Enzyme-assisted extraction of bioactive compounds from brown seaweeds and characterization

Sabeena Farvin K. Habeebullah[1] (ID) · Surendraraj Alagarsamy[1] · Zainab Sattari[1] · Sakinah Al-Haddad[1] · Saja Fakhraldeen[1] · Aws Al-Ghunaim[1] · Faiza Al-Yamani[1]

Abstract

Brown algae comprise the largest biomass producers in coastal waters and play important ecological roles. The complex nature of cell wall polysaccharides limits the extraction of bioactive compounds from these seaweeds. The aim of the study was to use enzyme-assisted extraction as a tool to release the bioactive compounds from seven brown seaweeds of Kuwait coast and characterization of the active extracts. The enzymatic extracts obtained by hydrolysing seaweeds with five carbohydrases and three proteases were screened for antioxidant and antimicrobial activity. Yield, total phenolics, and bioactivity were as a function of species difference in cell wall composition and specificity of the enzyme used. Among the six species of brown seaweeds studied, the enzymatic extracts obtained from *Sargassum boveanum*, *Sargassum angustifolium*, and *Feldmannia irregularis* showed high antioxidant activity in different assays. Though antimicrobial activities of the enzymatic extracts were low, Flavourzyme resulted in more number of seaweed extracts with antimicrobial activity against foodborne pathogens. In general, carbohydrases resulted in extracts with high radical scavenging activity whereas proteases resulted in extracts with high iron chelating activity. The extracts with highest antioxidant activity such as *S. boveanum*-Viscozyme and Alcalase extracts were further fractionated and characterized. The polyphenol and polysaccharide-rich fractions were responsible for the high radical scavenging and reducing power whereas the iron chelating activity and inhibition of lipid oxidation in liposome model system was mainly contributed by polysaccharide and protein-rich fractions. The results of study showed that enzyme-assisted extraction could be useful to make tailor-made seaweed extracts with specific bioactivity.

Introduction

Brown algae comprise more than 250 genera and 1500–2000 species and are one of the largest biomass producers in coastal regions (Silberfeld et al. 2010). They play vital ecological roles in marine communities as they provide home and food for many different sea animals. Brown seaweeds have been extensively studied for their biologically active polyphenolic derivatives called phlorotannins that are exclusively found in these seaweeds (Li et al. 2011). Depending on the species, phlorotannin content varies from 0.2 to 14% of dry weight of brown seaweeds (Holdt and Kraan 2011). These compounds have been reported to possess a number of bioactivities such as radioprotection, antioxidant, antidiabetic, antimicrobial, and anti-inflammatory properties (Li et al. 2011). Fucoxanthin is another major biofunctional pigment isolated and extensively studied from brown seaweeds and has been reported to possess antioxidant, anticancer, antiobesity and anti-inflammatory properties (Peng et al. 2011). In recent years, a family of fucose-containing sulphated polysaccharides from brown seaweeds has attracted attention due to their high bioactive properties and find their application in cosmetics, functional foods, and dietary supplements (Jiao et al. 2011; Wijesinghe and Jeon 2012; Deniaud-Bouët et al. 2017).

The brown seaweeds (Phaeophyceae) are characterized by their cell walls composed of cellulose, alginic acid, and various other polysaccharides, cellular inclusion of polyphenolic polymers known as physodes, and their main storage materials as laminarin (a β-1,3-glucan) with some species also producing mannitol, sucrose, glycerol, and oil as storage

✉ Sabeena Farvin K. Habeebullah
shabeebullah@kisr.edu.kw; sabeenafarvin@gmail.com

[1] Ecosystem Based Management of Marine Resources Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, PO Box 1638, 22017 Salmiya, Kuwait

reserves (Wehr 2002). The main structural components of cell walls of brown seaweeds are alginates and fucose-containing sulphated polysaccharides (FCSPs) including sulphated fucans and the later are cross linked to cellulose microfibrils (Deniaud-Bouët et al. 2014). The other components include proteins, phenolic, and halide compounds. Phenolic compounds are associated with alginates and proteins (Deniaud-Bouët et al. 2014). This complex nature of the cell wall acts as barrier for the extraction of bioactive compounds.

The use of enzyme for seaweed biorefinery is a promising biotechnological application that has been widely used to improve the extraction efficiency of bioactive components (Hardouin et al. 2014a, b; Puspita et al. 2017a; Vásquez et al. 2019). Conventional extraction techniques such as solvent extraction may not be always efficient in extracting phytochemicals as some of the compounds are either dispersed in cell cytoplasm or retained in the cell wall by hydrogen or hydrophobic bonding, which are not accessible to the solvents in a routine extraction process (Fleurence et al. 1995). Extraction techniques using digestive commercial enzymes such as carbohydrases and proteases were employed to degrade seaweed tissues and to help releasing a variety of bioactive compounds from the seaweeds (Heo et al. 2005; Jeon et al. 2011; Hardouin et al. 2014a, b). In addition, enzymes can convert the water-insoluble materials in the seaweeds to a water-soluble material which have the advantages over the use of organic solvents.

Arabian Gulf is bestowed with 282 species of seaweeds which include 70 species of brown seaweeds (John and Al-Thani 2014). The seaweeds of the Arabian Gulf are least studied and unexploited resources. In our earlier study on the chemical profile and antioxidant activity of 26 species of seaweeds from Kuwait's coast of the Arabian Gulf, we found that the seaweeds from this coast are unique in their phenolic composition and phytochemicals (Farvin et al. 2019). The water and ethanolic extracts of brown seaweeds such as *Sargassum* sp., *Canistrocarpus cervicornis*, and *Padina gymnospora* are rich in phenolics and had high antioxidant activity (Farvin et al. 2019). In the present study, seven brown algae which showed high antioxidant activity in the conventional solvent extractions were selected for the enzymatic extraction studies. The selected seaweeds include two Fucales (*Sargassum angustifolium* and *Sargassum boveanum*), two Dictyotales (*P. gymnospora* and *C. cervicornis*), and three Ectocarpales (*Colpomenia sinuosa*, *Feldmannia irregularis*, and *Iyengaria stellata*). In order to understand the suitable enzyme for proper hydrolysis of the selected seaweeds, five commercially available carbohydrases and three proteases were used in this study. The resulting enzyme extracts were screened for bioactivity such as antioxidant activity and antimicrobial activity. For antioxidant activity screening, four *in vitro* assays, viz., 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, metal chelating, reducing properties, and inhibition of lipid oxidation in liposome model system, were used. The antimicrobial activity was assessed against common foodborne and aquaculture pathogens by disk diffusion method. In order to understand the components responsible for the bioactivity, the enzyme extracts which showed high activity were further fractionated and characterized by method of Farvin and Jacobsen (2015).

Materials and methods

Algal materials

Seven brown seaweeds, viz., *Sargassum boveanum*, *Sargassum angustifolium* (Family: Sargassaceae), *Padina gymnospora*, *Canistrocarpus cervicornis* (Family: Dictyotaceae), *Colpomenia sinuosa*, *Iyengaria stellata* (Family: Scytosiphonaceae), and *Feldmannia irregularis* (Family: Acinetosporaceae), were collected between September 2016 and March 2017 from Kuwait's coast. *Sargassum boveanum* and *P. gymnospora* were collected from Fintas area in February 2017 and September 2016, respectively. *Feldmannia irregularis*, *C. sinuosa*, and *I. stellata* were collected from the Kuwait Tower area in December 2016. *Sargassum angustifolium* and *C. cervicornis* were collected from Salmiya beach and Julaia beach in January and February 2017, respectively. The collected samples were washed with seawater to remove any sand and epiphytes. The cleaned seaweeds were immediately frozen, freeze dried, powdered, and stored at − 86 °C under vacuum packing until further use.

