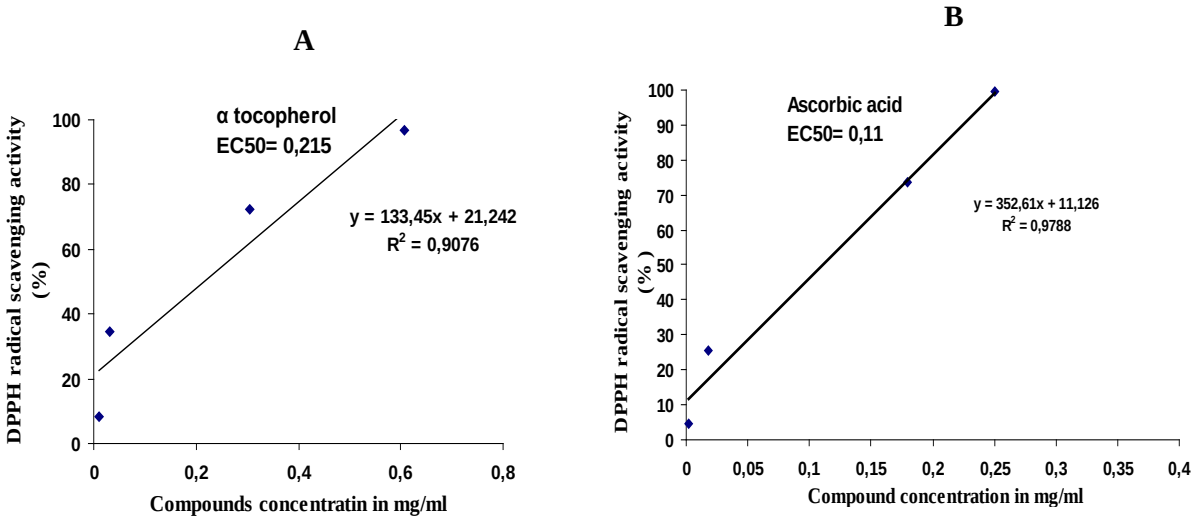

Figure 2: DPPH radical scavenging activition of reference compounds (n = 3)
A: α tocopherol; **B:** Ascorbic acid

