J Appl Phycol (2012) 24:1123–1132
DOI 10.1007/s10811-011-9742-8

Brown and red seaweeds as potential sources of antioxidant nutraceuticals

Antonio Jiménez-Escrig · Eva Gómez-Ordóñez · Pilar Rupérez

Received: 19 July 2011 / Revised and accepted: 10 October 2011 / Published online: 6 November 2011

Abstract Multifunctional antioxidant potential of several brown and red edible seaweeds was evaluated in organic and aqueous soluble extracts. The great reduction power and radical scavenging activity of *Bifurcaria bifurcata*—a Sargassaceae brown algal species—in both organic and aqueous extracts were emphasized. In addition, two Gigartinaceae red algal species, *Gigartina pistillata* and *Mastocarpus stellatus* showed relatively high reduction power in the aqueous extracts. When all of the variables of the aqueous extracts were combined in a principal component analysis, a clear differentiation pattern among the tested seaweeds was observed. In the Phaeophyceae, the correlation found among reduction power, radical scavenging activity and total phenolic content is in favour of the involvement of phenolic compounds in the antioxidant mechanisms, whereas in the case of the Florideophyceae, the role of sulphate-containing polysaccharides in reduction power is presumably shown. Nevertheless, the evidence of some taxonomy-based clustering (class and order levels) in this study may prove that polyphenol and sulphate content, besides multifunctional antioxidant profile, are related to specific groups of seaweeds. This evidence could help the search of suitable sources of phytochemicals from seaweeds for further nutraceutical applications.

Keywords Edible seaweed · Chemometrics · Polyphenols · Sulphated polysaccharides

A. Jiménez-Escrig (✉) · E. Gómez-Ordóñez · P. Rupérez
Metabolism and Nutrition Department, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN),
Consejo Superior de Investigaciones Científicas (CSIC),
José Antonio Novais 10, Ciudad Universitaria,
ES-28040 Madrid, Spain
e-mail: a.jimenez.escrig@csic.es

Introduction

Since ancient times, brown and red seaweeds have been part of the diet in Asian countries, especially China, Japan and Korea (Nisizawa et al. 1987). In European and other Western countries, seaweeds are utilized in pharmaceutical, cosmetic and food industries as a source of hydrocolloids such as agar, carrageenan and alginate (Juanes and Borja 1991; Marinho-Soriano and Bourret 2005). Furthermore, seaweeds are traditionally used as feed for animal nutrition (Ventura et al. 1994) or as amendments to increase crop production (Khan et al. 2009). Nowadays, around 18 million tonnes (wet weight) of seaweeds and other aquatic plants are produced/harvested annually with an estimated value of US $ 5,000 million (FAO 2011); however, seaweed species are often regarded as under-exploited bioresources (Khan et al. 2009; Cardozo et al. 2007). Over the past few decades, seaweeds also have been considered as promising organisms for providing both novel biologically active substances and essential compounds for human nutrition, with high potentially economical impact in food and pharmaceutical industry and public health (e.g., Cardozo et al. 2007; MacArtain et al. 2007; Smit 2004). However, much research, such as on their role in nutrition and disease prevention, remains to be done before science-based dietary recommendations can be given for edible seaweeds (Smit 2004). In this regard, systematic research on certain nutritional and health-promoting attributes of specific seaweeds commonly consumed in several European (Denis et al. 2010) and other countries (Gressler et al. 2010) has been recently reported as a prelude to future rational economic exploitation.

Seaweeds have to survive in a highly competitive environment, and therefore, they need to develop defence strategies that result in a tremendous diversity of antioxi-

dant compounds from different metabolic pathways such as carotenoids, phenols, minerals, sulphur compounds, vitamins, etc. (Cardozo et al. 2007). In addition, seaweeds are often located in the intertidal zones and consequently must be able, through protective antioxidant defence systems, to cope with a constantly changing environment, and fluctuations in light and oxygen (Blanchette 1997). In this context, the use of seaweeds as a source of antioxidants may be revitalized in the growing public consciousness in Western countries, regarding the health impact of consuming vegetables and fruits (MacArtain et al. 2007). Besides, consumers prefer natural to synthetic antioxidants, although safety limits of natural antioxidants are largely unknown (Pokorný 2007). However, there are few systematic studies reporting on the potential antioxidant activity of seaweeds.

In order to search for the health benefits or the efficacy of antioxidants in protecting food, the use of several *in vitro* models to screen for highly potential antioxidant activities of natural compounds could be a useful approach (Jeffery and Keck 2008). This search could be a necessary first step to select a specific antioxidant dietary source to assess more conclusive studies in cell, animal or human models (Mortensen et al. 2008). Therefore, the aim of this study was to screen for the potential antioxidant activity of several brown and red edible seaweeds commonly collected from the Northwestern Atlantic coast of Spain, by measuring the reduction power—ferric reducing/antioxidant power (FRAP)—and radical scavenging activity—2,2′-azinobis(3-ethylbenzothiazolin-6-sulphonate) (ABTS) and photochemiluminescent methods—in organic and aqueous soluble extracts. Polyphenol content, total carbohydrate, sulphate content and sulphation degree of polysaccharide were also determined. Furthermore, principal component analysis (PCA) was performed to simplify the data set and also to investigate if the parameters studied allowed grouping of the seaweeds according to their taxonomy.

Materials and methods

Nine seaweed species (Table 1) were provided by Porto-Muiños, a local food processing company (Cambre, A Coruña, Spain). Samples were collected from April 2008 to July 2009, at the intertidal zone at the Gulf of Artrabo, a marine bight formed by the Bay of A Coruña and the estuary of Ferrol and Ares in the Atlantic coastal region (latitude 43°22′ N, longitude 08°23′ W) of Galicia, Spain (Bode and Varela 1998). One of the brown seaweeds [*Saccharina latissima*] was cultivated under natural conditions. The seaweeds were rinsed with tap water to remove sand, epiphytes and encrusting material and then air-dried at 50°C. The dried samples were milled and passed through a 1-mm mesh sieve. At the laboratory, the milled seaweed samples were stored in plastic bags and kept at 2°C for further analysis.

