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Effect of Solvents on the Extractability of Phenolic Constituents and Their Antioxidant Capacity from Irish Seaweed

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ABSTRACT

Seaweeds are being used for human consumption in the Orient and help them live longer with low levels of hypertension, cancer and other ailments. The study aimed at extraction of principle constituents using a wide range of solvents and their mixtures on the basis of polarity, from Irish brown seaweeds namely *Himanthalia elongata*, *Laminaria saccharina* and *Laminaria digitata*. All the extracts were screened for total phenolic content (TPC) and their potential antioxidant capacity, using 2, 2´-diphenyl-1-picrylhydrazyl (DPPH) radical and ferric reducing antioxidant power (FRAP) assay. Among all the solvents tested, 60% methanolic extract and equi-volume mixture of chloroform, diethyl ether and n-hexane (Mix 4) extract exhibited the highest TPC which were in the range of 46.6 ± 2.8 to 156.0 ± 2.4 and 52.7 ± 1.9 to 128.2 ± 1.6 mg gallic acid equivalent/g respectively, among all the seaweed. Interestingly, the same extracts showed the highest antioxidant capacity wherein the value of FRAP ranged from 4.9 ± 0.13 to 11.7 ± 0.23 and 8.3 ± 0.23 to 26.3 ± 0.30 mg trolox equivalent/g, respectively, in all the seaweed studied. Results concluded that different solvents extract different amount of phenolic antioxidant compounds from seaweed. Thus, seaweed can be considered as potential source of natural antioxidants for food and pharmaceuticals purposes.

Keywords:Antioxidant capacity, phenolic compounds, Irish seaweed, solvent extraction

INTRODUCTION

The search for natural antioxidant and effort in order to extract and identify these compounds has received much attention for application in foods and pharmaceuticals. In this consequence, phenolic compounds have been suggested a promising source of natural antioxidants due to their role in the prevention of neurodegenerative disease. Epidemiological data has reviled that a polyphenolics rich diet may result in positive health benefits because of its antioxidant properties (Flora et al., 2007). Oxidation is a natural phenomenon in many living organisms which generates beneficial energy for biological reactions but at the same time it also produces some harmful free radicals and reactive oxygen species (ROS). The excessive production of these free radicals causes oxidative stress which leads to various disorders in human beings (Pong 2003). Therefore, phenolic antioxidants are vital entity which protects the body from these detrimental free radicals. Seaweed is very nutritious, and can be an interesting natural source of new compounds with biological activity that could be used as functional ingredients. These valuable functional ingredients can be extracted and used in other food products due to their strong antioxidant, antimicrobial, antiviral and anticancer properties. Additionally, due to the low content of lipids but high concentration of polysaccharides, minerals, polyunsaturated fatty acids and vitamins, marine algae are also considered to be a good source of healthy food. The diversity in the composition of seaweed could be due to the availability of these plants

in extreme harsh marine environment where they are exposed to a wide range of environmental stress such as light, temperatures and osmotic pressure. These factors can lead to the formation of free radicals and other ROS; therefore, seaweeds must produce a range of compounds in order to protect and survive any serious photodynamic damage (Chandini et al., 2008; Matsukawa et al., 1997). Thus, the aim of this study was to investigate and evaluate the phenolic content and antioxidant capacity of Irish seaweed. All the species were extracted with a range of solvents and their various combinations (based on polarity) in order to take out a range of compounds from them.

MATERIALS AND METHODS

Seaweed materials: Three Irish brown seaweed (*Phaeophyta*) used in the present study were *Himanthalia elongata*, *Laminaria saccharina* and *Laminaria digitata*. The seaweeds were purchased from Quality Sea Veg., Co Donegal, Ireland. Samples were washed thoroughly with fresh water to remove epiphytes, sand and debris and further stored them at -20°C.

Extraction procedure: Extraction of seaweed was carried out according to the method reported by Ganesan et al. (2008) using high polarity solvents such as water, methanol and mixtures thereof; and medium-to-low polarity solvents such as chloroform, diethyl ether, n-hexane and their various combinations. The selected solvents and their mixtures had a wide range of polarity (0 to 9), in order to extract a range of phenolic antioxidant compounds. All the extracts obtained were dried using a rotary evaporator (Buchi Syncore Polyvap, Mason Technology, Dublin, Ireland). Concentrations of 1000 ppm were used for TPC and FRAP analysis whereas 500 ppm was used for DPPH analysis.

Total phenolic content : The amount of TPC in the crude extracts was determined by the method of Taga et al. (1984). Briefly, an aliquot (100 µl) of extracts or standard solution of gallic acid was mixed with 2 ml of sodium carbonate, after 2 min, 100 µl of Folin-Ciocalteau's phenol reagent (Sigma-Aldrich, Germany) was added and mixed thoroughly. The reaction mixture was allowed to stand for 30 min in dark at ambient temperature. Absorbance of all the sample solution against reagent blank was determined at 720 nm with a spectrophotometer (Milton Roy Spectronic 1201). The analyses were done in triplicate and TPC of seaweeds was expressed as mg gallic acid (Sigma-Aldrich, Germany) equivalents (GAE)/ g of extracts (dry weight).