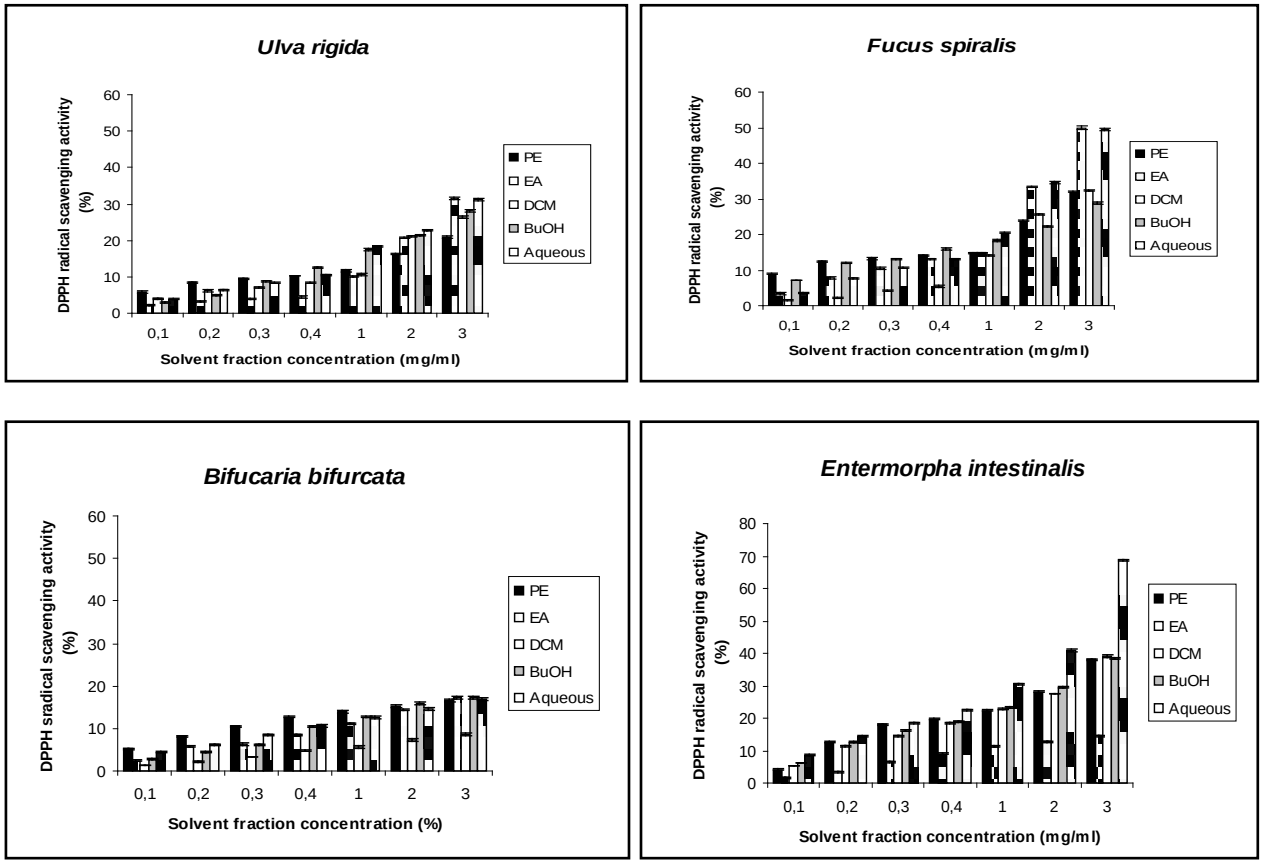

Figure 3: DPPH radical scavenging activity (%) of solvent fraction obtained for four seaweeds (n = 3)

Metal chelating activity

The IC_{50} of chelating effect of extract on Fe^{2+} and ferrozine complex formation was shown in Table 3. Metal chelating activities of all algal extracts were tested at the concentration of 0,01mg/ml to 1 mg/ml. EDTA-Na2 tested in this experiment was an excellent chelator for ferrous ions, and its chelating capacity was 96% at a concentration low as 0,05 mg/ml (IC_{50} = 0,02 mg/ml), but much higher than all the seaweeds extract (**Figure 4**).