Table 1 Taxonomy of tested seaweeds

Phylum Heterokontophyta, class Phaeophyceae	
Bifurcaria bifurcata R.Ross	Fucales, Sargassaceae
Himantalia elongata (Linnaeus) S.F.Gray	Fucales, Himanthaliaceae
Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders [formerly *Laminaria saccharina* (Linnaeus) J.V.Lamouroux)]	Laminariales, Laminariaceae
Phylum Rhodophyta, class Florideophyceae	
Chondracanthus acicularis (Roth) Fredericq	Gigartinales, Gigartinaceae
Dumontia contorta (S.G.Gmelin) Ruprecht	Gigartinales, Dumontiaceae
Gigartina pistillata (S.G.Gmelin) Stackhouse	Gigartinales, Gigartinaceae
Mastocarpus stellatus (Stackhouse) Guiry	Gigartinales, Phyllophoraceae
Nemalion helminthoides (Velley) Batters	Nemaliales, Liagoraceae
Osmundea pinnatifida (Hudson) Stackhouse	Ceramiales, Rhodomelaceae

Algaebase (2011)

Preparation of algal extracts

Organic extracts Half a gram of dried algal powder was placed in a test tube; 20 mL methanol/water (50:50 *v*/*v*) plus HCl were added to obtain a final pH of 2.0. The solution was thoroughly shaken at room temperature (1 h) and centrifuged (2,500×*g*, 10 min) and the supernatant was recovered. To the residue 20 mL acetone/water (70:30 *v*/*v*) was added, and shaking and centrifugation steps were repeated. Both organic extracts were combined (Jiménez-Escrig 2007) (Fig. 1).

Aqueous extracts Half a gram of dried algal powder was placed in a test tube; 50 mL water plus HCl (0.1 M final concentration) was added. The solution was thoroughly shaken at room temperature (1 h) and centrifuged (2,500×*g*, 10 min), and the supernatant was recovered (Fig. 1).

The solid content from the organic and aqueous extracts was determined by gravimetric measurement after drying an aliquot (2 mL) at 60°C overnight. From this data, the extraction yield was calculated as gram of dry extract per 100 g of dry algal powder. Total polyphenol content was estimated in the organic and aqueous extracts. Besides, total carbohydrate and anion sulphate content were measured in the aqueous extracts as described below. Both aqueous and organic extracts were used for the determination of antioxidant activities.

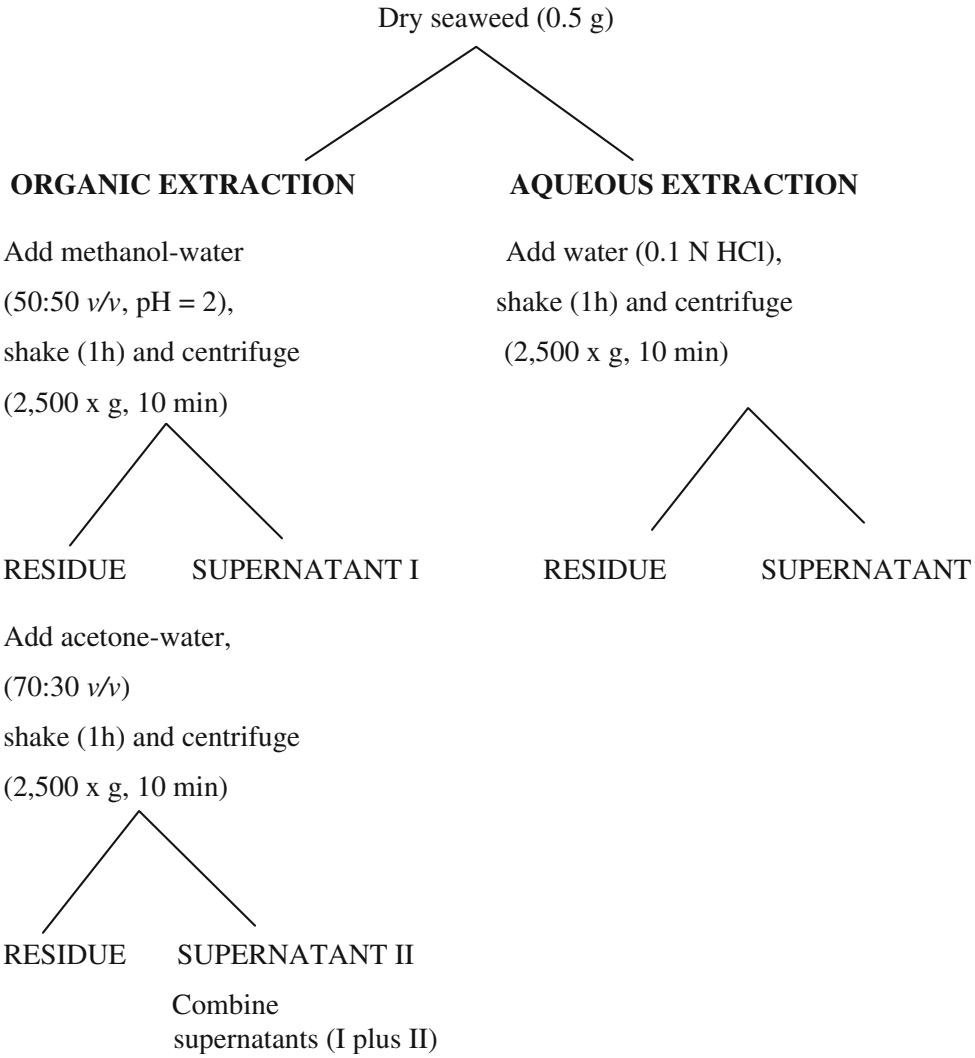

Fig. 1 Extraction procedure for the determination of bioactive compounds and antioxidant activities in selected seaweeds

Total polyphenol Total polyphenol content of the organic and aqueous extracts was determined spectrophotometrically according to the Folin–Ciocalteu procedure (Montreau 1972). A standard curve with phloroglucinol solutions (20–100 mg L^{-1}) was used for calibration (Jiménez-Escrig et al. 2001). Results were expressed as gram of phloroglucinol equivalents (PGE) per 100 g of seaweed dry extract.

Total carbohydrate Total carbohydrate content of the aqueous extracts was quantified colourimetrically according to the anthrone method (Loewus 1952) with minor modifications (Rupérez et al. 2002). A standard curve with glucose solutions (25–150 mg L^{-1}) was used for calibration. Results were expressed as gram of glucose equivalents (GE) per 100 g of seaweed dry extract.

