

Antioxidant Activity of the Brown Macroalgae *Fucus spiralis* Linnaeus Harvested from the West Coast of Ireland

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Abstract: The extraction and isolation of natural antioxidants with potential in reducing the incidences of oxidative stress in the body and their potential inclusion into functional foods is a major topic of research at present. In this study, the aim was to investigate food-friendly Accelerated Solvent Extraction[®] (ASE[®]) samples and a Viscozyme[®] hydrolysate of the brown macroalga *Fucus spiralis* Linnaeus for total phenolic content and antioxidant activities. Furthermore, the effect of ultra-filtration steps on the total phenolic content and antioxidant activities of the *Fucus spiralis* hydrolysate were also evaluated. *Fucus spiralis* ethanolic-aqueous and methanolic-aqueous ASE[®] extracts displayed high phenolic contents of 37.03±3.01 and 39.04±5.72 µg phloroglucinol equivalents mg/sample, respectively. Both the *Fucus spiralis* Viscozyme[®] hydrolysate and ASE[®] extracts displayed *in vitro* antioxidant activities. Our findings suggest that food-friendly extracts of *Fucus spiralis* show potential as alternative sources of antioxidants.

Keywords: ASE[®], enzymatic hydrolysis, *Fucus spiralis*, marine bioactives, peptides, phlorotannins, ultra-filtration

INTRODUCTION

Irish macroalgae tolerate harsh marine conditions and are required to defend themselves against various ecological hurdles (Connan *et al.*, 2007; Plaza *et al.*, 2008b; Smit, 2004). Macroalgae adapt to their environment by means of chemical protective mechanisms which include, among others, the production of antioxidants to limit oxidative damage (Chew *et al.*, 2008) and feeding-deterrents to prevent grazing (Manilal *et al.*, 2009). For these reasons, macroalgae may provide a source of novel marine bioactive compounds that have different bioactive characteristics to those found in terrestrial environments (Plaza *et al.*, 2008a). Brown macroalgae, in particular, have been reported to have high antioxidant capabilities (Vinayak *et al.*, 2011; Wang *et al.*, 2009). It has been suggested that the increased antioxidant capabilities of brown macroalgae, compared to red and green macroalgal classes (Wang *et al.*, 2009), are due to the presence of polyphenolic phlorotannin compounds (Nakai *et al.*, 2006). Polyphenolic phlorotannins consist of polymers of phloroglucinol units and tend to be broadly classified into small (<10-kDa) or large (>10-kDa) molecular size classes (Iken *et al.*, 2007). Phlorotannins are generally arranged into four main classes depending on their means of linkage, by aryl-aryl bonds and/or diaryl-ether bonds (Glombitza and Pauli, 2003), between the phloroglucinol units. They are namely the fuhalol/

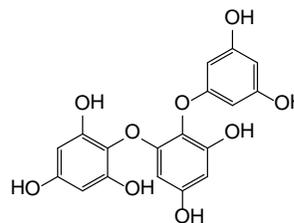


Fig. 1: Phlorotannin phlorethol

phlorethol class, fucol class, fucophlorethol class and eckol/carmalol class (Singh and Bharate, 2006). Figure 1 shows the compound triphlorethol, which is representative of the phlorethol class of phlorotannins, consisting of the phloroglucinol units linked by only aryl-ether bonds. Phlorotannins of the fucol and fucophlorethol classes, identified from *Fucus spiralis* collected in France, have shown greater DPPH[·] radical scavenging capability than ascorbic acid and the monomer phloroglucinol, two compounds used as positive controls for this antioxidant bioassay (Cérantola *et al.*, 2006).

Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are implicated in the development of some chronic diseases, such as heart-health complications and diabetes (Valko *et al.*, 2007). Radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors are antioxidant

compounds present in many foods with the potential to reduce free radical damage (Huang *et al.*, 2005). In the past, concerns have been raised about the possible toxic side-effects resulting from the use of synthetic antioxidants in food (Haigh, 1986). For this reason, the identification of antioxidant compounds from natural sources, such as macroalgae, is a priority area of research for the functional foods and beverages industry sectors. To date, there has been limited research activity aimed at exploiting Irish macroalgae as material for functional food ingredients with enhanced health benefits for the consumer. Functional foods can be defined as foods that beneficially affect; “one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of disease risk” (Diplock *et al.*, 1999).

The brown macroalga *Fucus spiralis* Linnaeus, of the order Fucales, is an inter-tidal species commonly found around the coasts of Ireland and Britain (White, 2008). Methanolic extracts of Irish brown macroalgae, *Laminaria digitata*, *Laminaria saccharina* and *Himanthalia elongata*, previously have displayed high Total Phenol Contents (TPC) and 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH[•]) radical scavenging capabilities (Cox *et al.*, 2010). Pinteus *et al.* (2009) reported that a *Fucus spiralis* methanol fraction from a Portuguese species had the highest Total Phenol Content (TPC) and DPPH[•] radical scavenging capability compared with the antioxidant activities of methanol, hexane and dichloromethane fractions from seven other macroalgal species.