Antioxidant capacity analysis

DPPH radical scavenging assay: This assay was carried out according to the original method of Yen and Chen (1995) and modified by Rajauria et al. (2010). The experiment was performed in a microtiter plate using with 1:1 ratio of 100 µl each of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical solution (165 µM) and samples and absorbance was recorded at 517 nm using a microplate reader (Powerwave, Biotek, Winooski, VT, USA). The ability to scavenge the DPPH radical was calculated using the following equation (Eq 1):

$$\text{Scavenging capacity } (\%) = \left[1 - \left(\frac{A_{sample} - A_{sample\ blank}}{A_{control}}\right)\right] \quad \text{[Eq 1]}$$

Ferric reducing antioxidant power (FRAP) assay: Total antioxidant reducing power of various extracts of seaweed were measured using FRAP assay according to the method reported by Benzie and Strain (1996) with some modifications (Jaiswal et al., 2012). FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), 40 mM hydrochloric acid and 20 mM ferric chloride (Sigma-Aldrich, Germany)) prepared separately

and further experiment was performed in a microtiter plate and absorbance was recorded at 593 nm using a microplate reader (Powerwave, Biotek, Winooski, VT, USA).Trolox (Sigma-Aldrich, Germany) was used as a standard and the results were expressed as mg trolox equivalents (TE)/g extract (dw).

RESULTS AND DISCUSSION

Total phenolic content: Phenolic compounds are plant secondary metabolites and play significant role in antioxidant capacity. In the present study, results indicated that all the extracts exhibited good amount of total phenolic content in all the species (Table 1). The 60% methanolic extract among the high polarity solvents and Mix 4 extract among the medium-to-low polarity solvents contained the highest amount of total phenol in all the species studied. Interestingly, high polarity solvents' extract of *H. elongata* exhibited the highest TPC (156.0 ± 2.4 mg GAE/g) compared to the medium-to-low polarity solvent extracts. On the other hand, in case of *L. saccharina* and *L. digitata*, medium-to-low polarity solvent extracts showed the highest TPC (79.4 ± 0.3 and 52.7 ± 1.9 mg GAE/g, respectively) compared to high polarity solvent extracts.

Table 1. Total phenolic content estimation of Irish seaweed

Extraction Solvets	Total Phenolic Content (mg GAE /g)		
	H. elongata	***L. saccharina***	***L. digitata***
High polarity solvents and their mixtures			
Water	54.2 ± 2.1	16.2 ± 1.4	13.8 ± 1.2
Methanol (20%)	95.2 ± 2.2	21.8 ± 1.9	17.5 ± 1.2
Methanol (40%)	118.6 ± 3.3	43.5 ± 1.9	28.6 ± 1.9
Methanol (60%)	156.0 ± 2.4	72.2 ± 2.1	46.6 ± 2.8
Methanol (80%)	91.7 ± 1.7	40.4 ± 2.3	25.9 ± 2.0
Methanol	37.8 ± 0.7	28.2 ± 1.5	21.5 ± 1.5
Medium-to-low polarity solvents and their mixtures			
Chloroform	31.2 ± 2.0	12.5 ± 0.3	15.2 ± 1.5
Diethyl ether	105.2 ± 1.8	53.4 ± 0.6	48.9 ± 2.4
N-hexane	14.1 ± 0.9	9.5 ± 1.3	7.7 ± 0.6
Mix 1	71.5 ± 2.3	31.1 ± 0.4	29.1 ± 1.5
Mix 2	62.3 ± 2.8	25.2 ± 1.1	27.5 ± 1.2
Mix 3	88.9 ± 2.6	39.8 ± 1.6	46.8 ± 2.0
Mix 4	128.2 ± 1.6	79.4 ± 0.3	52.7 ± 1.9

Mix 1: hexane and diethyl ether (1:1; v/v) ; Mix2: hexane and chloroform (1:1; v/v); Mix3: diethyl ether and chloroform (1:1; v/v); Mix4: hexane, diethyl ether and chloroform (1:1:1; v/v/v)

Antioxidant capacity analysis: Results from Fig. 1 show that the DPPH radical scavenging ability of all the seaweed samples can be ranked as 60% methanol > 40% methanol > 80% methanol > 20% methanol > water > methanol extracts for High polarity solvents. Similarly for the medium-to-low polarity solvents, the scavenging capacity ranked as Mix 4 > diethyl ether > Mix 2 > Mix 1 > Mix 3 > chloroform > n-hexane extract. In case of High polarity solvent extracts, DPPH radical scavenging capacity increased with the increment of methanol concentration in water upto 60% and then reduced. It is reported that polarity significantly affect the extraction, as smaller change in extractant polarity alters its efficacy to extract a specific group of antioxidant compounds along with its antioxidant properties (Zhou and Yu 2004). At the concentration of 500 ppm, 60% methanolic extract of *H. elongata* showed 100% scavenging while the same extract of *L. saccharina* and *L. digitata*

exhibited 82.9% and 33.0% scavenging against DPPH radical, respectively (Figure 1A). On the other hand, Mix 4 extract exhibited from 64.9 to 89.7% scavenging capacity at the same concentration, among all the seaweed studied (Figure 1B).