Anion sulphate (SO_4^{2-}) Anion sulphate content in aqueous extracts from selected seaweeds was determined by an ion chromatography method previously developed for inorganic anions in seaweeds (Gómez-Ordóñez et al. 2010). A Metrohm Advanced compact ion chromatographic instrument (IC-861 model, Metrohm AG, Switzerland) controlled using Metrodata IC Net 2.3 software and attached to an Advance Sample Processor (IC-838) with an injection valve unit (IC-812) with a 20-μL sample loop was used in all analyses. The instrument was also equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was with a conductivity detector (IC-819) Advanced from Metrohm. Separation was performed in a Metrosep A Supp 5-250 column (250×4 mm, 5 μm particle size). The carrier material was an anion-exchange polymer of polyvinyl alcohol with quaternary ammonium groups. All measurements were carried out at 32°C (column temperature) under the following elution conditions: 3.2 mM sodium carbonate/1 mM sodium hydrogen carbonate at 0.70 mL min^{-1} as mobile phase. In order to

adjust the baseline to 15 $\mu S\,cm^{-1}$, 50 mM sulphuric acid solution and ultrapure water (Milli-Q) were used for automatic chemical suppression. Results were expressed as gram of sulphate equivalents (SE) per 100 g of seaweed dry extract. The degree of substitution or degree of sulphation (DS), which indicated the average number of sulphonic groups attached to each glucose residue, was calculated from the sulphur content on the basis of Schöniger's formula (Tao et al. 2006), as follows: $DS = (1.62 \times S\%)/(32 - 1.02 \times S\%)$, where S% is the sulphate percentage in the samples, 32 the sulphur atomic weight, and 1.62×100 and 1.02×100 represent the molecular weight of the sugar residue and the attached sulphonic residue, respectively.

Multifunctional antioxidant activity

Reduction power The ability of seaweed—aqueous and organic extracts—to act as reducing compounds was assessed by means of the FRAP assay (Benzie and Strain 1996). This method is based on the principle that the reduction of ferric ion (FeIII) to ferrous ion (FeII) at low pH can form a coloured ferrous–2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) complex that absorbs maximally at 595 nm. The FRAP reagent contained 2.5 mL of a 10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1} hydrochloric acid, plus 2.5 mL of 20 mmol L^{-1} ferric chloride hexaaquo, plus 25 mL of 0.3 mol L^{-1} acetate buffer pH 3.6. Readings at the absorption maximum were taken every 15 s using a Beckman DU-640 spectrophotometer thermostatized at 37°C. The readings at 4 and 30 min were selected for calculation of reduction power (RP) values (Jiménez-Escrig et al. 2001). A standard curve with 6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid (Trolox) in methanol solution (2.5–200 μM) was used for calibration. Results were expressed as μmol of Trolox equivalents (TE) per g of algal dry extract.

Radical scavenging activity The ability of seaweed extracts to act as radical scavengers was tested by two assays: the ABTS decolourization assay and the automated photochemiluminescent (PCL) assay:

(a) *ABTS assay.* The analysis was performed using the ABTS decolourization protocol (Re et al. 1999) with some modifications (Sánchez-Alonso et al. 2008). ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS (66 mg) with a 2.45 mM solution of potassium persulphate (10 mL). The mixture was left in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with water to an absorbance of 0.70±0.02 at 658 nm on a Beckman DU-340 spectrophotometer. Radical scavenging activity (RSA) of the seaweed extracts was measured by mixing 0.1 mL of the sample extracts with 3.9 mL of diluted $ABTS^{\cdot+}$ solution, and the absorbance reading was taken at 1 min intervals for a total of 10 min. A standard RSA curve was prepared by reacting Trolox (10–600 μM) with $ABTS^{\cdot+}$ solution. Results were expressed as μmol of TE per g of algal dry extract.

(b) *PCL assay.* The principle of PCL is based on optical excitation (UV-A light, 365 nm) of a suitable photosensitizer (luminol), an excited state aminophthalate anion is produced, followed by chemiluminescence and generation of the superoxide radical $O_2^{\cdot-}$. All radical scavenging substances that are able to react with luminol or oxygen radicals affect the light output. The photosensitized chemiluminescence was measured with the device Photochem® (Popov and Lewin 2005). This system can be used for both water-soluble and lipid-soluble antioxidative substances. In the case of water-soluble antioxidants, a standard PCL curve was prepared with vitamin C (0.5–3.0 nM). Results are expressed as μmol of vitamin C equivalents (VCE) per g of algal dry extract. In the case of lipid-soluble antioxidants, a standard PCL curve was prepared with Trolox solution (0.5–3.0 nM). Results were expressed as μmol of TE per g of algal dry extract.

Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS 17.0). Results are expressed as mean values±standard deviation. Comparison of means of at least three independent extractions and measurements was performed by ANOVA. In order to test the null hypothesis, the significance of differences was defined at the 5% level ($P<0.05$). In addition, the multivariate analysis PCA was applied to summarize the information in a reduced number of principal components, selecting those values with eigenvalues larger than 1.0. Then, the factors were rotated using Varimax method to obtain the expected weight for each extraction factor.

Results and discussion

Extraction yield

There was a high variability in the extraction yields among different seaweed species (Table 2). Extractants also have an impact on the yield. The highest extraction yield was recorded for the water extract of *Nemalion helminthoides*,

Table 2 Yield of extraction (% *w/w* of seaweeds on a dry weight basis) with organic and aqueous solvents in selected brown and red seaweeds

Seaweeds	Organic extract	Aqueous extract
B. bifurcata	64.71 ± 0.52^{ah}	44.61 ± 1.29^{abi}
H. elongata	53.40 ± 0.79^{bh}	57.46 ± 035^{ch}
S. latissima	45.26 ± 0.48^{bh}	42.44 ± 0.22^{ah}
C. acicularis	24.25 ± 0.30^{dh}	29.26 ± 1.16^{di}
D. contorta	50.83 ± 0.72^{eh}	42.74 ± 0.54^{ai}
G. pistillata	33.39 ± 0.65^{fh}	28.59 ± 0.63^{di}
M. stellatus	34.76 ± 0.33^{fh}	44.43 ± 0.08^{abi}
N. helminthoides	42.02 ± 0.55^{gh}	68.02 ± 0.66^{ei}
O. pinnatifida	52.09 ± 0.75^{bei}	33.70 ± 0.27^{fi}