Recently, the successful extraction of bioactive compounds including phenolic acids, hydroxybenzaldehydes (Onofrejová *et al.*, 2010) and sulphated polysaccharides (Costa *et al.*, 2010) from various macroalgae was carried out. Accelerated Solvent Extraction[®] (ASE[®]) previously has been used to generate antioxidant extracts from *Himanthalia elongate* (Plaza *et al.*, 2010), *Bifurcaria bifurcata*, *Cystoseira tamariscifolia*, *Fucus ceranoides* and *Halidrys siliquosa* (Zubia *et al.*, 2009) and antiviral extracts from *Himanthalia elongate* (Santoyo *et al.*, 2010). ASE[®] has also been used to extract carotenoids, fatty acids and phenols from *Himanthalia elongate* (Plaza *et al.*, 2010). Enzymatic hydrolysis methods have also been carried out for the generation of antioxidant (Wang *et al.*, 2010; Heo *et al.*, 2005; Ahn *et al.*, 2004) and antihypertensive hydrolysates (Sato *et al.*, 2002; Suetsuna and Nakano, 2000). Wang *et al.* (2010) have generated crude polyphenol and polysaccharide fractions that exhibited *in vitro* antioxidant activity from an Umamizyme hydrolysate of *Palmaria palmata*. The di-peptides, Val-Tyr, Ile-Tyr, Phe-Tyr and Ile-Trp, have been isolated from a Protease S “Amano” hydrolysate from *Undaria pinnatifida* and, following synthesis of these peptides, *in vitro* ACE-I-

inhibitory activity and *in vivo* antihypertensive activity has been demonstrated in Spontaneously Hypertensive Rats (SHRs) (Sato *et al.*, 2002). This study aims to investigate how effective the Viscozyme[®] hydrolysis method and ASE[®] methods are for the generation of antioxidant extracts from *Fucus spiralis*.

MATERIALS AND METHODS

This study was carried out at the Nutraceutical facility within the Food BioSciences Department, Teagasc Food Research Centre, Ashtown, Dublin 15, between February 2009 and January 2011.

Figure 2 contains an experimental overview of the processes involved.

Chemicals: All chemicals used were reagent grade. 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox[®]) and Viscozyme[®] multi-enzyme complex were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium dihydrogen orthophosphate 1-hydrate and disodium hydrogen orthophosphate 2-hydrate were acquired from BDH (VWR International, West Chester, Pennsylvania, USA). Methanol (Hipersolv for HPLC) was purchased from VWR International. Water (ROMIL-SpS[™] Super Purity for HPLC) was obtained from Lennox (Naas Road, Dublin 12, Ireland). Diatomaceous earth was obtained from Dionex Corp. (Sunnyvale, CA, USA).

Apparatus: A New Brunswick[®] bio-reactor (New Brunswick Scientific, Edison, NJ, USA) was used for macroalgal hydrolysis. An Accelerated Solvent Extraction[®] (ASE[®]) 200 Extraction System from Dionex Corp. (Sunnyvale, CA, USA) was used to carry out the solvent extractions. Millipore[®] Prep/Scale[™] Tangential Flow Filtration (TFF) modules (Millipore[®], Billerica, MA, USA, 01824) were used for the ultra-filtration process. A Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) was used to measure the absorbance values in cuvette-based antioxidant assays. A FLUO star Omega micro plate reader system (BMG LABTECH GmbH, Offenburg, Germany) was used to measure the antioxidant absorbance values.

Macroalgal materials: Brown macroalgal samples used in this study were supplied by the Irish Seaweed Centre, National University of Ireland, Galway, (NUIG and ISCG) as part of the Marine Functional Foods Research Initiative/NutraMara project and were assigned specific collection numbers that related to the area of location and the date of sample collection. *Fucus spiralis* (ISCG0022) was collected near Spiddal, Co. Galway on February 2nd 2009. *Cystoseira*

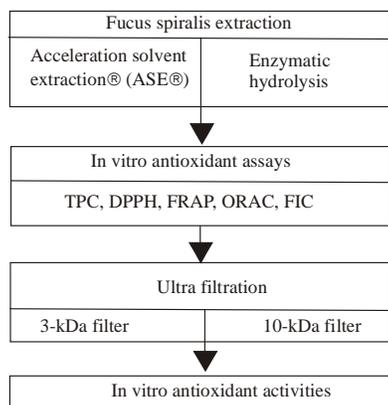


Fig. 2: Experimental overview

tamariscifolia (ISCG0039) was collected near Finavarra, Co. Clare on May 7th 2009.

Hydrolysis of *Fucus spiralis* ISCG0022 with Viscozyme®: A sample of freeze-dried *Fucus spiralis* ISCG0022, (15 g) was ground into small particles using a pestle and mortar. Ground samples were mixed with 500 mL of HPLC-grade water. This mixture was autoclaved at 110°C for 10 min to denature endogenous macroalgal enzymes. Viscozyme® (Sigma), a multi-enzyme complex containing a wide-range of carbohydrases, was used for the hydrolysis process. Hydrolysis was carried out in a New Brunswick® bio-reactor for 8 h at a pH of 5.5 and an agitation rate of 200 rpm and at a temperature of 50°C. Viscozyme® was subsequently added to the macroalgal water mixture (enzyme: substrate ratio; 1:10 w/v). A control hydrolysis process was also carried out where no enzyme was added to the macroalgal mixture. Following hydrolysis, enzymes were deactivated at 98°C for 15 min. The hydrolysate was clarified by centrifugation at 3000 rpm, for 20 min at 4°C. Hydrolyses were carried out in triplicate. Hydrolysates were subsequently freeze-dried and stored at -60°C until further use.

Ultra-filtration of *Fucus spiralis* hydrolysate: The *Fucus spiralis* enzymatic hydrolysates were filtered using the Millipore® ultra-filtration system and the manufacturer's instructions. Hydrolysates were dissolved in HPLC-grade water and clarified by centrifugation at 3000 rpm for 15 min at 4°C. Prep/Scale™ TFF ultra-filtration modules were used to obtain 3-kDa and 10-kDa ultra-filtrates. Ultra-filtrates were freeze-dried and stored at -60°C until further use.