Enzyme-assisted extraction of seaweeds

The enzymes used in this study were kindly donated by Novozyme (Novozyme Nordisk, Bagsvaerd, Denmark). The enzymatic conditions and characteristics of the enzymes are given in Table 1. Ten grams of seaweed powder was weighed into a 2 L reaction vessel; to this, about 1 L of corresponding buffer was added. The reaction vessel was connected to an overhead stirrer and placed inside a water bath maintained at optimal temperature needed for the enzyme action. When the optimal temperature has reached, 1 mL (0.1% enzyme) was added to the mixture and stirred at 350 rpm for 20 h in water bath. After the incubation, the enzyme was inactivated by heating at 100 °C for 10 min and cooled in ice. The mixture was centrifuged at 1500×g for 10 min to collect the supernatant. The extract thus obtained was freeze dried, weighed for calculating yield, and reconstituted in a known quantity of water. The extracts were stored at − 86 °C freezer until further use. A water extract was also made in a similar manner for comparison.

Table 1 Optimum hydrolysis conditions and characteristics of enzymes used in this study

Enzyme	Optimum conditions		Buffer used	Characteristics
	pH	Temperature		
Carbohydrases				
Viscozyme L	4.5	50 °C	0.1 M acetate buffer	A multi-enzyme complex (containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase)
AMG 300 L	4.5	60 °C	0.1 M acetate buffer	Exo-1,4-α-D-glucosidase
Celluclast 1.5 L FG	4.5	50 °C	0.1 M acetate buffer	Cellulase
Termamyl 120 L	6	60 °C	0.1 M phosphate buffer	A heat-stable α-amylase
Ultraflo L	6	40 °C	0.1 M phosphate buffer	A heat-stable multi-β-glucosidase
Proteases				
Flavourzyme 500MG	7	50 °C	0.1 M phosphate buffer	Containing both endoprotease and exopeptidase
Alcalase 2.4 L FG	8	50 °C	0.1 M phosphate buffer	Endopeptidase
Neutrase 0.8 L	8	50 °C	0.1 M phosphate buffer	Metalloendoprotease

Total phenolic content

The total phenolic content of the different enzymatic extracts was determined by the Folin-Ciocalteu method (Farvin and Jacobsen 2015). In brief, an aliquot (100 μL) of extract in Eppendorf was mixed with 0.75 mL of Folin-Ciocalteu reagent (1:10 diluted) and allowed to stand at room temperature for 5 min. Sodium bicarbonate (6%, 0.75 mL) was added to the mixture and incubated at room temperature for 90 min. Two hundred fifty microlitres of this reaction mixture was transferred to microplates and the absorbance was measured at 725 nm using a microplate reader (Variscan Lux, Thermo Scientific, Finland). A standard curve was plotted using different concentrations of gallic acid (Sigma, Germany) and the amount of total phenolics was expressed as gallic acid equivalents (mg GAE g^{-1} extract).

Bioactivity screening of enzymatic extracts

The enzymatic extracts were screened for antioxidant and antimicrobial activity. The enzymatic extracts at different concentrations were screened for antioxidant activity by using four *in vitro* tests, viz., DPPH radical scavenging, iron chelating property, reducing power, and inhibition of lipid oxidation in phospholipid liposome model system. All the analysis was performed in triplicate unless otherwise specified.

DPPH free radical scavenging effect was measured according to the method of Farvin et al. (2014) in a microplate reader (Varioscan Lux, Thermo Scientific, Finland). DPPH solution (0.1 mL, 0.1 mM in 95% ethanol) was mixed with 0.1 mL of extracts (at a concentration of 0.1, 1, 5, and 10 mg mL^{-1}) in an Eppendorf tube. The mixture was shaken and left for 30 min at room temperature. The reaction mixture was transferred to microplates, and the absorbance of the resulting solution was measured at 517 nm using a microplate reader. A blank with distilled water instead of sample and a sample control with sample and 95% ethanol were also made. The effective concentration EC_{50} (concentration of extracts required to scavenge 50% of DPPH radicals) was calculated. The results are expressed as antiradical power, which is $1/EC_{50}$.

The iron chelating property of the extracts was determined according to method of Farvin et al. (2014) in a microplate reader (Variscan Lux, Thermo Scientific). In brief, to 100 μL of the enzymatic extracts (at a concentration of 0.1, 1, 5, and 10 mg mL^{-1}) in Eppendorf tubes, 135 μL of deionized water and 5 μL of 2 mM ferrous chloride were added. After 3 min, 5 mM ferrozine (10 μL) was added. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm in the microplate reader. A blank with distilled water and a sample control without adding ferrozine was also made. The effective concentration EC_{50} (concentration of extracts required to chelate 50% of iron) was calculated. The results are expressed as EC_{50} values.

The reducing power of the extracts was measured according to the method of Farvin et al. (2014) in a microplate reader (Varioscan Lux, Thermo Scientific). In brief, to 100 μL of enzymatic extracts (at a concentration of 0.1, 1, 5, and 10 mg mL^{-1}) in Eppendorf tube, 100 μL 0.2 M phosphate buffer (pH 6.6) and 100 μL of 1% potassium ferricyanide were added. The mixture was incubated at 50 °C for 20 min and 100 μL of 10% TCA was added into this reaction mixture. An aliquot of 144 μL from the incubation mixture was mixed with 144 μL of distilled water and 25 μL of 0.1% ferric chloride in an Eppendorf tube. After 10 min, the absorbance of the solution was measured at 700 nm in the microplate reader. The reducing power was expressed as absorbance at 700 nm. Increased absorbance (A_{700}) of the reaction mixture indicated increased reducing power.

Inhibition of lipid peroxidation in a liposome model system was done according to the method of Farvin et al. (2014). Liposomes were prepared from soybean phosphatidylcholine by extrusion method as described by Habeebullah et al. (2010). Lipid oxidation was performed in a model system containing 0.1 mg of phosphatidylcholine liposomes per mL of phosphate-buffered saline (PBS) (3.4 mM Na_2HPO_4-NaH_2PO_4, 0.15 M NaCl, pH 7.0) and enzymatic extracts at a concentration of 5 mg mL^{-1}. Lipid oxidation was initiated by iron redox cycling using 50 μM $FeCl_3$ and 100 μM ascorbate. The order of addition was buffer, extracts, liposome, ferric chloride, and ascorbic acid. The reactants were mixed by vortexing for 2 s and incubated at 37 °C in a water bath for 1 h. The liposome assay solution with distilled water was used as the control. Lipid oxidation was measured by determining the concentration of thiobarbituric acid reactive substance (TBARS) formed according to the method of Buege and Aust (1978). The amount of TBA-reactive substances (malondialdehyde or MDA) released per mg phospholipid (PL) was calculated using the molar extinction coefficient of MDA as 1.56×10^5. The % inhibition of TBARS formation was calculated as follows.

$$\%\text{inhibition} = \frac{T_c - T_s}{T_c} \times 100$$

where T_c is the μmoles of MDA released by the control (Liposome alone) and T_s is the μmoles of MDA released by the samples.

The antibacterial activities of the enzyme extracts were also determined by disk diffusion method as described by Murray et al. (1995). Briefly, the seaweed extracts were filtered through a 0.2 μm syringe filter. Paper disks impregnated with 10 μL of extracts were used for antimicrobial activity assay using the conventional diffusion plate method in Mueller-Hinton agar (MHA) (BD, Difco, USA). Inhibition zones around the disk indicated antibacterial activity, which was measured after 24 h of incubation at 37 °C for bacterial strain and 48 h for yeast and fungi isolates. Different antibiotics (ampicillin (AM-10), sulfamethoxazole with trimethoprim (SXT), gentamicin (GM-120) were used as positive controls in the plates. The test organisms used were *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Aeromonas hydrophila* (ATCC 35654), *Bacillus cereus* (ATCC 11778), enterohaemorrhagic *Escherichia coli* (EHEC), enterotoxigenic *E. coli* (ETEC)*12652* (obtained from NICED, India), *Candida albicans* (ATCC 10231), and *Saccharomyces cerevisiae* (ATCC 9763). Commercial BD BBL™ Sensi-Disks antimicrobial susceptibility test disks were used. The determinations were performed at least twice and the averages of the values reported.