Results are expressed as mean±standard deviation (n=3). Column wise values of same letters (a–g) of this type indicate no significant difference (P<0.05) among seaweed species. Row wise values of same letters (h–i) of this type indicate no significant difference (P< 0.05) between extracts

whereas the lowest was for the organic extract of *Chondracanthus acicularis*. When comparison was made with literature values, a similar variability in yield of extraction is given in the aqueous or acetone extracts of several Icelandic seaweed species (44.7% to 10.5%) (Wang et al. 2009). In contrast, lower yield (2.85% to 5.01%) is given in methanol extracts from selected Indian red seaweeds (Ganesan et al. 2008). In our work, the extraction yield percentage could not be attributed to the brown or red type of seaweed. In the case of aqueous extracts, extraction yields were significantly correlated to the anion sulphate content in both red and brown seaweeds (P<0.058, r= 0.448; P<0.079, r=0.453, respectively). This may be explained by the relatively high solubility in water of the polysaccharides to which sulphate groups are linked. Consistently, it is described that sulphation improves the water solubility of polysaccharides in the mushroom *Ganoderma lucidum* (Liu et al. 2010).

Total phenolic content

The concentration of polyphenols in seaweeds depends on many variables such as habitat, season of harvesting and environmental conditions (light, temperature and salinity). In addition, the distribution of phenolics varies with the species (Rodríguez-Bernaldo de Quirós et al. 2010). Significant differences were found in total polyphenol content among different seaweed species (Fig. 2). The brown seaweeds showed higher total polyphenol contents than red seaweeds. Among the species studied, two Fucales, *Bifurcaria bifurcata* and *Himantalia elongata*, displayed the highest polyphenol content. This feature was in agreement with previous studies in some Phaeophyceae from Brittany coasts (Zubia et al. 2009), reporting the Fucales as those among brown seaweeds with the highest polyphenol content. Also, a higher polyphenol content in brown than in red seaweeds has been reported (Jiménez-Escrig et al. 2001). In our work, except for *S. latissima* cultured in natural conditions, brown seaweeds showed on average 4.6-fold higher total polyphenol content than red seaweeds.

The organic solvent was more efficient than aqueous extraction for polyphenolic compounds in all species tested. This was in agreement with previous studies reporting the aqueous mixtures of methanol, ethanol or acetone as the more effective extractants of polyphenol compounds (Wang et al. 2009; Koivikko et al. 2005). In this sense, acetone is shown to break down hydrogen bonds formed between phenolic and protein carboxylic groups during extraction, leading to an increase in yield of extraction (Kallithraka et al. 1995).

Total carbohydrate and anion sulphate content

Polysaccharides are generally present in the cell walls of terrestrial plants and seaweeds, showing structural, matrix or storage functions. Seaweed phycocolloids are used as thickening, stabilizing or gelling agents in food and cosmetics industry (Jiménez-Escrig and Sánchez-Muniz 2000). Marine algae are the most important source of non-animal sulphated polysaccharides. Those sulphated polysaccharides demonstrate a wide spectrum of biomedical properties, i.e. as phytochemical analogues of heparin,

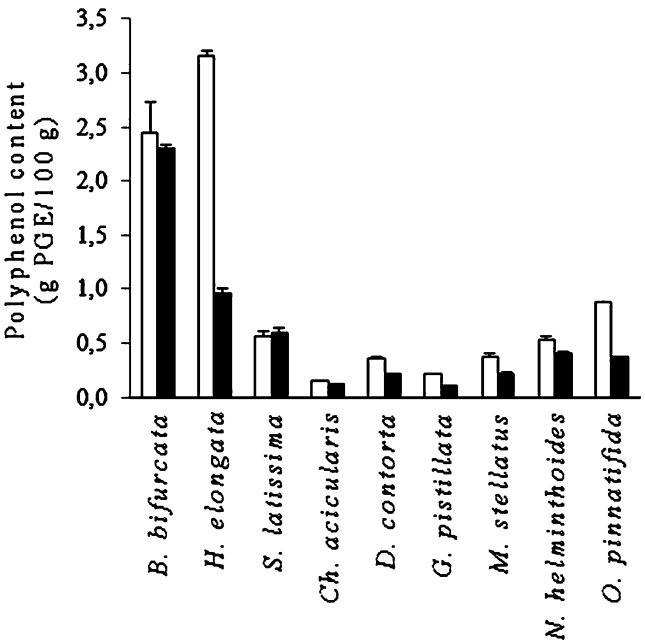

Fig. 2 Total phenolic content (g PGE 100 g^{-1}) in organic (*white*) and aqueous (*black*) extracts from selected seaweeds

which acts as an anticoagulant drug in mammalians (Preetha and Devaraj 2010) or as potential antioxidants (Rupérez et al. 2002). Total carbohydrate and sulphate content of aqueous extracts from the nine seaweeds tested is given in Figs. 3 and 4a. In addition, in Fig. 4b, the degree of substitution by sulphonic groups or degree of sulphation (DS) in each sugar residue, calculated according to Schöniger's formula (Tao et al. 2006), is shown. Interestingly, a strong correlation was found between carbohydrate, sulphate content and DS in brown seaweeds ($P<0.0001$, $r=0.829743$). In contrast, no correlation was found in red seaweeds. Therefore, it could be deduced that the distribution of sulphated polysaccharides among the six red seaweeds tested is heterogeneous.