Accelerated solvent extraction® (ASE®): Freeze-dried *Fucus spiralis* ISCG0022 and *Cystoseira tamariscifolia* ISCG0039 samples were ground into small particles using a pestle and mortar. Solvent extractions of the macroalgal samples were performed using an

Accelerated Solvent Extractor (ASE® 200) equipped with a solvent controller unit. Macroalgal samples were mixed with silica, at a sample: silica ratio of 1:2 w/w and then loaded into cells packed with diatomaceous earth. The cells were filled with the solvent(s) and an initial heat-up step was employed. A static extraction was performed and the cell was rinsed with extraction solvent. The solvent was purged from the cell with N₂ gas and finally depressurization occurred. Between extractions, a rinse of the complete system was carried out using isopropyl alcohol to avoid carry-over of extract. *Fucus spiralis* was extracted using an ethanol water (80:20 v/v) mixture at 100°C and a methanol water (60:40 v/v) solvent mixture at 90°C. *Cystoseira tamariscifolia* was extracted using an ethanol water (80:20 v/v) mixture at 100°C. For both types of ASE® solvent mixtures, the pressure was set at 1000 psi, the flush volume was 75% of the cell volume and the purge times applied were 90 sec for 22 mL cells and 200 sec for 33 mL cells. All extractions were carried out in triplicate. Extracts were dried using a Labconco® centrifugal vacuum concentrator set at 40°C. Dried extracts were stored at -60°C until further use.

Determination of total phenol content: The Total Phenol Content (TPC) of the *Fucus spiralis* ISCG0022 ASE® extracts, hydrolysates, ultra-filtrates and *Cystoseira tamariscifolia* ISCG0039 (positive control) ASE® extracts were assessed using the method of Singleton and colleagues (Singleton *et al.*, 1999). Phloroglucinol was used as the standard for assessment of total phenol content. An aqueous phloroglucinol stock solution (120 mg/L) was diluted with different volumes of distilled water to make a serial dilutions set (100, 80, 60, 40, 20 mg/mL, respectively) for the generation of a standard curve. For the analysis, 100 µL of macroalgal sample, diluted to 0.75 mg/mL, or standard solution, 100 µL of methanol, 100 µL of Folin-Ciocalteu reagent and 700 µL of 20% sodium carbonate were added to micro-centrifuge tubes. The samples were vortexed immediately and tubes were incubated in the dark for 20 min at room temperature. After incubation, all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer. All measurements were carried out in triplicate. The measurement values were compared to the calibration curve generated using the phloroglucinol standard and expressed as milligram phloroglucinol equivalents (PE) per gram of sample (mg PE/g). *Cystoseira tamariscifolia* ISCG0039 ASE® extracts were used as a positive control as this species was previously reported as having a high phenolic content (10.91±0.07% dry weight) and DPPH· radical scavenging activity (EC₅₀ of 0.49 mg/mL) (Zubia *et al.*, 2009). Zubia and colleagues (Zubia *et al.*, 2009) employed a dichloromethane: methanol (1:1, v/v)

solvent mixture for the extraction, whereas in this study *Cystoseira tamariscifolia* was extracted with an aqueous-ethanol solvent mixture.

Antioxidant assays:

DPPH[•] radical scavenging ability: A modified version of the DPPH[•] assay method of Goupy and co-workers (Goupy *et al.*, 1999) was used to determine the DPPH[•] radical scavenging capabilities of *Fucus spiralis* ASE[®] extracts, hydrolysates, ultra-filtrates and *Cystoseira tamariscifolia* ASE[®] extracts (positive control). Briefly, a working DPPH[•] solution (0.048 mg/mL) was prepared by making a 1 in 5 dilution of the methanolic DPPH[•] stock solution (2.38 mg/mL). Prior to analysis, serial dilutions of the *Fucus spiralis* and *Cystoseira tamariscifolia* stock samples (1.5 mg/mL) were prepared at concentrations of 0.75, 0.3, 0.15, 0.075, 0.03 mg/mL, respectively. In all experiments Trolox[®] was used as the standard. The standard/diluted sample (500 µL) and DPPH[•] working solution (500 µL) were added to a micro-centrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature. The absorbance was then measured against methanol (blank solution) at 515 nm. The decrease in absorbance of the sample extract was calculated by comparison to a control (500 µL sample extraction solvent and 500 µL DPPH[•]). The relative decrease in absorbance (PI) was calculated as follows:

$$PI (\%) = [1 - (A_e/A_b)] \times 100$$

where,

A_e = Absorbance of sample extract

A_b = Absorbance of control

The PIs used to calculate the related antioxidant activity were superior (PI₁) and inferior (PI₂) to the value estimated at the 50% decrease of DPPH[•] absorbance (Ollanketo *et al.*, 2002). Antioxidant activity was expressed as Antiradical Power (ARP), which is the reciprocal of the IC₅₀ (mg/mL). The IC₅₀ is defined as the concentration of sample extract that produces a 50% reduction of the DPPH[•] radical absorbance (Ollanketo *et al.*, 2002). The higher the ARP value the stronger the radical scavenging activity of a sample (Brand-Williams *et al.*, 1995).

Ferric reducing antioxidant power: The Ferric Reducing Antioxidant Power (FRAP) of *Fucus spiralis* ASE[®] extracts, hydrolysates, ultra-filtrates and *Cystoseira tamariscifolia* ASE[®] extracts (positive control) was assayed according to a previously described method (Stratil *et al.*, 2006) with slight modifications. The oxidant in the FRAP assay consisted of a reagent mixture that was prepared fresh on the day of use by mixing acetate buffer (pH 3.6), ferric chloridesolution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in the ratio of 10:1:1,

respectively. The FRAP reagent was heated, while protected from light using aluminum foil, until it had reached a temperature of 37°C. A Trolox[®] standard curve, using standard concentrations of 0.1 mM-0.4 mM and two-fold dilutions of the *Fucus spiralis* stock samples (1.5 mg/mL), were prepared. The assay mixture contained 20 µL of MeOH (blank), diluted sample or standard and 180 µL of FRAP reagent in a microplate. The absorbances were measured at 593 nm on the automated FLUOstar Omega microplate reader system. The Trolox[®] standard curve was used to calculate the antioxidant activity of the samples in relation to Trolox[®] and was expressed as milligram Trolox[®] equivalents (TE) per gram of sample (mg TE/g).