Fractionation of the active enzymatic extracts

The preliminary screening of the enzymatic extracts on different antioxidant activity assays and total phenolic compounds revealed that some of the seaweed extracts digested with particular enzymes showed high bioactivity. In order to understand the components responsible for this bioactivity, these extracts were further fractionated into phenolic-rich (PhR fraction), protein-rich (PrR fraction), polysaccharide-rich (PsR fraction), and low molecular weight fractions (LMW fractions) according to the method of Farvin and Jacobsen (2015). In brief, about 10 g of freeze dried enzymatic extracts prepared by above described procedure was dissolved in 150 mL of distilled water and was extracted three times with 100 mL ethyl acetate. The ethyl acetate phase was collected and the aqueous phase was extracted with ethyl acetate after adjusting to pH 5 and subsequently to pH 2. The ethyl acetate phase was pooled into the same bottle and concentrated by using a rotary evaporator and this formed the PhR fraction. The pH precipitated fraction obtained by the centrifugation of aqueous layer at 1500×g for 10 min was considered as PrR fraction. The supernatant was precipitated with three times absolute ethanol and was subjected to low-temperature precipitation overnight. It was centrifuged at 1500×g for 10 min and the supernatant was collected. Ethanol in the supernatant was removed by rotary evaporation and freeze dried. This formed the low molecular weight fraction (LMW fraction). The precipitate obtained after centrifugation was dissolved in distilled water and was de-proteinated by liquid-liquid partition with Sevag reagent (butanol:chloroform, 1:5) (Staub 1965) several times, then precipitated with three volumes of absolute ethanol. The precipitate obtained after centrifugation at 1500×g for 10 min was freeze dried and this forms the PsR fraction. The yield of each fraction obtained were expressed as g (100 g)$^{-1}$ extract.

Characterization and bioactivity screening of the fractions

The four fractions (PhR, PrR, PsR, and LMW fractions) obtained from the enzymatic extracts were evaluated for chemical composition. The total phenolic content (TPC) of the different fractions were determined by the Folin-Ciocalteu method (Farvin and Jacobsen 2013). The soluble protein content of the LMW and PrR fractions were determined by Bradford microassay method with bovine serum albumin (BSA) as standard (Bradford 1976). Total reducing sugars and soluble carbohydrates for the LMW and PsR fractions were analysed by the dinitrosalicylic acid (DNS) (Miller 1959) and phenol-sulphuric acid method (Masuko et al. 2005) respectively, with glucose as the standard. These individual fractions were further screened for antioxidant activity as mentioned earlier.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) of triplicate analysis. The data on the various assays were subjected to two-way analysis of variance (ANOVA) using GraphPad Prism 7.04 (GraphPad Software Inc., USA). Tukey's multiple comparison test was used for comparison of the group means. P value < 0.05 was considered as statistically significant. In order to choose the extract for fractionation, the complex data on the four antioxidant assays, total phenolic content of the different enzymatic extracts were subjected to principal component analysis (PCA) by using Unscrambler version 10.0 (Camo, Norway).

Results and discussion

Yield and total phenolic content of the enzymatic extracts

The yield of enzymatic extracts from brown seaweeds were compared with water extracts (Table 2). The yield of the enzymatic extracts varied with seaweed species and also the enzyme used for the extraction. The overall yield showed that the enzymatic extraction recovered about 1.2 to 3 times more cell wall material compared to control water extraction in different seaweeds (Table 2). The enzymes break down the algal cell walls and complex inner storage materials, thereby releasing compounds into solution which may be the reason for the higher yield of enzymatic extracts over the conventional water extracts. The yield of the enzymatic extracts of different seaweeds ranged from 30.3 ± 1.3 to $92.9 \pm 2.2\%$ while the yield of water extracts ranged from 18.7 ± 0.3 to $50.8 \pm 1.3\%$. Except *P. gymnospora* and *C. cervicornis*, the protease and carbohydrase treatments yielded high soluble materials indicating extraction of more compounds to the solution by breaking the important interlinks in the cell walls. The enzymatic extracts of brown seaweeds *F. irregularis* and *C. sinuosa* showed higher yield when compared to other species. The enzymatic extracts of *P. gymnospora* had the lowest yield. This species may contain less digestible protein and polysaccharides when compared to the other seaweeds. In general, proteases showed higher yield than carbohydrases. Among the five carbohydrases, Ultraflo showed high yield in all the brown seaweeds, and in proteases, Flavourzyme showed the highest yield.

Ultraflo L is a heat-stable multi-β-glucosidase from the fungus *Humicola insolens*. Ultraflo L might have broken down the laminarin (β-1,3-glucan), the main storage polysaccharide in brown algae and contributed to the higher yield. Unlike Alcalase (endo-peptidase) and Neutrase (metallo-endoprotease), Flavourzyme is an enzyme complex containing both endoprotease and exopeptidase which might have broken down different peptide bonds in the cell wall proteins as well as the proteins inside the cells leaving more soluble fractions. In addition to this, some species showed high yield in certain enzymes; for example, *S. angustifolium*, *S. boveanum*, and *C. cervicornis* showed high yield in Alcalase and Neutrase treatment whereas *F. irregularis* showed significantly ($P < 0.001$) high yield in Termamyl and Neutrase and *I. stellata* in Alcalase and Celluclast treatments. The breaking down of cell walls by the enzymes used for the hydrolysis have different specificities for the different block structures of cell wall and hence resulted in a mixture of compounds with different yield.

Total phenolic content also varied with species and enzyme used for extraction (Table 2). *S. angustifolium*, *S. boveanum*, and *F. irregularis* showed high total phenolic content in almost all the enzymatic extracts. In *S. angustifolium*, the highest phenolic content was for Flavourzyme followed by Viscozyme, whereas *S. boveanum* showed the highest phenolic content in AMG, Termamyl, and Alcalase. Viscozyme followed by Alcalase released more phenolic content in *F. irregularis*. Lowest TPC was observed in *I. stellata* which was followed by *C. sinuosa*, *C. cervicornis*, and *P. gymnospora*. In *C. cervicornis* and *P. gymnospora*, Alcalase extracts showed more phenolic compounds. In all of these brown seaweeds, the control with water extraction showed significantly ($P < 0.001$) lower TPC when compared to the enzymatic extracts.

The cell walls of brown seaweeds are made of cellulose, sulphated fucans, and alginates. The sulphated fucan and alginates form the main portion of the cell walls which comprise up to 45% of algal dry weight while cellulose accounts for a small portion (1–8% of algal dry weight) depending upon the species (Michel et al. 2010). Halogenated and/or sulphated phenolic compounds known as phlorotannins and halide compounds as iodide are additional components in brown algal cell walls which form strong complexes with proteins either by reversible hydrogen bonding or by irreversible covalent condensation (Deniaud-Bouët et al. 2014). In traditional water extraction, the presence of these compounds in the cell wall limits the accessibility of water to bioactive compounds, thereby reducing the extraction efficiency. The different enzymes used in the study might have cleaved the cell wall-associated polysaccharides and proteins at different positions depending upon the enzyme specificity, thereby releasing the bound compounds, which resulted in higher yield and total phenolic content of the enzymatic extracts than the control water extracts. The difference in yield and total phenolic content of the enzymatic extracts in different species stems from their cell wall composition. In the present study, the yield and total phenolics obtained for the enzymatic extracts of *S. boveanum* and *S. angustifolium* were far higher than the yield and total phenolics of other S*argassum* spp. reported by Heo et al. (2003) from Korea and Sánchez-Camargo et al.