Multifunctional antioxidant activity

The antioxidant activity of plants includes several multifunctional mechanisms. Thus, to evaluate this potential antioxidant activity, it is necessary to use several antioxidant assays that include different antioxidant mechanisms (Sánchez-Moreno 2002). A useful way of viewing the interactions among various antioxidants is to take into account oxidation–reduction potentials, measuring the reduction power (RP). Another mechanism commonly used is the radical scavenging activity (RSA). Therefore, in order to evaluate the total antioxidant activity of seaweed extracts, three different functional systems were chosen: FRAP based on the standard redox potential of Fe(III)/Fe(II) (0.77 V), ABTS and PCL assays based on the transfer of one hydrogen to the synthetic radical $ABTS^{\cdot+}$ or the biological superoxide radical $O_2^{\cdot-}$, respectively, by the tested antioxidant. These systems measure the RP of reductants with an ionization potential above 0.77 V and the RSA of antioxidants containing an RH

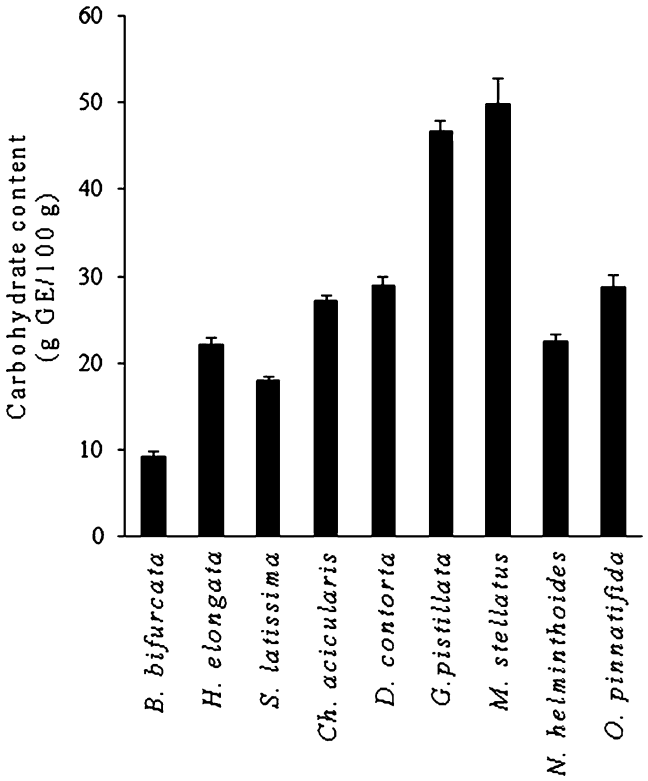

Fig. 3 Total carbohydrate (g GE 100 g^{-1}) of polysaccharides in aqueous extracts from selected seaweeds

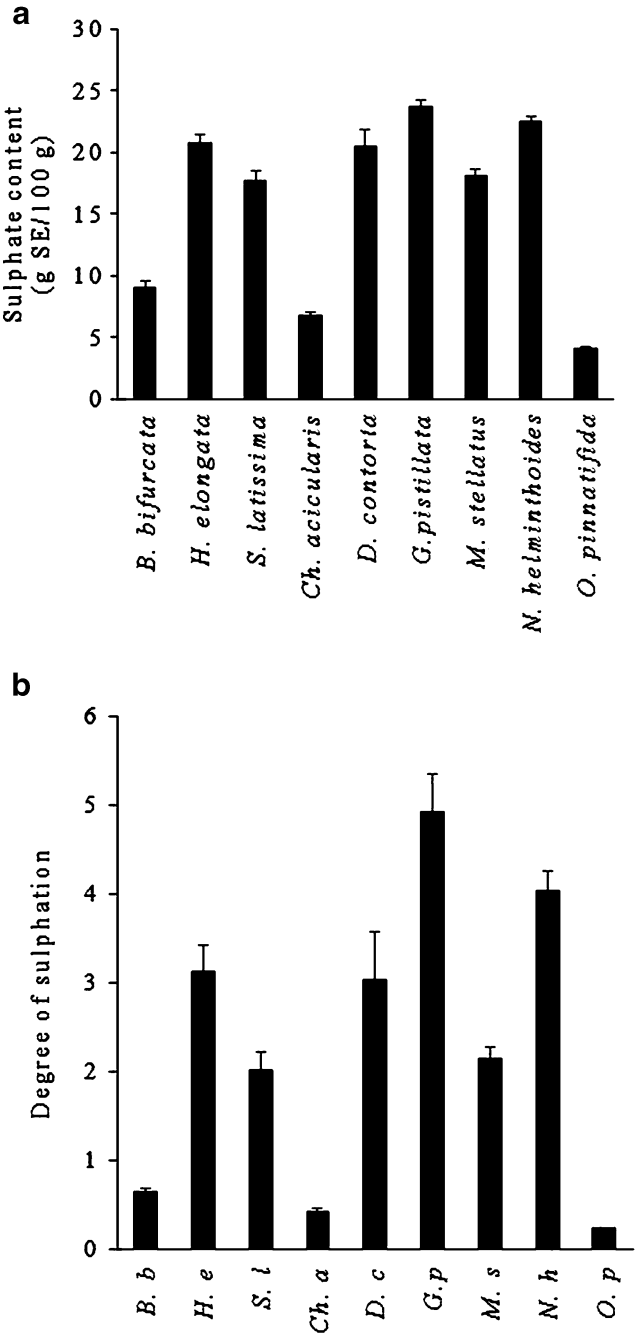

Fig. 4 **a** Total sulphate content (g SE 100 g^{-1}) and **b** DS of polysaccharides in aqueous extracts from selected seaweeds

group with an adequate enthalpy difference ($\Delta H_1 < 0$) in the scavenging reaction towards the radical (Trouillas et al. 2008).

Reduction power To study the kinetics of the organic (Table 3) and aqueous extracts (Table 4) in the FRAP assay, RP values at different times (4 and 30 min) were recorded and the ratio between 30 and 4 min (F30/F4) values was calculated. A significant difference in F30/F4 ratio between both types of seaweeds was found either in organic ($P<0.0034$), or in aqueous ($P<0.0001$) extracts. On average, F30/F4 ratio in organic extracts was 1.86±0.24 and 1.35±0.32 for brown and red seaweeds, respectively, whereas in aqueous extracts, it was 1.92±0.37 and 1.26±0.12 for brown and red seaweeds, respectively, indicating that brown seaweeds showed slower kinetics than red seaweeds for the RP assay in the organic extracts. This feature was independent of the polar nature of the extracts. Previous studies report the same characteristic of brown seaweeds in organic extracts (Jiménez-Escrig et al. 2001). The peculiar kinetics of brown seaweeds could be due to its content in polyphenols, which was relatively higher than that in red seaweeds (Fig. 2).