Oxygen Radical Antioxidant Capacity (ORAC): This assay was carried out according to the method of Ou *et al.* (2001) using fluorescein as the fluorescent probe. The assay mixture consisted of 10 nM fluorescein (150 µL), 240 mM AAPH (2, 2'-azo-bis (2-amidinopropane) dihydrochloride, 25 µL) and 25 µL of either standard solution, *Fucus spiralis* ASE[®] extracts, hydrolysates, ultra-filtrates or *Cystoseira tamariscifolia* ASE[®] extracts (used as the positive control). The blank for this experiment consisted of 10 mM phosphate buffer (pH 7.4), which was added instead of sample or standard. Trolox[®], at different concentrations (5-60 µM), was used to obtain a standard curve and to compare ORAC values of various samples (expressed as mg TE/g). The fluorescence of the assay mixtures was recorded at 90-second intervals with the automated FLUOstar Omega microplate reader system. The area between the fluorescence decay curve of the blank and the sample extract is used to calculate the ORAC values of the samples. The data was analyzed using MARS data analysis software, linked to the Omega reader control software.

Ferrous Ion Chelating (FIC) capability assay: The Ferrous Ion Chelating (FIC) capability of *Fucus spiralis* ASE[®] extracts, hydrolysates, ultra-filtrates and *Cystoseira tamariscifolia* ASE[®] extracts (positive control) was assessed using a previously described method (Decker and Welch, 1990), as modified by Wang and co-workers (Wang *et al.*, 2009). *Fucus spiralis* samples (100 µL) were mixed with distilled water (135 µL) and 2 mM Iron (II) chloride (5 µL) in a microplate. Five millimolarferrozine (10 µL) was then added to start the reaction. The solution in the wells was mixed and allowed to stand for 10 min at room temperature. The absorbance was measured at 562 nm using the automated FLUOstar Omega microplate reader system. Distilled water (100 µL) was added instead of sample solution as a negative control for this experiment. The blank consisted of distilled water (10 µL), which was added instead of ferrozine. Ethylene Diamine Tetra Acetic acid disodium salt (EDTA/Na₂)

was used as the reference standard. Measurements were performed in triplicate. The ferrous ion-chelating ability was calculated as follows:

$$\text{Ferrous ion-chelating ability (\%)} = [(A_0 - (A_1 - A_2)) / A_0] \times 100$$

where,

A_0 : The absorbance of the control

A_1 : The absorbance of the sample or standard

A_2 : The absorbance of the blank

Statistical analysis: All samples were prepared and analysed in triplicate, unless otherwise stated. Measurement values are presented in means±their standard deviation. One-way Analysis of Variance (ANOVA), followed by the Tukey post hoc comparison test, was carried out to test for difference between seaweed extracts in the statistical program Minitab® Release 15 for Windows. The Pearson correlation coefficient (r) and probability-value (p) were used to show correlations and their significance. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Total phenolic content of extracts and ultra-filtrates:

Table 1 shows that, within the total phenol content (TPC) results, the *Fucus spiralis* and *Cystoseira tamariscifolia* ASE® extracts have significantly higher TPC values compared to the *Fucus spiralis* Viscozyme® hydrolysate, ultra-filtrates and control. This may indicate that the ASE® method, which employed high temperatures and aqueous organic solvent mixtures, is a more effective technique for the extraction of polyphenols than enzymatic hydrolysis. In recent years, ASE® has been identified and widely used as an efficient method for the extraction of phenolic compounds from natural sources (Alonso-Salces *et al.*, 2001), including seaweed (Onofrejová *et al.*, 2010).

In this study the *Fucus spiralis* Viscozyme® hydrolysate did not have significantly different TPC to the *Fucus spiralis* control where no Viscozyme® enzyme was added. This suggests that the carbohydrase Viscozyme® did not enhance the extraction of polyphenols during hydrolysis. It has been suggested that the potential level of polyphenols extracted from macroalgae by enzymatic hydrolysis may be species and/or enzyme-dependent (Wang, 2009). For instance, proteases, unlike carbohydrases, can degrade large proteins into smaller peptides and amino acids and, therefore, can reduce the complexes that often occur between proteins and polyphenols, resulting in an increase in unbound polyphenols (Wang, 2009). Notably, the ultra-filtration of the *Fucus spiralis* Viscozyme® hydrolysate did not result in a significant difference of total phenol content in both the 3-kDa and 10-kDa ultra-filtrates. Therefore, if phlorotannins or other classes of polyphenols present in the *Fucus spiralis* hydrolysate were contributing to the antioxidant activity, they are possibly present in the low molecular weight range and with a small degree of polymerization.

Antioxidant activity of extracts and ultra-filtrates:

The DPPH· radical scavenging assay showed the *Fucus spiralis* aqueous ethanol (80:20 v/v) extract had the highest ARP (10.90±2.4) and that this value was significantly higher than those for the corresponding *Fucus spiralis* Viscozyme® hydrolysate (6.14±0.46), 3-kDa ultra-filtrate (6.18±2.16) and the *Fucus spiralis* aqueous methanol extract (80:20 v/v), (6.87±0.74). The DPPH· radical scavenging activity did not differ significantly between the *Fucus spiralis* Viscozyme® hydrolysate, the control, or the 3-kDa and 10-kDa ultra-filtrates and these similar activities may correspond to similar total phenol contents.