Table 2   Yield and total phenolic content of the enzymatic extracts from different brown seaweeds

Enzymes	S. angustifolium	S. boveanum	P. gymnospora	C. cervicornis	C. sinuosa	F. irregularis	I. stellata
% Yield (g (100 g)$^{-1}$ dried seaweed)							
Carbohydrases							
Viscozyme	59.9 ± 1.1 [b,y]	59.8 ± 3.2 [b,y]	37.7 ± 1.5 [b,x]	62.2 ± 2.6 [b,y]	72.2 ± 2.0 [b,z]	72.7 ± 3.1 [b,z]	60.0 ± 2.9 [b,y]
AMG	53.2 ± 1.4 [b,y]	53.6 ± 1.7 [b,y]	38.5 ± 1.0 [b,x]	57.8 ± 2.0 [b,y]	72.3 ± 2.5 [b,z]	75.8 ± 4.6 [b,z]	65.4 ± 2.2 [b,y]
Celluclast	64.8 ± 1.2 [b,y]	63.9 ± 1.6 [b,y]	33.7 ± 1.0 [b,x]	64.8 ± 1.1 [b,y]	70.7 ± 2.1 [b,z]	74.7 ± 4.6 [b,z]	74.3 ± 2.6 [c,z]
Termamyl	55.6 ± 2.0 [b,x]	62.7 ± 1.5 [b,x]	30.3 ± 1.3 [b,w]	56.5 ± 2.1 [b,x]	71.0 ± 2.3 [b,y]	85.4 ± 4.0 [b,c,z]	65.6 ± 3.0 [b,x,y]
Ultraflo	75.6 ± 1.0 [c,x]	75.4 ± 2.0 [c,x]	36.2 ± 1.0 [b,w]	77.6 ± 1.0 [c,x]	82.5 ± 3.2 [c,y]	95.1 ± 2.0 [c,z]	72.7 ± 1.3 [c,x]
Proteases							
Flavourzyme	92.3 ± 2.0 [d,z]	90.2 ± 1.1 [d,z]	59.1 ± 1.2 [c,x]	82.7 ± 2.0 [d,y]	83.8 ± 2.2 [c,y]	92.9 ± 2.2 [c,z]	82.0 ± 2.9 [d,y]
Alcalase	72.6 ± 1.5 [c,y]	70.2 ± 1.0 [a,c,y]	34.4 ± 1.0 [b,x]	72.6 ± 2.0 [c,y]	74.2 ± 4.5 [b,y]	77.9 ± 2.0 [b,y]	70.8 ± 1.0 [b,c,y]
Neutrase	70.8 ± 1.0 [b,c,x,y]	74.4 ± 1.5 [c,y]	33.6 ± 1.0 [b,w]	75.1 ± 2.0 [c,y]	67.8 ± 2.0 [a,b,x]	92.0 ± 2.9 [c,z]	64.6 ± 2.0 [b,x]
Control (water)	36.9 ± 0.3 [a,y]	29.1 ± 1.1 [a,y]	18.7 ± 0.3 [a,x]	46.1 ± 0.9 [a,z]	50.8 ± 1.3 [a,z]	44.1 ± 1.1 [a,z]	39.3 ± 0.6 [a,y,z]
Total phenolic content (mg GAE g^{-1} extract)							
Carbohydrases							
Viscozyme	72.5 ± 3.7 [d,y]	70.8 ± 1.2 [c,d,y]	22.3 ± 2.3 [a,w]	32.2 ± 3.2 [a,x]	21.4 ± 1.6 [a,w]	82.5 ± 3.0 [e,z]	24.4 ± 0.5 [b,w]
AMG	66.3 ± 1.3 [c,y]	74.8 ± 1.2 [d,z]	27.0 ± 0.2 [a,w]	33.9 ± 5.8 [a,w]	23.1 ± 1.1 [a,w]	46.1 ± 1.2 [b,x]	19.8 ± 0.5 [b,w]
Celluclast	59.2 ± 1.6 [c,x]	58.3 ± 2.2 [c,x]	29.5 ± 1.7 [a,w]	26.1 ± 1.1 [a,w]	24.4 ± 0.3 [a,w]	54.8 ± 1.0 [b,x]	22.2 ± 0.1 [b,w]
Termamyl	61.1 ± 1.2 [c,y]	74.8 ± 1.8 [d,z]	33.4 ± 1.4 [a,b,w]	32.9 ± 2.3 [a,w]	22.7 ± 1.1 [a,v]	50.5 ± 1.2 [b,x]	22.7 ± 2.2 [b,v]
Ultraflo	62.4 ± 1.2 [c,z]	65.4 ± 5.0 [c,z]	36.5 ± 2.0 [a,b,x]	30.1 ± 0.5 [a,x]	21.7 ± 0.5 [a,w]	48.7 ± 1.9 [b,y]	20.4 ± 0.3 [b,w]
Proteases							
Flavourzyme	84.0 ± 6.7 [e,z]	61.2 ± 1.0 [c,y]	41.3 ± 2.8 [a,b,x]	31.2 ± 0.5 [a,w]	27.7 ± 4.5 [a,w]	62.6 ± 0.7 [c,y]	22.0 ± 0.9 [b,w]
Alcalase	69.3 ± 2.1 [c,d,y]	74.8 ± 1.5 [d,z]	63.8 ± 3.4 [b,y]	43.0 ± 2.6 [b,x]	32.0 ± 2.5 [b, w]	75.0 ± 0.7 [d,z]	38.4 ± 1.3 [b,w]
Neutrase	50.0 ± 3.7 [b,y]	50.5 ± 2.9 [b,y]	32.3 ± 1.9 [a,b,x]	29.8 ± 1.6 [a,x]	24.4 ± 2.0 [a,x]	65.7 ± 0.6 [c,z]	25.5 ± 0.4 [b,x]
Control (water)	22.9 ± 5.0 [a,y]	32.4 ± 3.9 [a,z]	24.5 ± 3.6 [a,y]	37.3 ± 5.1 [b,z]	16.7 ± 1.7 [a,x]	28.2 ± 4.2 [a,y]	9.5 ± 1.9 [a,x]

Values are as mean ± SD (n = 3). Tukey's test. The letters a, b, c, d, and e show the significant difference among enzymatic extracts of particular seaweed species and the letters w, x, y, and z show the significant difference among the extracts of the different species. Same letters show no significant difference

(2016) from France. This might be due to difference in species as well as the geographical location of the seaweeds. Kuwait coast is a unique environment with high salinity (34 to 45 ppt), temperature (30–35 °C), and UV index (8–9). During low tides, about 2–3 km of the intertidal areas is exposed to sunlight; hence, the algae in this coast have to be exposed to dehydration for 3–4 h during the low tides. As an adaptation, these seaweeds might produce rigid cell walls incorporating more phenolics and sulphated polysaccharides. Studies have shown that when brown algae were transplanted from low saline to high saline waters, the genes responsible for the production of sulphated polysaccharides and phenolic polymers were upregulated in order to change the plasticity of cell wall composition in relation to habitat (Megías et al. 2007; Dittami et al. 2012).

Antioxidant activity of enzymatic extracts

The enzymatic extracts obtained from different brown seaweeds were screened for antioxidant activity by using four *in vitro* assays, viz., radical scavenging, metal chelating, reducing power, and inhibition of lipid oxidation in liposome model system. Table 3 shows the antiradical power ($1/EC_{50}$) of the different enzymatic extracts. The antiradical power of the different enzymatic extracts ranged from 0.5 ± 0.01 to 12.5 ± 0.6 and varied with species and also with the enzyme used for the extraction. In general, the carbohydrases showed high radical scavenging activity when compared to proteases, and in the proteases, Neutrase showed highest radical scavenging activity. *S. angustifolium* and *S. boveanum* followed by *F. irregularis* showed significantly ($P < 0.001$) high antiradical power. The highest antiradical power among all the species studied was for *S. angustifolium* and *S. boveanum* when hydrolysed with Viscozyme and AMG. These species also showed high antiradical power when hydrolysed with Termamyl, Ultraflo, and Neutrase. *F. irregularis* showed high antiradical power in Neutrase and Ultraflo extracts. The antiradical power of the different enzyme extracts of *P. gymnospora*, *C. cervicornis*, *C. sinuosa*, and *I. stellata* were significantly ($P < 0.001$) low when compared to *Sargassum* species studied (Table 3). The control water extracts showed significantly ($P < 0.001$) low antiradical power except for *C. cervicornis* where the water extracts showed significantly ($P < 0.001$) high antiradical power than the enzymatic extracts

(Table 3). The water extracts of this species also showed high phenolic content (Table 2).