The RP (expressed as μm TE g^{-1}) of the organic (Table 3) and the aqueous extracts (Table 4) showed activity for all the seaweeds tested. The brown seaweed *B. bifurcata* showed the highest RP either in organic (464 μm TE g^{-1}) or in aqueous (195 μm TE g^{-1}) extracts. The second highest RP value among tested seaweed in aqueous extracts was shown by the red seaweed *Gigartina pistillata* (85.85 μm TE g^{-1}), whereas in organic extracts it was shown by the brown *H. elongata* (44.89 μm TE g^{-1}). In the case of *N. helminthoides*, the great viscosity of the aqueous extracts interfered with the measurement of the activity, and no value could be obtained. The organic extracts showed significantly higher or similar RP than the aqueous extracts for brown seaweeds, whereas the opposite tendency was shown for red seaweeds, in which the aqueous extracts of all the tested seaweeds, except for *N. helminthoides*, showed higher RP.

Reduction power versus polyphenol content *B. bifurcata* with the relatively highest polyphenol content in both tested extracts (Fig. 2) showed the highest RP value (Tables 3 and 4). In contrast, in the case of organic extracts, the brown seaweed with the highest polyphenol content (*H. elongata*) did not show the highest RP value. This could indicate qualitative differences in the polyphenol composition of *B. bifurcata* versus *H. elongata.* It is worth mentioning that literature data are controversial about relationships between polyphenol contents and antioxidant activity in seaweeds. Whereas, a positive correlation in certain seaweeds is observed (Jiménez-Escrig et al. 2001), this correlation is not systematic and clear because of the putative implication of phenol structures in the antioxidant activity and especially of polyphenol polymerization degree; an inverse correlation between polymerization degree and bioactivity in phlorotannins from seaweeds is observed (Connan et al. 2007). Apart from polyphenols, the presence of polar acyclic diterpenoids has been described in the case of *B. bifurcata* (Ortalo-Magné et al. 2005), which potential role as antioxidants in the organic extracts should not be ruled out.

Reduction power versus total carbohydrate and anion sulphate content High correlations were found among RP values and total carbohydrate ($P<0.0448$, $r=0.887091$) or sulphate ($P<0.0036$, $r=0.6820551$) content in red seaweeds. Indeed, a significant correlation between RP value and degree of substitution of sulphate in polysaccharides was found ($P<0.0768$, $r=0.837383$) in red seaweeds. The red seaweed with the highest sulphate content and DS (*G. pistillata*) showed the second highest RP among all seaweeds tested. Consistently, the possibility to enhance the RP of polysaccharides through sulphation is described. Introduction of sulphate group might enhance the electron cloud density of active hydroxyl groups and enhance the

Table 3 Multifunctional antioxidant activity of organic extracts from brown and red seaweeds

Seaweed	RP		RSA	
	4 min	30 min	ABTS	PCL
B. bifurcata	269±4[a]	464±5[a]	334±0.8[a]	20.62±0.32
H. elongata	20.39±0.59[b]	43.55±0.71[b]	62.88±3.90[b]	1.36±0.09[a]
S. latissima	14.07±0.94[c]	24.38±1.42[c]	40.57±2.28[c]	0.75±0.04[b]
C. acicularis	9.42±0.82[d]	9.61±0.79[d]	nd	nd
D. contorta	12.34±0.53[ec]	20.62±0.64[e]	nd	nd
G. pistillata	21.15±1.09[b]	29.07±1.93[f]	nd	nd
M. stellatus	5.76±0.41[f]	9.62±0.48[d]	nd	nd
N. helminthoides	4.41±0.93[f]	6.73±0.81[g]	nd	nd
O. pinnatifida	12.56±1.02[ec]	20.62±0.74[e]	nd	nd

Results are expressed as mean± standard deviation ($n=3$). Column wise values of same letters (a–g) indicate no significant difference ($P<0.05$). RP, ABTS and PCL=μmol Trolox equivalent g^{-1} algal dry extract

nd not detected

Table 4 Multifunctional antioxidant activity of aqueous extracts from brown and red seaweeds

Seaweed	RP		RSA	
	4 min	30 min	ABTS	PCL
B. bifurcata	117±5.9[a]	195±13.6[a]	89.76±2.30[a]	1.80±0.13[a]
H. elongata	26.50±2.21[b]	44.89±1.20[b]	10.95±0.82[b]	1.88±1.10[a]
S. latissima	11.70±0.85[c]	22.24±1.32[c]	38.77±2.27[c]	0.32±0.02[b]
C. acicularis	7.67±0.35[c]	10.45±0.22[d]	nd	nd
D. contorta	20.43±1.78[d]	25.28±1.88[d]	nd	nd
G. pistillata	77.76±5.02[e]	85.85±5.20[e]	nd	nd
M. stellatus	44.92±2.01[f]	54.62±2.14[f]	nd	nd
N. helminthoides	nd	nd	nd	nd
O. pinnatifida	12.97±0.53[g]	22.80±1.69[c]	nd	nd

Results are expressed as mean± standard deviation (n=3). Column wise values of same letters (a–g) indicate no significant difference (P<0.05). RP and ABTS=μmol Trolox equivalent g^{-1} algal dry extract. PCL =μmol VCE g^{-1} algal dry extract

nd not detected

molecular electron-withdrawing activity, which can eliminate free radicals and terminate radical-mediated oxidative chain reactions (Liu et al. 2010). Also, it has been shown that sulphation of polysaccharides increases its RP activity in the mushroom *G. lucidum* (Liu et al. 2010). Thus, DS could modulate the relatively high RP found in the aqueous extracts of *G. pistillata*.

Radical scavenging activity

Regarding RSA, only brown seaweeds have shown activity towards the ABTS or the PCL systems in both solvent extracts (Tables 3 and 4). In general, these algae showed higher RSA in organic than in aqueous extracts. Among the nine seaweeds tested, *B. bifurcata* showed the highest RSA in the ABTS model, either in the organic (334 μm TE g^{-1}), or aqueous (89.76 μm TE g^{-1}) extracts, as in the case of RP assay. Also, the organic extracts of this seaweed showed the highest RSA in the PCL assay (20.62 μm TE g^{-1}). Consistently, *B. bifurcata* shows relatively high RSA (DPPH stable free radical) and RP among ten seaweeds tested (Zubia et al. 2009). *B. bifurcata* belongs to the Sargassaceae which have been extensively studied because of their natural diversity in bioactive compounds. In our case, no RSA was found in the aqueous extracts containing sulphated polysaccharides from red seaweeds. Algal polysaccharides, mainly from brown seaweeds, have been reported to play an important role as free radical scavengers for the prevention of oxidative damage in living organisms. This activity depends on several structural parameters, such as the type of sugar and glycosidic branching, the molecular weight, the degree and position of sulphation (Kumar et al. 2008).