The *Fucus spiralis* Viscozyme® hydrolysate 10-kDa ultra-filtrate (27.96±3.14 µg TE/mg sample), the aqueous methanol extract (80:20 v/v) (25.63±0.63 µg TE/mg sample) and the *Cystoseira tamariscifolia*

Table 1: The Total Phenol Content (TPC), DPPH· radical scavenging activity, Ferric Reducing Power (FRAP) capacity, Oxygen Radical Absorbance Capacity (ORAC) and Ferrous Ion Chelating (FIC) ability values for *Fucus spiralis* Viscozyme® hydrolysate, ultra-filtrates, control, ASE® extracts and *Cystoseira tamariscifolia* ASE® extract

Extract/fraction	TPC ^a	DPPH ^b	FRAP ^c	ORAC ^c	FIC ^d
<i>Fucus spiralis</i> Hydrolysate	20.64±1.04 x	6.14±0.46 x	20.88±1.97	15.85±1.99 x e	n/t
<i>Fucus spiralis</i> 10-kDa ultra-filtrate	18.06±2.16 x	6.91±1.55	27.96±3.14 x	6.83±0.70 y	72.60±2.97 x
<i>Fucus spiralis</i> 3-kDa ultra-filtrate	16.29±4.88 x	6.18±2.16 x	12.12±4.59 y	5.72±0.34 y	50.67±5.58 y
<i>Fucus spiralis</i> Control (no enzyme)	22.31±1.36 x	8.01±0.45	18.23±2.52 y, z	15.89±1.4 x	46.42±2.36 y
<i>Fucus spiralis</i> ASE®-EtOH/water	37.03±3.01 y	10.90±2.4 y	20.64±2.19	16.57±0.51 x	15.51±8.70 z
<i>Fucus spiralis</i> ASE®-MeOH/water	39.04±5.72 y	6.87±0.74 x	25.63±0.63 x, z	25.68±0.77 z	52.74±6.46 y
<i>Cystoseira tamariscifolia</i> ASE®-EtOH/water	38.56±6.32 y	7.98±1.89	29.52±6.83 x	9.08±3.61 y	18.18±2.34 z

n/t: Not tested; ^a: µg phloroglucinol equivalents/mg sample; ^b: Antiradical Power (ARP); The reciprocal of the IC₅₀; ^c: µg trolox equivalents/mg sample; ^d: % inhibition of ferrous ion; ^e: Duplicate extracts measured in triplicate; x, y, z: Column-wise values with different letters of this type indicate significant difference (p<0.05)

aqueous ethanol extract (80:20 v/v) (20.64±2.19 µg TE/mg sample) possessed the highest ferric reducing antioxidant power. The 10-kDa ultra-filtrate displayed significantly higher antioxidant activity in this assay than the *Fucus spiralis* Viscozyme® hydrolysate, the control and the 3-kDa ultra-filtrate, suggesting components that have strong antioxidant activities in this assay were concentrated by ultra-filtration with the 10-kDa membrane filter, but were possibly larger in size than 3-kDa.

The *Fucus spiralis* aqueous-methanolic extract displayed the highest antioxidant activity (25.68±0.77 µg TE/mg sample) in the ORAC assay, relative to the positive control (9.08±3.61 µg TE/mg sample). In accordance with results found in the TPC and DPPH· radical scavenging assays, the *Fucus spiralis* Viscozyme® hydrolysates and control have similar activities in the ORAC assay and gave values of 15.85±1.99 and 15.89±1.4 µg TE/mg, respectively.

The 10-kDa *Fucus spiralis* ultra-filtrate had the highest metal (Fe²⁺) chelating ability with a value of 72.60±2.97% inhibition. Interestingly, both the *Fucus spiralis* and *Cystoseira tamariscifolia* aqueous-ethanolic (80:20 v/v) solvent extracts had significant, low Fe²⁺ metal chelating abilities with value of 15.51±8.7 and 18.18±2.34%, respectively. Conflicting reports exist as to how effective macroalgal polyphenols are as metal chelators. Some studies have found that polyphenols are powerful ferrous ion chelators (Wang *et al.*, 2009; Senevirathne *et al.*, 2006), while others have claimed that metal chelation is not a significant function of polyphenol antioxidant activity (Rice-evans *et al.*, 1996). It is widely thought that the potential of polyphenols to act as metal chelators depends on their structure and the proximity of their hydroxyl groups (Morel *et al.*, 1993; Laughton *et al.*, 1991). A study of four flavonoids, baiclein, luteolin, naringenin and quercetin, assessed their ability to inhibit the Fenton reaction of the iron-ATP complex and suggested that the greater iron chelating activity of quercetin and luteolin, compared to baiclein and naringenin, was due the 3', 4'-dihydroxy (catechol) moiety on the B ring of the compounds (Cheng and Breen, 2000). In fact, the presence of *ortho*-dihydroxyl groups, such as 3'-4', 7-8 dihydroxy groups, along with 5-OH and/or 3-OH in conjunction with a C4 keto group and a large number of OH groups, are apparently very important factors for iron-binding of polyphenols (Khokhar and Owusu-Apenten, 2003).

In this study, 3-kDa and 10-kDa membrane filters were used in an attempt to decipher the possible molecular weights of the components responsible for the antioxidant activities of the *Fucus spiralis* Viscozyme® hydrolysates. Membrane filtration methods have been employed previously as means of concentrating bioactive fractions from natural resources

Table 2: Correlation coefficients, *R*, for relationships between assays

	TPC ^a	DPPH ^b	FRAP ^c	FIC ^d
DPPH	0.594*			
FRAP	0.554*	0.317		
FIC	-0.625*	-0.580*	-0.051	
ORAC	0.572*	0.205	0.181	-0.124

*: Indicates that the correlation is significant with p-value <0.05

(Li and Chase, 2010), including macroalgae (Denis *et al.*, 2009). Ye and colleagues (Ye *et al.*, 2008) have purified polysaccharide fractions, from *Sargassum pallidum*, which displayed *in vitro* anti-tumoral activities, with the aid of a Molecular-Weight Cut-Off (MWCO) -membrane. The purification of a mycosporine-like amino acid, porphyra-334, from *Porphyra yezoensis* involved employing a 3-kDa membrane filter (Yoshiki *et al.*, 2009).