Similar to our results, studies involving enzymatic extraction of other *Sargassum* species also showed a high radical scavenging activity. Heo et al. (2005) reported a high free radical scavenging of *S. correanum* when hydrolysed by Viscozyme. Similarly, *S. horneri* and *S. muticum* also showed high antioxidant activity including high radical scavenging activity when hydrolysed with Viscozyme and Alcalase (Park et al. 2004; Sánchez-Camargo et al. 2016). Though the extracts which showed high radical scavenging activity also contained high total phenolics, some extracts with high total phenolic content such as Flavourzyme extracts of *S. angustifolium*, Alcalase extracts of *S. boveanum*, *P. gymnospora*, and *F. irregularis* did not show high radical scavenging activity. This indicated that phenolic compound alone was not responsible for the antiradical power, and other co-extracted compounds such as proteins, peptides, and oligosaccharides also contributed to radical scavenging activity.

In contrast to antiradical power, the iron chelating property was high for protease-digested extracts when compared to carbohydrases (Table 3). The seaweeds extracted with the Flavourzyme and Alcalase showed significantly ($P < 0.001$) high iron chelating activity when compared to other enzymatic extractions by having low EC_{50} values. *Padina gymnospora*-Flavourzyme extracts showed the highest iron chelating activity. Water extracts of *C. sinuosa*, *C. cervicornis*, and *I. stellata* showed significantly high EC_{50} values when compared to enzymatic extracts indicating a very low iron chelating activity. Water extracts of *S. angustifolium* showed iron chelating activity similar to that of Termamyl and Ultraflo whereas the iron chelating activity of *S. boveanum* was similar to Viscozyme and Celluclast extracts. The high iron chelating activity of the proteases indicated that small proteins and peptides released during the enzymatic hydrolysis of the seaweeds are responsible for the iron chelating activity. Megías et al. (2007) reported a notable copper chelation of Alcalase and Flavourzyme hydrolysates of chick pea proteins and they isolated copper chelating peptides from these extracts.

Table 4 shows the reducing power of the different enzymatic extracts of seaweeds. In general, the reducing power was

Table 3 The antiradical power and iron chelating activity of different brown seaweed enzymatic extracts

Enzymes	S. angustifolium	S. boveanum	P. gymnospora	C. cervicornis	C. sinuosa	F. irregularis	I. stellata
Antiradical power ($1/EC_{50}$)							
Carbohydrases							
Viscozyme	12.5 ± 0.6 [d,z]	12.5 ± 0.4 [d,z]	0.8 ± 0.1 [a,w]	3.3 ± 0.1 [b,c,x]	1.1 ± 0.1 [a,w]	7.1 ± 0.3 [c,y]	1.0 ± 0.1 [a,w]
AMG	12.5 ± 0.2 [d,z]	11.1 ± 0.2 [c,z]	0.6 ± 0.1 [a,w]	2.3 ± 0.2 [b,x]	0.8 ± 0.1 [a,w]	4.0 ± 0.2 [b,y]	0.5 ± 0.1 [a,w]
Celluclast	0.8 ± 0.1 [a,w]	4.0 ± 0.1 [b,x]	3.7 ± 0.2 [b,x]	1.5 ± 0.1 [a,w]	1.1 ± 0.1 [a,w]	3.7 ± 0.3 [b,x]	0.71 ± 0.1 [a,w]
Termamyl	11.1 ± 0.2 [c,z]	11.1 ± 0.2 [c,z]	4.2 ± 0.1 [b,y]	3.8 ± 0.1 [c,y]	1.7 ± 0.2 [a,b,x]	4.1 ± 0.0 [b,y]	1.0 ± 0.3 [a,x]
Ultraflo	11.1 ± 0.1 [c,z]	11.1 ± 0.3 [c,z]	3.3 ± 0.1 [b,x]	3.8 ± 0.3 [c,x]	1.7 ± 0.1 [a,b,w]	8.3 ± 0.4 [d,y]	1.4 ± 0.1 [a,b,w]
Proteases							
Flavourzyme	2.3 ± 0.1 [b,y]	1.7 ± 0.1 [a,x]	0.8 ± 0.1 [a,x]	2.8 ± 0.2 [b,y]	1.1 ± 0.1 [a,x]	6.7 ± 0.2 [c,z]	0.9 ± 0.1 [a,x]
Alcalase	2.5 ± 0.1 [b,y]	2.4 ± 0.1 [a,y]	0.7 ± 0.1 [a,x]	2.9 ± 0.1 [b,y]	0.5 ± 0.01 [a,x]	1.0 ± 0.1 [a,x]	1.1 ± 0.1 [a,x]
Neutrase	11.1 ± 0.3 [c,z]	10.0 ± 0.2 [c,y]	3.5 ± 0.4 [b,w]	4.5 ± 0.3 [c,x]	2.5 ± 0.4 [c,v]	11.1 ± 0.2 [e,z]	1.6 ± 0.1 [b,v]
Control (water)	0.7 ± 0.01 [a,x]	1.2 ± 0.01 [a,x]	1.4 ± 0.01 [a,x]	9.1 ± 0.1 [d,z]	0.7 ± 0.01 [a,x]	2.5 ± 0.01 [a,y]	0.5 ± 0.01 [a,x]
Iron chelating activity (EC_{50}, mg mL^{-1})							
Carbohydrases							
Viscozyme	4.8 ± 0.1 [d,x]	4.3 ± 0.1 [d,w]	2 ± 0.1 [c,u]	7.4 ± 0.1 [f,y]	9.4 ± 0.3 [f,z]	2.8 ± 0.1 [b,v]	4 ± 0.4 [d,w]
AMG	2.8 ± 0.1 [b,w]	3.2 ± 0.1 [b,c,x]	2.4 ± 0.1 [c,w]	2.7 ± 0.1 [d,w]	5.6 ± 0.1 [d,y]	3.3 ± 0.1 [c,x]	3.6 ± 0.2 [c,x]
Celluclast	4.3 ± 0.2 [d,x]	4.2 ± 0.2 [d,x]	2.4 ± 0.2 [c,w]	4.0 ± 0.3 [e,x]	8.6 ± 0.4 [e,y]	3.9 ± 0.2 [d,x]	4.2 ± 0.2 [d,x]
Termamyl	3.6 ± 0.2 [c,y]	2.8 ± 0.3 [b,x]	1 ± 0.1 [a,b,w]	3.3 ± 0.1 [e,y]	3.5 ± 0.2 [b,y]	2.4 ± 0.1 [b,x]	2.7 ± 0.1 [b,x]
Ultraflo	3.2 ± 0.1 [c,y]	2.4 ± 0.2 [b,x]	0.6 ± 0.1 [a,w]	3.4 ± 0.1 [e,y]	8 ± 0.3 [e,z]	3 ± 0.2 [c,y]	3.4 ± 0.2 [c,y]
Proteases							
Flavourzyme	0.4 ± 0.1 [a,x]	0.6 ± 0.1 [a,x]	0.2 ± 0.1 [a,x]	1.0 ± 0.1 [b,y]	0.7 ± 0.1 [a,x]	0.6 ± 0.1 [a,x]	0.7 ± 0.1 [a,x]
Alcalase	0.6 ± 0.2 [a,x]	0.6 ± 0.1 [a,x]	0.4 ± 0.1 [a,x]	0.6 ± 0.1 [a,x]	0.6 ± 0.1 [a,x]	0.5 ± 0.1 [a,x]	0.7 ± 0.1 [a,x]
Neutrase	2 ± 0.2 [b,y]	2.1 ± 0.2 [b,y]	0.9 ± 0.1 [a,x]	2 ± 0.1 [c,y]	4.4 ± 0.3 [c,z]	2.5 ± 0.3 [b,y]	2.8 ± 0.2 [b,y]
Control (water)	3.9 ± 0.2 [c,x]	4.0 ± 0.1 [d,x]	1.6 ± 0.1 [b,v]	> 10 [g,z]	> 10 [g,z]	2.2 ± 0.1 [b,y]	8.8 ± 0.3 [e,y]