The presence in the extracts of other bioactive compounds apart from polyphenols or sulphated polysaccharides, such as peptides or pigments, which might be involved in the antioxidant activity of seaweeds, could also be possible.

Principal component analysis

Principal component analysis (PCA) identifies patterns in data, expressing them in such a way as to highlight similarities and differences. Thus, PCA was carried out on the aqueous extracts to investigate the relation between the bioactive compounds and the antioxidant activities (RP) in the species tested. PCA was carried out by selecting those PCs with eigenvalues larger than 1.0; the higher eigenvalue, the higher percentage of the total variance explained. The first two components explained 84.96% of the total variance in the data set (eigenvalues=2.708 and 2.390, respectively). The first component (PC 1) accounted for 45.13% of the variance and correlated positively with all the variances except for total phenolics. The second component (PC 2) accounted for 39.83% of the total variance and correlated positively with the total phenolics and the reduction power, and negatively with the rest of the variances. Using the rotated component matrix (Table 5), we could infer that the variables related to

Table 5 Rotated component matrix for the analysis of the relation between bioactive compounds and reduction power in seven species of seaweeds

Variable	Component	
	PC 1	PC 2
Total phenolics	−0.162	0.900
Total carbohydrate	0.343	−0.833
Anion sulphate content	0.920	−0.101
Degree of sulphation	0.936	−0.147
Reduction power at 4 min	0.636	0.639
Reduction power at 30 min	0.538	0.770

Factors were extracted with the principal component analysis method using the Varimax rotation

PC principal component

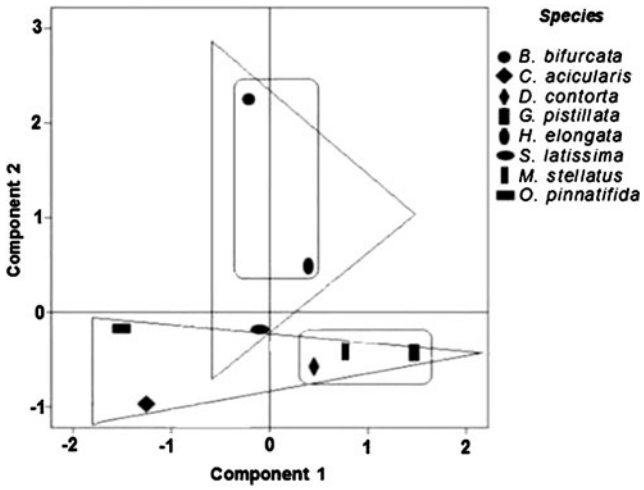

Fig. 5 PCA scatter plot using six variables in the study of the bioactivity of aqueous extracts in eight species of seaweeds. Principal component 1 accounted for 45.13% of the variance and is related to the variables related to the presence of sulphate linked to the sugar residue and to the activity of reduction power. Principal component 2 accounted for 39.83% of the variance and it is related to the polyphenol content and to the activity of reduction power. *Triangle* class: *up* Phaeophyceae, *down* Florideophyceae. *Rectangle* order: *up* Fucales, *down* Gigartinales

the presence of sulphate in the sugar residue and to the reduction power of the aqueous extracts had the highest weightings in the PC 1. Accordingly, by referring to the PCA scatter plot (Fig. 5), the red seaweeds *Dumontia contorta*, *M. stellatus* and *G. pistillata*, and the brown *H. elongata* with the highest DS, sulphate content and RP (mainly RP-4 min value) were located in the positive part of the PC 1, with *G. pistillata* located further to the right along PC 1. Consequently, this red seaweed showed the highest values among all tested seaweeds in the three variables described previously defining the PC 1. On the other hand, *C. acicularis* and *Osmundea pinnatifida*, with rather relatively low DS and sulphate content, as well as weak RP, were located on the negative/opposite site of the PC 1. In the PC 2, the highest weightings were for polyphenol content and RP (mainly PR-30 min value), showing firstly that polyphenol content was unrelated to the presence of sulphates and secondly that RP attributed to polyphenols was more related to the PR-30 value than to the PR-4 value. Accordingly, two brown seaweeds (*B. bifurcata* and *H. elongata*) were placed in the positive part of the PC 2, indicating their relatively high polyphenol content and RP value. *H. elongata* was the only seaweed among those tested placed in the positive part of both PC 1 and PC 2; this peculiar position indicated the contribution of this brown seaweed to all the variances which defined both components. Regarding *B. bifurcata*, it was placed on the top position of the PC scatter plot, specifically at the positive part of PC 2 and at the negative part of PC 1. This position is justified because this seaweed exhibited the highest polyphenol content and the highest RP (PC 2), along with a relatively low DS and sulphate content (PC 1) among all of the seaweeds tested. Plotting the scores according to their class and order drew some distinctions between the different seaweeds tested. The Phaeophyceae appeared close to the vertical axis (PC 1=0) indicating the low influence of the variables defining PC 1 (mainly sulphate content) whereas the Florideophyceae appeared on the negative side of PC 2, indicating the weak influence of the variables defining PC 2 (polyphenol and RP-30). More specifically, the brown Fucales appeared on the positive side of PC 2 indicating the strong influence of polyphenol content and RP-30 in this order, whereas red *Gigartinales* appeared mostly at the far right of the positive side of PC 1 indicating the strong influence of the sulphate content and RP-4 in this order. Other factors such as seasonal changes, exact localization, and cultural practices are likely to play important roles in defining the sulphate and phenolic content and the resulting antioxidant properties of the seaweed. Nevertheless, the evidence of some taxonomy-based clustering in this study may prove that polyphenol and sulphate content, besides multifunctional antioxidant profile, are related to specific taxa of seaweeds. The tightness of the clustering could be improved by adding more seaweed samples to these data.

In summary, this novel approach on the search of nutraceuticals, taking into account different aspects such as multifunctional antioxidant activity, polyphenol and sulphated polysaccharide content, in brown and red seaweeds, can allow a more complete picture of the potential bioactive compounds involved than the usual approaches based only on polyphenols and antioxidant properties. In general, the correlation found between marked radical scavenging activities, reduction power and total polyphenol content is in favour of the involvement of phenolic compounds in the antioxidant mechanisms in Phaeophyceae, whereas in the case of Florideophyceae, the involvement of sulphate-containing polysaccharides in the reduction power is presumably evidenced.