Previously, it has been demonstrated that lower molecular cut-off membranes yielded fractions with higher bioactivities compared to higher molecular cut-offs (He *et al.*, 2006; Ranathunga *et al.*, 2006). From the analysis of the means obtained for the TPC and DPPH· radical scavenging activities assays, the 3-kDa and 10-kDa ultra-filtrates of *Fucus spiralis* did not possess significantly different phenolic content or antioxidant activities relative to the *Fucus spiralis* Viscozyme® hydrolysate and *Fucus spiralis* control with no Viscozyme® enzyme added. However, in the FRAP and FIC assays the 10-kDa ultra-filtrate had significantly higher reducing power and chelating ability than the 3-kDa ultra-filtrate and control. A recent study assessed the antioxidant activities of various MWCO fractions (>100-kDa, 30-100-kDa, 10-30-kDa, 5-10-kDa and <5-kDa) of *Fucus vesiculosus* (Wang, 2009). No correlation between the molecular size of macroalgal phlorotannins and *in vitro* antioxidant activity observed (Wang, 2009).

TPC and antioxidant activity correlation analyses:

Significant correlations, though weak, exist between the TPC of all *Fucus spiralis* macroalgal extracts and Viscozyme® hydrolysate ultra-filtrates and their antioxidant activities, which were assessed using the assays listed in Table 2. This outcome may indicate that other bioactive compounds, in addition to algal polyphenols, are contributing to the antioxidant activities assessed using DPPH·, FRAP, Fe²⁺ metal chelating and ORAC assays of the samples. Vinayak *et al.* (2011) and co-workers, as well as Zubia and colleagues (Zubia *et al.*, 2009) found weak correlations ($r = 0.396794$, $p < 0.005$ and $r = -0.399$, $p < 0.01$, respectively) between the TPC and the DPPH· radical scavenging activities of macroalgal extracts. It is often assumed that if the R² values from such correlation analyses are low, it indicates that non-phenolic compounds present are mostly responsible for an extract's antioxidant activity (Babbar *et al.*, 2011; Patthamakanokporn *et al.*, 2008). However, it is

important to also take into consideration the type and quantity of different polyphenols potentially present within the extract. For instance, individual polyphenols may have substantial antioxidant potential, but there may be synergistic or antagonistic interactions between phenolic and non-phenolic compounds that could be affecting the bioactivity (Babbar *et al.*, 2011).

Significant weak negative correlations were found between the TPC and FIC assays and also between DPPH· radical scavenging activity and FIC assay. These observed correlations may imply that phenols are not the principal chelators in some of the *Fucus* samples and that perhaps other compounds such as peptides, which are recognized as effective chelating agents (Pal and Rai, 2010; Seth and Mahoney, 2000), may be responsible for the activities observed. These observed correlations may also indicate that phenolic compounds responsible for the radical scavenging activity are different to those responsible for the metal chelation. For instance, although polyphenols have been reported to be efficient chelators (Kim *et al.*, 2008), this is largely dependent on the specific orientations of suitable functional groups as discussed earlier (Andjelković *et al.*, 2006; Van Acker *et al.*, 1996) and those orientations could possibly negatively affect their radical scavenging capabilities. Previous studies have found no clear correlations between TPC and FIC ability of macroalgal extracts (Wang *et al.*, 2009, 2010) or plant extracts (Ghasemi *et al.*, 2009; Ebrahimzadeh *et al.*, 2009). No other significant correlations were observed.

CONCLUSION

In conclusion, the *Fucus spiralis* ASE[®] extracts generated in this study were shown to possess equivalent Total Phenol Content (TPC) when compared to the reference antioxidant extracts of *Cystoseira tamariscifolia* which were used as positive controls in this study. *Fucus spiralis* ASE[®] ethanolic aqueous extract possessed a high phenolic content and superior DPPH· antiradical power. As ethanol is Generally Recognized As Safe (GRAS) for use as an extraction solvent by the Food and Drug Administration (FDA) it bodes well for the future, potential use of *Fucus spiralis* ethanol extracts as antioxidant, functional food ingredients. It is possible that phlorotannins within the *Fucus spiralis* samples are partially responsible for the antioxidant activity observed, as they are abundant in brown macroalgal species and have a broad range of reported bioactivities. Recently, an enriched phlorotannin fraction that showed anti-diabetic properties was purified from a brown macroalgal *Ascophyllum nodosum* extract (Nwosu *et al.*, 2011). Three phlorotannins, trifucodiphlorethol A, trifucotriphlorethol A and fucotriphlorethol A, displaying chemo-protective potential, through selected

cytochrome P450 enzyme inhibition and antioxidant mechanisms, were also isolated from an ethanol extract of *Fucus vesiculosus* (Parys *et al.*, 2010).

The next steps involved in this research will include isolation and complete chemical characterization of the bioactive compounds responsible for the observed antioxidant activities.

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ABBREVIATIONS

ASE [®]	= Accelerated Solvent Extraction [®]
DPPH	= 2, 2-diphenyl-1-picrylhydrazyl
EtOH	= Ethanol
FIC	= Ferrous ion chelating
FRAP	= Ferric reducing antioxidant power
kDa	= Kilo-Daltons
MeOH	= Methanol
MWCO	= Molecular-weight cut-off
ORAC	= Oxygen radical antioxidant capacity
PGE	= Phloroglucinol equivalents
RNS	= Reactive nitrogen species
ROS	= Reactive oxygen species
TPC	= Total phenol content
TE	= Trolox equivalents

REFERENCES

- Ahn, C.B., Y.J. Jeon, D.S. Kang, T.S. Shin and B.M. Jung, 2004. Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphonlomentaria* by electron spin resonance spectrometry. *Food Res. Int.*, 37: 253-258.
- Alonso-salces, R.M., E. Korta, A. Barranco, L.A. Berrueta, B. Gallo and F. Vicente, 2001. Pressurized liquid extraction for the determination of polyphenols in apple. *J. Chromatogr. A*, 933: 37-43.
- Andjelković, M., J. Van Camp, B. De Meulenaer, G. Depaemelaere, C. Socaciu, M. Verloo and M. Verhe, 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.*, 98: 23-31.
- Babbar, N., H.S. Oberoi, D.S. Uppal and R.T. Patil, 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Res. Int.*, 44: 391-396.