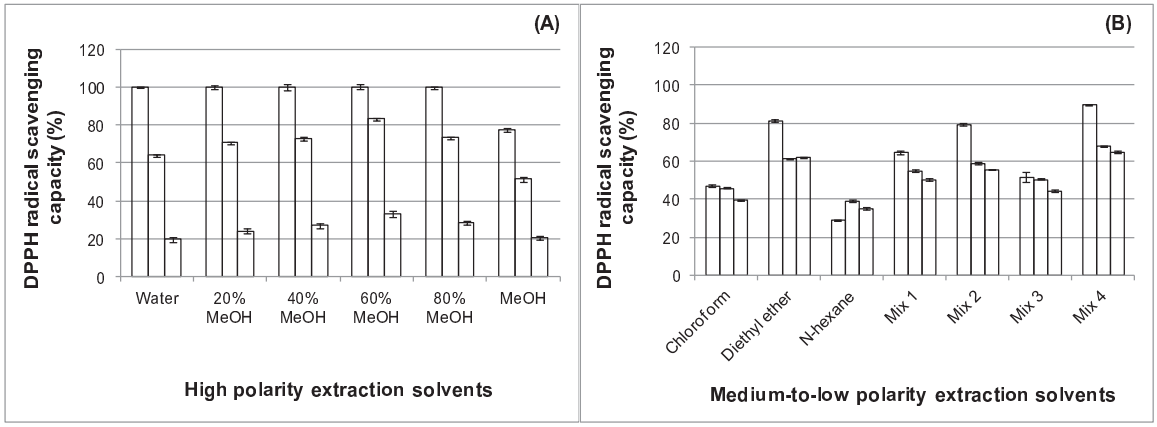

Figure 1. DPPH radical scavenging capacity of Irish seaweed. [MeOH: methanol; Mix 1: hexane and diethyl ether; Mix2: hexane and chloroform; Mix3: diethyl ether and chloroform and Mix4: hexane, diethyl ether and chloroform].

The reducing power of brown seaweeds was in the range of 4.7 ± 0.1 to 11.7 ± 0.2 in *H. elongata*, 4.0 ± 0.2 to 6.4 ± 0.2 in *L. saccharina* and 2.4 ± 0.2 to 4.9 ± 0.1 in *L. digitata* extracts, with the varying concentration of methanol in water (Figure 2A). Among all the tested concentrations of methanol from 0–100% in water, extract from 60% methanol exhibited highest reducing power among all the species studied. Moreover, all the extracts from medium-to-low polarity solvents exhibited the highest reducing power in *H. elongata* (5.5 ± 0.2 to 26.3 ± 0.3 mg TE/g) followed by *L. saccharina* (1.6 ± 0.1 to 10.9 ± 0.3 mg TE/g) and *L. digitata* (1.7 ± 0.1 to 8.3 ± 0.2 mg TE/g) (Fig. 2B). Interestingly, Mix 4 extract showed the highest FRAP value compared to the medium-to-low polarity as well as high polarity solvents' extract in all the seaweed studied (Figure 2).

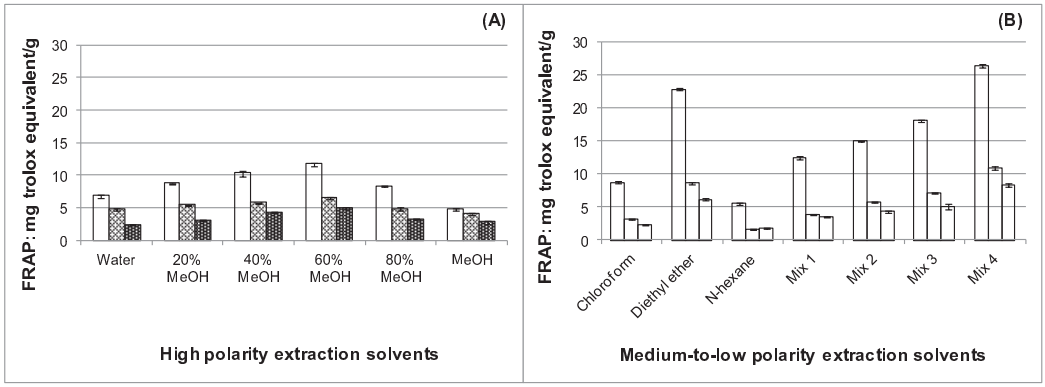

Figure 2: Ferric antioxidant reducing power of Irish seaweed. [MeOH: methanol; Mix 1: hexane and diethyl ether; Mix2: hexane and chloroform; Mix3: diethyl ether and chloroform and Mix4: hexane, diethyl ether and chloroform].

CONCLUSIONS

The above findings indicate that the recovery of phenolic compounds extensively depend on the polarity or dielectric constant of solvents used. Varying amount of TPC and antioxidant capacity indicate that seaweed contain a range of phenolic compounds and recovery of those compounds solely depends upon the selection of best extractant. Additionally, antioxidant capacity analysis indicates that seaweed could be a potential source of novel natural phenolic compounds.

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