Acknowledgements The Spanish Ministry of Science and Innovation through Project AGL2008-00998 ALI supported this research. Thanks are given to Porto-Muiños for the seaweed samples provided.

References

Algaebase (2011). http://www.algaebase.org. Accessed 15 June 2011

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal Biochem 239:70–76

Blanchette CA (1997) Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. Ecology 78:1563–1578

Bode A, Varela M (1998) Mesoscale estimations of primary production in shelf waters: a case study in the Golfo Artabro (NW Spain). J Exp Mar Biol Ecol 229:111–131

Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E (2007) Metabolites from algae with economical impact. Comp Biochem Physiol - C Toxicol Pharmacol 146:60–78

Connan S, Deslandes E, Gall EA (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

Denis C, Morançais M, Li M, Deniaud E, Gaudin P, Wielgosz-Collin G, Barnathan G, Jaouen P, Fleurence J (2010) Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). Food Chem 119:913–917

FAO (2011). http://www.fao.org/fishery/statistics/en. Accessed 26 Sept 2011

Ganesan P, Kumar CS, Bhaskar N (2008) Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Biores Technol 99:2717–2723

Gómez-Ordóñez E, Alonso E, Rupérez P (2010) A simple ion chromatography method for inorganic anion analysis in edible seaweeds. Talanta 82:1313–1317

Gressler V, Yokoya NS, Fujii MT, Colepicolo P, Filho JM, Torres RP, Pinto E (2010) Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chem 120:585–590

Jeffery EH, Keck A (2008) Translating knowledge generated by epidemiological and in vitro studies into dietary cancer prevention. Mol Nutr Food Res 52:S7–S17

Jiménez-Escrig A (2007) Multifunctional in vitro antioxidant evaluation of strawberry (*Fragaria virginiana* Dutch.). Int J Food Sci Nutr 58:629–636

Jiménez-Escrig A, Sánchez-Muniz FJ (2000) Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. Nutr Res 20:585–598

Jiménez-Escrig A, Jiménez-Jiménez I, Pulido R, Saura-Calixto F (2001) Antioxidant activity of fresh and processed edible seaweeds. J Sci Food Agric 81:530–534

Juanes JA, Borja A (1991) Biological criteria for the exploitation of the commercially important species of *Gelidium* in Spain. Hydrobiologia 221:45–54

Kallithraka S, Garcia-Viguera C, Bridle P, Bakker J (1995) Survey of solvents for the extraction of grape seed phenolics. Phytochem Anal 6:265–267

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009) Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul 28:386–399

Koivikko R, Loponen J, Honkanen T, Jormalainen V (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J Chem Ecol 31:195–212

Kumar KS, Ganesan K, Rao PVS (2008) Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty—an edible seaweed. Food Chem 107:289–295

Liu W, Wang H, Yao W, Gao X, Yu L (2010) Effects of sulfation on the physicochemical and functional properties of a water-insoluble polysaccharide preparation from *Ganoderma lucidum*. J Agric Food Chem 58:3336–3341

Loewus FA (1952) Improvement in the anthrone method for determination of carbohydrates. Anal Chem 24:219

MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR (2007) Nutritional value of edible seaweeds. Nutr Rev 65:535–543

Marinho-Soriano E, Bourret E (2005) Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). Biores Technol 96:379–382

Montreau FR (1972) Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin-Ciocalteau. Connaiss Vigne Vin 24:397–404

Mortensen A, Sorensen IK, Wilde C, Dragoni S, Mullerová D, Toussaint O, Zloch Z, Sgaragli G, Ovesná J (2008) Biological models for phytochemical research: from cell to human organism. Br J Nutr 99(ES 1):ES118–ES126

Nisizawa K, Noda H, Kikuchi R, Watanabe T (1987) The main seaweed foods in Japan. Hydrobiologia 151–152:5–29

Ortalo-Magné A, Culioli G, Valls R, Pucci B, Piovetti L (2005) Polar acyclic diterpenoids from *Bifurcaria bifurcata* (Fucales, Phaeophyta). Phytochemistry 66:2316–2323

Pokorný J (2007) Are natural antioxidants better—and safer—than synthetic antioxidants? Eur J Lip Sci Technol 109:629–642

Popov I, Lewin G (2005) Photochemiluminescent detection of antiradical activity. VII. Comparison with a modified method of thermo-initiated free radical generation with chemiluminescent detection. Luminescence 20:321–325

Preetha SP, Devaraj H (2010) Role of sulphated polysaccharides from *Sargassum wightii* in the control of diet-induced hyperlipidemia and associated inflammatory complications in rats. Eur J Inflammation 8:23–30

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26:1231–1237

Rodríguez-Bernaldo de Quirós A, Lage-Yusty MA, López-Hernández J (2010) Determination of phenolic compounds in macroalgae for human consumption. Food Chem 121:634–638

Rupérez P, Ahrazem O, Leal JA (2002) Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. J Agric Food Chem 50:840–845

Sánchez-Alonso I, Jiménez-Escrig A, Saura-Calixto F, Borderías J (2008) Antioxidant protection of white grape pomace on restructured fish products during frozen storage. LWT-Food Sci Technol 41:42–50

Sánchez-Moreno C (2002) Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Technol Int 8:121–137

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Tao Y, Zhang L, Cheung PCK (2006) Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohyd Res 341:2261–2269

Trouillas P, Marsal P, Svobodová A, Vostálová J, Gažák R, Hrbáč J, Sedmera P, Křen V, Lazzaroni R, Duroux J, Walterová D (2008) Mechanism of the antioxidant action of silybin and 2,3-dehydrosilybin flavonolignans: a joint experimental and theoretical study. J Phys Chem A 112:1054–1063

Ventura MR, Castañon JIR, McNab JM (1994) Nutritional value of seaweed (*Ulva rigida*) for poultry. Animal Feed Sci Technol 49:87–92

Wang T, Jónsdóttir R, Ólafsdóttir G (2009) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem 116:240–248

Zubia M, Fabre MS, Kerjean V, Lann KL, Stiger-Pouvreau V, Fauchon M, Deslandes E (2009) Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. Food Chem 116:693–701