- Brand-williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
- Cérantola, S., F. Breton, E.A. Gall and E. Deslandes, 2006. Co-occurrence and antioxidant activities of fucol and fucophlorethol classes of polymeric phenols in *Fucus spiralis*. Bot. Mar., 49: 347-351.
- Cheng, I.F. and K. Breen, 2000. On the ability of four flavonoids, baiclein, luteolin, naringenin and quercetin, to suppress the Fenton reaction of the iron-ATP complex. Biometals, 13: 77-83.
- Chew, Y.L., Y.Y. Lim, M. Omar and K.S. Khoo, 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci. Technol., 41: 1067-1072.
- Connan, S., E. Deslandes and E.A. Gall, 2007. Influence of day-night and tidal cycles on phenol content and antioxidant capacity in three temperate intertidal brown seaweeds. J. Exp. Mar. Biol. Ecol., 349: 359-369.
- Costa, L.S., G.P. Fidelis, S.L. Cordeiro, R.M. Oliveira, D.A. Sabry, R.B.G. Câmara, L.T.D.B. Nobre, M.S.S.P. Costa, J. Almeida-Lima, E.H.C. Farias, E.L. Leite and H.A.O. Rocha, 2010. Biological activities of sulfated polysaccharides from tropical seaweeds. Biomed. Pharmacother., 64: 21-28.
- Cox, S., N. Abu-Ghannam and S. Gupta, 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int. Food Res. J., 17: 205-220.
- Decker, E.A. and B. Welch, 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem., 38: 674-677.
- Denis, C., A. Massé, J. Fleurence and P. Jaouen, 2009. Concentration and pre-purification with ultrafiltration of a R-phycoerythrin solution extracted from macro-algae *Grateloupiaturuturu*: Process definition and up-scaling. Sep. Purif. Technol., 69: 37-42.
- Diplock, A.T., P.J. Aggett, M. Ashwell, F. Borneo, E.B. Fern and M.B. Roberfroid, 1999. Scientific concepts of functional foods in Europe: Consensus document. Br. J. Nutr., 81: s1-s27.
- Ebrahimzadeh, M.A., S.M. Nabavi and S.F. Nabavi, 2009. Correlation between the *in vitro* iron chelating activity and poly phenol and flavonoid contents of some medicinal plants. Pak. J. Biol. Sci., 12: 934-938.
- Ghasemi, K., Y. Ghasemi and M.A. Ebrahimzadeh, 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak. J. Pharm. Sci., 22: 277-281.
- Glombitza, K.W. and K. Pauli, 2003. Fucols and phlorethols from the brown Alga *Scytothamusaustralis* Hook. et Harv. (Chnoosporaceae). Bot. Mar., 46: 315-320.
- Goupy, P., M. Hugues, P. Boivin and M.J. Amiot, 1999. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric., 79: 1625-1634.
- Haigh, R., 1986. Safety and necessity of antioxidants: EEC approach. Food Chem. Toxicol., 24: 1031-1034.
- He, H., X. Chen, C. Sun, Y. Zhang and P. Gao, 2006. Preparation and functional evaluation of oligopeptide-enriched hydrolysate from shrimp (*Acetes chinensis*) treated with crude protease from *Bacillus* sp. SM98011. Bioresour. Technol., 97: 385-390.
- Heo, S.J., E.J. Park, K.W. Lee and Y.J. Jeon, 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour. Technol., 96: 1613-1623.
- Huang, D., B. Ou and R.L. Prior, 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem., 53: 1841-1856.
- Iken, K., C.D. Amsler, J.M. Hubbard, J.B. McClintock and B.J. Baker, 2007. Allocation patterns of phlorotannins in Antarctic brown algae. Phycologia, 46: 386-395.
- Khokhar, S. and R.K. Owusu-Apenten, 2003. Iron binding characteristics of phenolic compounds: Some tentative structure-activity relations. Food Chem., 81: 133-140.
- Kim, E.Y., S.K. Ham, M.K. Shigenaga and O. Han, 2008. Bioactive dietary polyphenolic compounds reduce nonheme iron transport across human intestinal cell monolayers. J. Nutr., 138: 1647-1651.
- Laughton, M.J., P.J. Evans, M.A. Moroney, J.R.S. Hoult and B. Halliwell, 1991. Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives: Relationship to antioxidant activity and iron-reducing ability. Biochem. Pharmacol., 42: 1673-1681.
- Li, J. and H. Chase, 2010. Applications of membrane techniques for purification of natural products. Biotechnol. Lett., 32: 601-608.
- Manilal, A., S. Sujith, G.S. Kiran, J. Selvin, C. Shakir, R. Gandhimathi and M.V.N. Panikkar, 2009. Biopotentials of seaweeds collected from southwest coast of India. J. Mar. Sci. Technol., 17: 67-73.
- Morel, I., G. Lescoat, P. Cognel, O. Sergent, N. Padelop, P. Brissot, P. Cillard and J. Cillard, 1993. Antioxidants and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem. Pharmacol., 45: 13-19.
- Nakai, M., N. Kageyama, K. Nakahara and W. Miki, 2006. Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. Mar. Biotechnol., 8: 409-414.