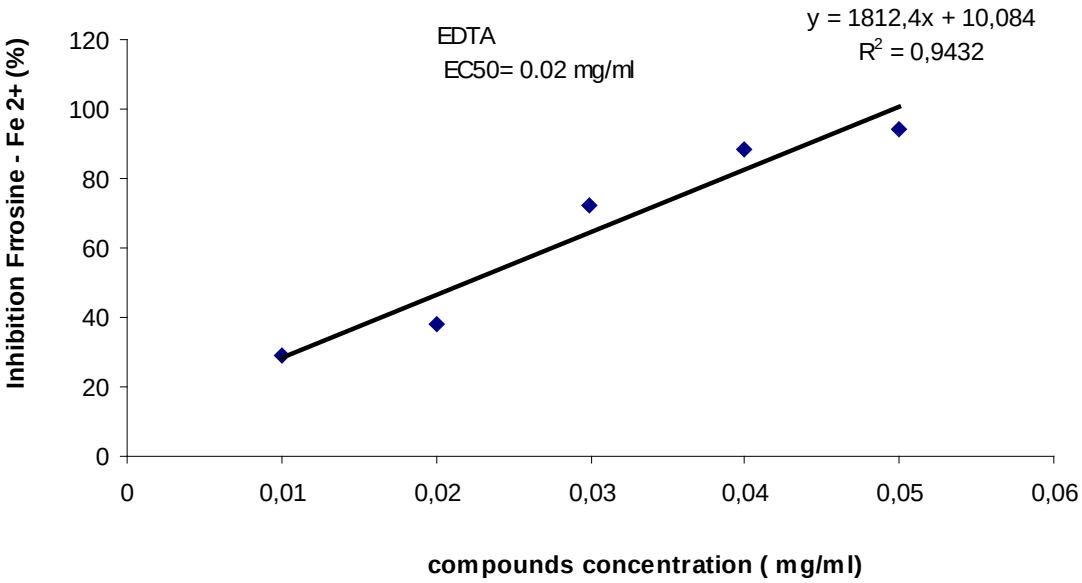

Figure 4: Chelation of ferrous ion by EDTA compound

As shown in table 3, the methanolic extract obtained from *Ulva rigida*, *Fucus spi*ralis and *Bifucaria bifucarta* showed 50% at the concentration of 0,5mg/ml, 0,45 mg/ml and 0,75 mg/ml respectively. *Enteromorpha intestinalis* had rather weak chelating capacity (IC_{50} =1,12 mg/ml). In accordance with DPPH results, potent chelation abilities were again detected in methanolic extract and its solvent fraction. All solvent and aqueous fraction of *Ulva rigida* and *Fucus spi*ralis exhibited equivalent chelating effects as compared to EDTA-Na2 standard compound. The IC_{50} was lower than 0,05mg/ml. The other fractions of *Bifucaria bifurcata* and *Enteromorpha intestinalis* showed less potent chelating effect as compared to other seaweeds (IC_{50} was high than 0,4mg/ml).Interestingly, however aqueous fraction from *Enteromorpha intestinalis* with lower polyphenol level and high scavenging activity exhibited the lower chelation effects (IC_{50} =1,70 mg/ml).

Tableau 3. Chelation of ferrous ions by methanolic extract and solvent fraction obtained for four seaweeds (n = 3)

				IC_{50} (mg/ml)		
Seaweeds	**Total ME**	**PE**	**EA**	**DCM**	**BuOH**	**Aqueous**
Ulva rigida	**0,5 0 [c] ± 0,04**	**0,02 [p] ± 0,00**	**0,01 [p] ± 0,00**	**0,045 [q] ±0,01**	**0,05 [q] ± 0,01**	**0,01[p] ± 0,00**
Fucus spiralis	**0,45 [c] ± 0,01**	**0,015 [p] ± 0,01**	**0,015 [p] ± 0,01**	**0,01 [p] ± 0,00**	**0,04 [q] ± 0,01**	**0,03[q] ± 0,00**
Bifucaria bifurcate	**0,75 [b] ± 0,05**	**0,55[s] ± 0,12**	**0,60 [s] ± 0,10**	**1,5 [u] ± 0,16**	**0,40 [r] ± 0,14**	**1,10 [t] ± 0,08**
Enteromorpha intestinalis	**1,12 [a] ± 0,07**	**0,55 [s] ±0,14**	**2[w] ± 0,20**	**1[t] ± 0,12**	**1,5[u] ± 0,10**	**1,70[v] ± 0,15**

All the values are mean ± SD; SD: standard deviation

ME: methanol; PE: petroleum ether; EA: ethyl acetate; DCM: dichloromethane; BuOH: butanol

a-c column wise values with same superscripts of this type, indicate no significant difference (P ‹ 0,05)

p-w Row wise values with different superscript of this, indicate significant difference (P ‹ 0,05)

Discussion

As compared to the results of the present study, Wang et al., 2009 showed considerable variations in extraction yield among different seaweeds species (Green, red and brown algae). The highest extraction yield was recorded for the water extract of *Ulva lactuca* (44,7%) whereas the lowest extraction was recorded for 70% acetone extract of *Chondrus crispus* (10,5%). For seaweed species tested, the extraction yields of water extracts were higher than those of solvents extracts which indicates that most of the soluble components in seaweeds were high in polarity. In our study, significant difference was observed in the phenolic content of four seaweeds tested. As compared to our result, Yan et al., 2009 observed significant differences in total phenolic content among different seaweeds species (Brown, red and green algae). Brown algae generally contained higher amounts of polyphenols than red and green algae. This is agreement with our study. Another study of Connan et al., 2006 showed high levels of total phenolic content fucoid seaweed species with the highest amount of 70% acetone extract of *Fucus vesiculosus* (24,2 mg gallic acid equivalent/g DW extract). For most seaweed species tested, 70% aqueous acetone was more efficient to extract polyphenolic compounds compared to water. In our study, the organic solvents such as EA, DCM, BuOH are more efficient to extract polyphenolic compounds compared to water. The phenolic compounds are generally more soluble in polar organic solvent than in water. On the contrary, other compounds such as water soluble polysaccharides, proteins and organic acid were simultaneously extracted when using water as the only extractant (Chirinos et al., 2007). Phenolic compounds are commonly found

in plants and have been reported to have several biological activities including antioxidant proprieties. Another report revealed that polyphenol of marine seaweeds have antioxidant activity (Yan and al., 1999; Kuda et al., 2005). However, the major active compounds in different seaweeds extracts have been reported to be phlorotanins and fucoxanthin (Yan et al., 1999; Yan et al.,1996).