Values are expressed as mean ± SD ($n = 3$). Tukey's test. The letters a, b, c, d, and e show the significant difference among enzymatic extracts of particular seaweed species and the letters w, x, y, and z show the significant difference between extracts of the different species. Same letters indicate no significant difference

Table 4 The reducing power and inhibition of lipid peroxidation in liposomal model system of the different enzymatic extracts of brown seaweeds

Enzymes	S. angustifolium	S. boveanum	P. gymnospora	C. cervicornis	C. sinuosa	F. irregularis	I. stellata
Reducing power at 1 mg concentration (10 mg mL^{-1})							
Carbohydrases							
Viscozyme	1.0 ± 0.0 [e,z]	1.0 ± 0.08 [e,z]	0.2 ± 0.01 [a,w]	0.4 ± 0.0 [c,x]	0.1 ± 0.0 [a,v]	0.8 ± 0.0 [e,y]	0.2 ± 0.0 [b,w]
AMG	0.5 ± 0.02 [b,y]	0.8 ± 0.01 [d,z]	0.3 ± 0.0 [b,w]	0.4 ± 0.0 [c,x]	0.2 ± 0.0 [b,v]	0.4 ± 0.01 [b,x]	0.1 ± 0.0 [a,u]
Celluclast	0.4 ± 0.02 [b,y]	0.5 ± 0.01 [a,z]	0.2 ± 0.02 [a,w]	0.2 ± 0.0 [a,w]	0.2 ± 0.01 [b,w]	0.3 ± 0.0 [a,x]	0.1 ± 0.0 [a,v]
Termamyl	0.6 ± 0.0 [c,z]	0.6 ± 0.01 [b,z]	0.3 ± 0.0 [b,x]	0.4 ± 0.01 [c,y]	0.2 ± 0.01 [b,w]	0.4 ± 0.01 [b,y]	0.2 ± 0.0 [b,w]
Ultraflo	0.7 ± 0.01 [d,z]	0.6 ± 0.01 [b,y]	0.3 ± 0.01 [b,w]	0.4 ± 0.01 [c,x]	0.2 ± 0.01 [b,v]	0.4 ± 0.01 [b,x]	0.2 ± 0.0 [b,v]
Proteases							
Flavourzyme	0.9 ± 0.01 [e,z]	0.6 ± 0.02 [b,y]	0.4 ± 0.0 [c,w]	0.3 ± 0.01 [b,v]	0.2 ± 0.01 [b,u]	0.5 ± 0.01 [c,x]	0.2 ± 0.0 [b,u]
Alcalase	0.6 ± 0.02 [c,y]	0.7 ± 0.01 [c,z]	0.5 ± 0.01 [d,x]	0.4 ± 0.03 [c,w]	0.2 ± 0.01 [b,u]	0.6 ± 0.01 [d,y]	0.3 ± 0.01 [c,v]
Neutrase	0.5 ± 0.0 [b,z]	0.5 ± 0.02 [a,z]	0.3 ± 0.09 [b,x]	0.4 ± 0.0 [c,y]	0.2 ± 0.0 [b,w]	0.4 ± 0.0 [b,y]	0.2 ± 0.0 [b,w]
Control (water)	0.1 ± 0.01 [a,v]	0.6 ± 0.03 [b,y]	0.3 ± .01 [b,x]	0.6 ± 0.01 [d,y]	0.2 ± 0.01 [b,w]	0.3 ± 0.0 [a,x]	0.2 ± 0.0 [b,w]
Inhibition of TBARS formation in liposomes (at 5 mg mL^{-1})							
Carbohydrases							
Viscozyme	24.2 ± 1.4 [b,y]	50.6 ± 5.0 [b,z]	30.6 ± 0.3 [b,c,y]	17.4 ± 1.8 [c,x]	17.0 ± 0.9 [b,x]	25.5 ± 8.2 [b,y]	13.0 ± 1.4 [b,x]
AMG	41.7 ± 3.8 [d,y]	62.5 ± 2.5 [c,z]	49.6 ± 3.1 [d,y]	20.3 ± 2.3 [c,x]	16.5 ± 3.1 [b,x]	40.5 ± 9.2 [c,y]	15.5 ± 1.4 [b,x]
Celluclast	16.9 ± 2.1 [b,x]	17.9 ± 5.9 [a,x]	44.0 ± 1.3 [d,y]	19.8 ± 1.6 [c,x]	15.0 ± 0.0 [b,x]	36.7 ± 1.1 [b,y]	20.3 ± 2.9 [b,c,x]
Termamyl	35.2 ± 5.6 [c,y]	56.9 ± 2.3 [b,z]	8.9 ± 1.2 [a,x]	3.6 ± 0.4 [b,x]	1.8 ± 0.9 [a,x]	23.4 ± 1.8 [b,y]	− 1.1 ± 3.8 [a,w]
Ultraflo	45.2 ± 2.8 [d,z]	52.5 ± 5.7 [b,z]	9.4 ± 2.2 [a,x]	− 3.8 ± 1.3 [a,w]	3.5 ± 1.0 [a,x]	21.6 ± 1.4 [b,y]	4.7 ± 0.9 [b,x]
Proteases							
Flavourzyme	29.4 ± 5.4 [b,y]	48.1 ± 4.4 [b,z]	26.9 ± 2.3 [b,c,y]	13.1 ± 1.3 [c,x]	21.8 ± 4.4 [b,y]	23.3 ± 3.4 [b,y]	5.6 ± 1.0 [b,w]
Alcalase	47.2 ± 2.5 [d,z]	56.5 ± 1.1 [b,z]	16.7 ± 0.8 [b,x]	19.9 ± 1.3 [c,x]	29.6 ± 4.1 [c,y]	31.8 ± 1.3 [b,y]	23.2 ± 1.8 [b,c,x]
Neutrase	8.4 ± 4.1 [a,x]	51.1 ± 2.9 [b,z]	15.0 ± 3.6 [b,x,y]	8.7 ± 1.1 [b,x]	2.0 ± 0.3 [a,x]	17.3 ± 1.1 [a,x]	9.1 ± 1.6 [b,x]
Control (water)	18.2 ± 2.7 [b,z]	18.0 ± 3.7 [a,z]	6.9 ± 0.9 [a,y]	− 1.1 ± 2.3 [a,x]	6.1 ± 0.3 [a,y]	10.1 ± 0.5 [a,y]	1.8 ± 0.2 [b,x]

Values are expressed as mean ± SD ($n = 3$). Tukey's test. The letters a, b, c, d, and e show the significant difference among enzymatic extracts of particular seaweed species and the letters w, x, y, and z show the significant difference between extracts of the different species. Same letters indicate no significant difference

low in all the extracts including control and enzymatic extracts when compared to ascorbic acid (Farvin et al. 2019). Among the different seaweeds, Viscozyme extracts of *S. angustifolium* and *S. boveanum* showed the highest reducing power (Table 4). In all the seaweeds, the control water extracts also showed low reducing power and there is no significant difference between the enzymatic extracts. Several authors have correlated the reducing property of the extracts and its total phenolic content (Paixão et al. 2007; Dudonné et al. 2009; Chakraborty et al. 2015). In the present study, though the Viscozyme extracts of *Sargassum* sp. which showed the highest reducing power also contain high total phenolic content, other enzyme extracts which contained high total phenolic content did not have high reducing power. This indicated that reducing power depends on the compounds released by the enzyme which in turn varies with seaweed species and cell wall composition. Viscozyme is a multi-enzyme complex containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase (Novozyme Nordisk, Denmark). When S*argassum* sp. was hydrolysed by this multi-enzyme complex, it released a combination of different poly- or

oligosaccharides along with phenolics depending upon the cell wall composition, which may be responsible for the high reducing property of these extracts. Similarly, Viscozyme hydrolysates of wine making by-products have been reported to release more bound phenolics and were shown to have high reducing power (Camargo et al. 2016). Viscozyme released higher amounts of some phenolics compared to pronase treatment and certain phenolics such as *p*-coumaric, caffeic acids, and procyanidin dimer B were extracted only with Viscozyme but not with pronase treatment (Camargo et al. 2016). Thus, the high reducing power of Viscozyme hydrolysate of *Sargassum* sp. might be due to the release of specific compounds present in these seaweeds.