- Nwosu, F., J. Morris, V.A. Lund, D. Stewart, H.A. Ross and G.J. McDougall, 2011. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chem.*, 126: 1006-1012.
- Ollanketo, M., A. Peltoketo, K. Hartonen, R. Hiltunen and M.L. Riekkola, 2002. Extraction of sage (*Salvia officinalis*) by pressurized hot water and conventional methods: Antioxidant activity of the extracts. *Eur. Food Res. Technol.*, 215: 158-163.
- Onofrejová, L., J. Vasícková, B. Klejdus, P. Stratil, L. Misurcová, S. Krácmár, J. Kopecký and J. Vacek, 2010. Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. *J. Pharm. Biomed. Anal.*, 51: 464-470.
- Ou, B., M. Hampsch-Woodill and R.L. Prior, 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.*, 49: 4619-4626.
- Pal, R. and J.P. Rai, 2010. Phytochelatin: Peptides involved in heavy metal detoxification. *Appl. Biochem. Biotechnol.*, 160: 945-963.
- Parys, S., S. Kehraus, A. Krick, K.W. Glombitza, S. Carmeli, K. Klimo, C. Gerhauser and G.M. König, 2010. In vitro chemopreventive potential of fucophloretols from the brown alga *Fucusvesiculosus* L. by anti-oxidant activity and inhibition of selected cytochrome P450 enzymes. *Phytochem.*, 71: 221-229.
- Patthamakanokporn, O., P. Puwastien, A. Nitithamyong and P.P. Sirichakwal, 2008. Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. *J. Food Comp. Anal.*, 21: 241-248.
- Pinteus, S., S. Azevedo, C. Alves, T. Mouga, A. Cruz, C. Afonso, M.M. Sampaio, A.I. Rodrigues and R. Pedrosa, 2009. High antioxidant potential of *Fucus spiralis* extracts collected from Peniche coast (Portugal). *New Biotechnol.*, 25: 296-296.
- Plaza, M., A. Cifuentes and E. Ibáñez, 2008a. In the search of new functional food ingredients from algae. *Trends Food Sci. Technol.*, 19: 31-39.
- Plaza, M., A. Cifuentes and E. Ibáñez, 2008b. In the search of new functional food ingredients from algae. *Trends Food Sci. Technol.*, 19: 31-39.
- Plaza, M., S. Santoyo, L. Jaime, G. García-Blairsy Reina, M. Herrero, F.J. Señoráns and E. Ibáñez, 2010. Screening for bioactive compounds from algae. *J. Pharm. Biomed. Anal.*, 51: 450-455.
- Ranathunga, S., N. Rajapakse and S.K. Kim, 2006. Purification and characterization of antioxidative peptide derived from muscle of conger eel (*Conger myriaster*). *Eur. Food Res. Technol.*, 222: 310-315.
- Rice-Evans, C.A., N.J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.*, 20: 933-956.
- Santoyo, S., M. Plaza, L. Jaime, E. Ibáñez, G. Reglero and J. Señoráns, 2010. Pressurized liquids as an alternative green process to extract antiviral agents from the edible seaweed *Himantalia elongata*. *J. Appl. Phycol.*, pp: 1-9, DOI: 10.1007/s10811-010-9611-x,
- Sato, M., T. Hosokawa, T. Yamaguchi, T. Nakano, K. Muramoto, T. Kahara, K. Funayama, A. Kobayashi and T. Nakano, 2002. Angiotensin I-converting enzyme inhibitory peptides derived from wakame (*Undariapinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. *J. Agric. Food Chem.*, 50: 6245-6252.
- Senevirathne, M., S.H. Kim, N. Siriwardhana, J.H. Ha, K.W. Lee and Y.J. Jeon, 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Technol. Int.*, 12: 27-38.
- Seth, A. and R.R. Mahoney, 2000. Iron chelation by digests of insoluble chicken muscle protein: The role of histidine residues. *J. Sci. Food Ag.*, 81: 183-187.
- Singh, I.P. and S.B. Bharate, 2006. Phloroglucinol compounds of natural origin. *Nat. Prod. Rep.*, 23: 558-591.
- Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-CIOCALTeu reagent. *Methods Enzym.*, 299: 152-178.
- Smit, A.J., 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J. Appl. Phycol.*, 16: 245-262.
- Stratil, P., B. Klejdus and V. Kuban, 2006. Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J. Agric. Food Chem.*, 54: 607-616.
- Suetsuna, K. and T. Nakano, 2000. Identification of an antihypertensive peptide from peptic digest of wakame (*Undariapinnatifida*). *J. Nutr. Biochem.*, 11: 450-454.
- Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, 39: 44-84.
- Van Acker, S.A., B.E. Van Den, D.J. Berg, M.N.J.L. Tromp, D.H. Griffioen, W.P. Van Bennekom, W.J.F. Van Der Vijgh and A. Bast, 1996. Structural aspects of antioxidant activity of flavonoids. *Free Radical Bio. Med.*, 20: 331-342.
- Vinayak, R.C., A.S. Sabu and A. Chatterji, 2011. Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. *Evid. Based Complement. Alternat. Med.*, 2011, Doi: 10.1093/ecam/neq024.

- Wang, T., 2009. Enhancing the Quality of Seafood Products through New Preservation Techniques and Seaweed-based Antioxidants: Algal Polyphenols as Novel Natural Antioxidants. School of Health Sciences, University of Iceland, Reykjavik, ISBN: 978-9979-9928-3-7.
- Wang, T., R. Jónsdóttir and G. Ólafsdóttir, 2009. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.*, 116: 240-248.
- Wang, T., R. Jónsdóttir, H.G. Kristinsson, G.O. Hreggvidsson, J.Ó. Jónsson, G. Thorkelsson and G. Ólafsdóttir, 2010. Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmariapalmata*. *LWT-Food Sci. Technol.*, 43: 1387-1393.
- White, N., 2008. *Fucus spiralis*. Spiral wrack. Marine Life Information Network (MarLIN): Biology and Sensitivity Key Information Sub-programme [online]. Marine Biological Association of the United Kingdom.
- Ye, H., K. Wang, C. Zhou, J. Liu and X. Zeng, 2008. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem.*, 111: 428-432.
- Yoshiki, M., K. Tsuge, Y. Tsuruta, T. Yoshimura, K. Koganemaru, T. Sumi, T. Matsui and K. Matsumoto, 2009. Production of new antioxidant compound from mycosporine-like amino acid, porphyra-334 by heat treatment. *Food Chem.*, 113: 1127-1132.
- Zubia, M., M.S. Fabre, V. Kerjean, K.L. Lann, V. Stiger-Pouvreau, M. Fauchon and E. Deslandes, 2009. Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. *Food Chem.*, 116: 693-701.