DPPH test has been used extensively as a free radical to evaluate reducing substances (Cotelle et al., 1996) and is a useful reagent for investigating the free radical scavenging activity of compounds (Duan et al., 2006). Because of different extraction, measurement methods and units used in various antioxidant activity studies on seaweed reported in the literature, direct comparison of our results on radical scavenging activity of seaweed extracts with other studies is not feasible. However, similar tendency was observed by Wang et al., 2009. In this study, screening of potential antioxidant activities of water and 70% acetone extracts from ten species (brown, red and green algae) of Icelandic seaweed using antioxidant assay was done. The DPPH radical scavenging activity of water and 70% acetone seaweed extracts increased in a concentration-dependent manner. The highest scavenging activity was recorded in Fucus species. In the case of a red seaweed species, Ganesan et al., 2007 reported that antioxidant activities of methanol extracts of all algae dependency increases with increasing concentration of the extract. It was observed that the extracts containing high levels of polyphenol were not potent DPPH radical scavenging, suggesting that algal polyphenol was not the principal constituent responsible for the antiradical properties of the extracts. As pointed out by some researchers, change in extractant polarity alters its efficacy to extract a specific group of antioxidant compounds and influence the antioxidant properties of the extracts. Other classes of antioxidant compounds such as fucoxanthin and sterols could be partially and simultaneously extracted and thus may have contributed to the overall activities.

The solvent and aqueous fraction of *Ulva rigida* and *Fucus spi*ralis were the most active extract which interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. There was not a direct relation between chelatory activity and the phenolic content. EA fraction from *Enteromorpha intestinalis* with high phenolic content (24,44 mg gallic acid / gDW extract) showed the low chelating of Fe2+. In contrast, the solvent fraction of *Ulva rigida* and *Fucus spi*ralis with low phenolic content showed good chelating activity. The high binding capacities to different heavy metals of algal dietary fibres such as alginate, fucoidan from brown algae and carrageenan, agar from red algae and ulvan from green algae are well documented. However, there are contradictory reports in the literature regarding metal

chelating capacities of polyphenols. Some studies have demonstrated that polyphenol derived from seaweeds are potent ferrous ion chelators (Chew et al., 2008; Senevirathne et al., 2006) and metal chelating potency of phenolic compounds are dependant upon their unique phenolic structure and the number and location of the hydroxyl groups (Santaso et al., 2004). However, our result agrees with other authors which have claimed that meal chelation of some polyphenols play a minor role in the overall antioxidant activities (Rice-Evans and al., 1996). The study conducted by Toth and Pavia (2000), showed that other compounds such as polysaccharids or phylochelatins were more effective than phlorotanins for the detoxification and resistance to copper accumulation in *Ascophyllum nodosum*. In addition, some peptides as well as proteins have also been reported to possess the abilities to chelate metal ions (Saiga et al., 2003).

Conclusion

It can be concluded that crude extracts and fraction obtained from marine algae which was tested (*Ulva rigida, Enteromorpha intestinalis, Fucus spiralis* and *Bifurcaria bifurcate)* exhibit antioxidant activity. The result indicates that methanolic extract and its solvent fraction have more potent chelation abilities of ferrous ion in accordance with DPPH scavenging activity. Different solvent fraction obtained from total (methanol) extract exhibit higher antioxidant activities as compared to the total extract. Thus, this could be due to the fact that crude (methanol) extract tend to have more interfering substances as compared to fractions. Furthermore, no direct relationship was found between antioxidant activity and the total phenolic content, suggesting that polyphenol play a minor role in the metal chelating ability and other compounds such as polysaccharides, protein, organic acid which may contribute in the overall antioxidant activities. The finding of this work are useful for further research to identify, isolate and characterize the specific compound which is responsible for higher antioxidant activity and which may promote their use as natural sources of antioxidants.

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