Table 4 also shows the inhibition of TBARS formation of different enzymatic extracts in liposome model system. The inhibition of lipid oxidation among the different brown seaweeds varied from − 3.8 ± 1.3 to 62.5 ± 2.5% depending upon the enzyme used for hydrolysis and the species of seaweeds. *S. boveanum* showed the highest inhibition of lipid oxidation in almost all the enzyme extracts except Celluclast. This was followed by Ultraflo, Alcalase, and AMG extracts of

S. angustifolium, AMG and Celluclast extracts of *P. gymnospora*, and AMG extracts of *F. irregularis*. All the other extracts showed low inhibition. Water and Ultraflo extracts of *C. cervicornis* and Teramyl extracts of *I. stellata* showed pro-oxidant activity. The control water extracts showed significantly ($P < 0.001$) low inhibition of lipid oxidation when compared to enzymatic extracts. Water extracts of *S. boveanum* and *S. angustifolium* were similar to Celluclast extracts. The antioxidant activity of the different extracts in the liposome model system did not correlate with antiradical power, iron chelating, and reducing power. Liposome system is a prototype for the real lipid containing systems. So the extracts which showed high inhibition in liposome model systems may work well in real food system containing lipids (Farvin and Jacobsen 2015). Similar to our observation, Heo et al. (2003) also reported that there was no correlation between antiradical power and inhibition of lipid oxidation of the enzymatic extracts in linoleic acid oxidation. The difference in antioxidant activity of different enzymatic extracts might come from the different compounds released from the seaweeds depending upon the seaweed composition and specificity of the enzyme. The bioactivities of polysaccharides were reported to vary according to their structural makeup, monosaccharide composition, sulphate content, position of sulphate ester groups, and molecular weight (Li et al. 2008).

Antimicrobial properties of the different enzymatic extracts

Table 5 shows the antimicrobial activity of the enzymatic extracts of different brown seaweeds. In general, the digestion with Flavourzyme resulted more number of seaweed extracts with antibacterial activity. The proteases, Alcalase, and Neutrase yielded no antibacterial extracts in any of the seaweeds (data not shown) and no extract was effective against any of the pathogenic *E. coli* (EPEC, ETEC, EHEC) and *C. albicans* (data not shown). *F. irregularis* showed antibacterial activity against *S. aureus* when hydrolysed with Viscozyme, Celluclast, and Flavourzyme. *C. sinuosa* when hydrolysed with Flavourzyme showed high antibacterial activity against *E. faecalis* and low activity against *B. cereus*. *P. gymnospora* showed antibacterial activity against *E. faecalis* and *B. cereus* when digested with Celluclast and Ultraflo respectively. AMG extracts of *C. cervicornis* showed antibacterial activity against *A. hydrophila* and *S. angustifolium* showed antibacterial activity against *S. cerevisiae*. *I. stellata* showed antibacterial activity against *B. cereus* when hydrolysed with Flavourzyme. However, the inhibition zones of most of these extracts were very low except *P. gymnospora* Celluclast and *C. sinuosa* Flavourzyme extracts. The EC$_{50}$ value of these extracts was 5 mg mL^{-1}. As the zone of inhibition from the enzymatic extracts were very low, we can infer

that the extracts from these seaweeds are poor antimicrobial agents.

Phlorotannins isolated from brown algae were reported to possess antibacterial activity against a number of foodborne pathogens including *S. aureus* and *B. cereus* (Eom et al. 2012). Nagayama et al. (2002) reported that the interactions of phlorotannins with bacterial enzymes and proteins may play an important role in the bactericidal action. However, in the present study, most of the extracts which showed the antibacterial activity contained less total phenolics when compared to other extracts which did not show any antibacterial activity. So phlorotannin alone is not responsible for the antibacterial activity. It is interesting to note that antimicrobial activity was species dependent and also on the hydrolysing enzymes used. The seaweeds when hydrolysed with particular proteases and carbohydrases will liberate a series of poly- or oligosaccharides, proteins, and peptides along with the phenolic compounds depending on the specificity of the enzyme and the nature of cell wall composition of seaweeds. Thus, the composition and the bioactivity of the enzyme extracts differ with seaweeds and enzymes used for hydrolysis. In the present study, Alcalase and Neutrase, an endopeptidase and metalloendoprotease respectively, when acted on seaweeds, the resulting extracts do not have any antibacterial activity. However, Flavourzyme, an enzyme complex containing both endoprotease and exopeptidase, when acted on seaweed might have liberated some peptides which showed antibacterial activity. Similarly, Taha et al. (2013) reported that when sunflower protein isolate hydrolysed with a mixture of Alcalase and Flavourzyme, it showed antibacterial activity against all of the tested microorganisms but not when digested with individual enzymes. Likewise, peptides obtained by the trypsin hydrolysis of the seaweed *Saccharina longicruris* were shown to have antibacterial against *S. aureus* (Beaulieu et al. 2015).

Puspita et al. (2017b) studied the antimicrobial and antioxidant activity of enzymatic extracts from three species of tropical *Sargassum*. They found that the *Sargassum* sp. showed no antibacterial activity when hydrolysed by Viscozyme or protamax. In our study also, when *Sargassum* digested with Viscozyme and other proteases, it did not yield any antibacterial activity against the test organisms. However, *S. angustifolium* when digested with AMG showed activity against yeast *S. cerevisiae*. AMG, an exo-1,4-α-D-glucosidase from *Aspergillus niger*, might have cleaved the sulphated fucans at different positions and released the fucoidans or low molecular weight fucans with antimicrobial activity. The fucoidans and low molecular weight fucan from different seaweed have been reported to possess antibacterial activity against foodborne pathogens (Huang et al. 2018; Jun et al. 2018).

Fucans from brown algae are reported to have backbones which contain either long stretches of alternating α-(1–3)- and

Table 5 The antimicrobial properties of different enzymatic extracts

| Enzymes	Species	S. aureus	E. faecalis	S. cerevisiae	B. cereus	A. hydrophila
Viscozyme	S. boveanum	–	–	–	–	–
	S. angustifolium	–	–	–	–	–
	P. gymnospora	–	–	–	–	–
	C. cervicornis	–	–	–	–	–
	C. sinuosa	–	–	–	–	–
	F. irregularis	7.0 ± 0.8	–	–	–	–
	I. stellata	–	–	–	–	–
AMG	S. boveanum	–	–	–	–	–
	S. angustifolium	–	–	7.5 ± 0.7	–	–
	P. gymnospora	–	–	–	–	–
	C. cervicornis	–	–	–	–	7. 0 ± 0. 6
	C. sinuosa	–	–	–	–	–
	F. irregularis	–	–	–	–	–
	I. stellata	–	–	–	–	–
Celluclast	S. boveanum	–	–	–	–	–
	S. angustifolium	–	–	–	–	–
	P. gymnospora	–	15.0 ± 1.3	–	–	–
	C. cervicornis	–	–	–	–	–
	C. sinuosa	–	–	–	–	–
	F. irregularis	7.0 ± 0.5	–	–	–	–
	I. stellata	–	–	–	–	–
Ultraflo	S. boveanum	–	–	–	–	–
	S. angustifolium	–	–	–	–	–
	P. gymnospora	–	–	–	7.0 ± 0.4	–
	C. cervicornis	–	–	–	–	–
	C. sinuosa	–	–	–	–	–
	F. irregularis	–	–	–	–	–
	I. stellata	–	–	–	–	–
Flavourzyme	S. boveanum	–	–	–	–	–
	S. angustifolium	–	–	–	–	–
	P. gymnospora	–	–	–	–	–
	C. cervicornis	–	–	–	–	–
	C. sinuosa	–	22.0 ± 1.5	–	7.0 ± 0.5	–
	F. irregularis	8.0 ± 0.6	–	–	–	–
	I. stellata	–	–	–	7.0 ± 0.3	–

The symbol "–" represents no activity. Values are in mm and expressed as mean ± SD ($n = 2$)

α-(1–4)-L-fucose residues bearing one sulphate group in C2 or C4, or backbones based on a α-(1–3)-linked L-fucose residues bearing one or two sulphate groups in C2 and C4 (Deniaud-Bouët et al. 2017). However, some brown alga shows difference in structure by having substitutions such as acetyl groups and/or side chains of distinct lengths, sugar compositions, and at various bonding positions (Nagaoka et al. 1999; Ale and Meyer 2013; Deniaud-Bouët et al. 2014), which increases the number of possible structure. This may be the reason for the difference in bioactivity of different enzymatic extracts.

Isolation and characterization of specific compounds of interest

Results of the screening revealed that antimicrobial activities of the seaweed enzymatic hydrolysates are very low. Hence, the antioxidant activity and total phenolic content was used as a criterion for selecting the extracts for characterization. The data on the four antioxidant assays and TPC were very difficult to compare as there are 63 extracts. Hence, a PCA plot was made on the complex data of different antioxidant activities and total phenolic content of the enzyme extracts
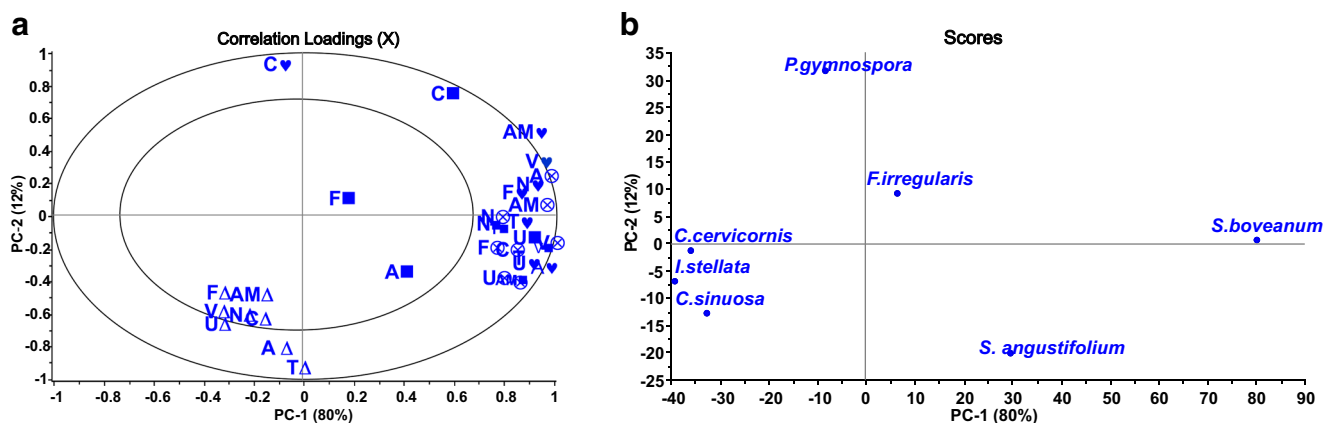
Fig. 1 a The correlation loadings for the different variables. The symbols represent the following: ■: antiradical power; △: iron chelating; ⊗: reducing power; and ♥: inhibition of lipid oxidation in liposomes. The letters represent the following: F: Flavourzyme; A: Alcalase; N: Neutrase; AM: AMG; U: Ultraflo; V: Viscozyme; T: Termamyl. **b** The score plot PCA of different brown algae

(Fig. 1a, b). From the PCA plot, it could be inferred that *S. boveanum* showed high antioxidant activity especially when it was hydrolysed with Viscozyme and Alcalase. Hence, *S. boveanum*-Viscozyme and *S. boveanum*-Alcalase extracts were selected for further fractionation and characterization. Each enzymatic extract was further fractionated into polysaccharide-rich fraction (PsR fraction), polyphenol-rich fraction (PhR fraction), protein-rich fraction (PrR fraction), and low molecular weight fraction (LMW fraction) as described in the "Materials and methods" section.

Chemical composition and bioactivity of the fractions from brown seaweeds

Sargassum boveanum when digested with Viscozymes and Alcalase yielded more of low molecular weight fraction which was followed by PrR and PsR fractions (Table 6). It is obvious that the multi-enzyme complex Viscozyme (containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase) and the endopeptidase Alcalase might have acted on polysaccharides and proteins at different positions which resulted in high levels of low molecular weight oligosaccharides and peptides. The total phenolic content was significantly ($P < 0.001$) high for the phenolic-rich fractions in both Alcalase and Viscozyme extracts (Table 6). However, in Alcalase extracts, the PrR fractions showed significantly high total phenolics and other fractions in both the extracts also showed low levels of phenolics indicating the presence of bound phenolic compounds. Phlorotannins have been reported to form strong complexes with proteins, either reversible by hydrogen bonding or irreversible by covalent condensation (Stern et al. 1996). This might be the reason for the high TPC of the protein-rich fractions from Alcalase hydrolysates.

In *S. boveanum* Viscozyme extracts, the PsR fractions showed significantly ($P < 0.001$) high reducing sugar when compared to LMW fractions whereas the total soluble carbohydrate was significantly high in LMW fraction than the PsR fraction. In the case of *S. boveanum* Alcalase extracts, the total soluble carbohydrates were significantly ($P < 0.001$) high for the PsR fraction than the LMW fractions. However, there was no significant difference ($P > 0.001$) in reducing sugar in PsR and low molecular weight fractions of Alcalase extracts. This shows that when hydrolysing *S. boveanum* with multi-enzyme complex such as Viscozymes, a number of poly- or oligosaccharide with free aldehyde or keto group have formed which might act as reducing agents. This may also be the reason for the high reducing power of Viscozyme extracts of *S. boveanum* and *S. angustifolium* (Table 4). Viscozyme hydrolysis of sugar beet pulp also reported to have high content of reducing sugars when compared to other enzymes (Berlowska et al. 2018). Both Alcalase and Viscozyme extracts showed no significant difference in soluble protein content and were low for both in PrR fraction and LMW fractions. The low solubility of proteins could be due to the formation of protein phenolic complex since both LMW and PrR fractions contained phenolic compounds. Thus, when the algal cell wall was disrupted by proteases or carbohydrases, the intracellular constituents including proteins were prone to form complex with polyphenols leading to aggregation or precipitation which leads to low protein solubility (Siriwardhana et al. 2008).

The antioxidant activities of different fractions are shown in Fig. 2a–d. The bioactivity of different fractions varied with the enzyme used for hydrolysis. In the case of Viscozyme extracts, the antiradical power was significantly ($P < 0.001$) high for both PhR and PsR fractions when compared to other fractions and there was no significant ($P > 0.001$) difference between PhR and PsR fractions. In Alcalase extracts, PhR fractions showed significantly ($P < 0.001$) high radical power which was followed by PsR and PrR fractions (Fig. 2a). The

Table 6 The yield and chemical constituents of different fractions from *S. boveanum,* Viscozyme, and Alcalase extracts

|	Yield (g (100 g)$^{-1}$ extract)	TPC (mg GAE g^{-1})	Total soluble carbohydrate (g Glu kg^{-1})	Reducing sugar (g Glu kg^{-1})	Soluble protein (g kg^{-1})
S. boveanum-Viscozyme					
PsR fraction	6.1 ± 1.0 [a]	55.4 ± 2.6 [a]	76.2 ± 3.9 [a]	302.7 ± 41.5 [b]	ND
PrR fraction	21.6 ± 5.0 [b]	71.4 ± 3.9 [a]	ND	ND	2.1 ± 0.8 [a]
PhR fraction	0.8 ± 0.1 [a]	295.1 ± 28.9 [b]	ND	ND	ND
LMW fraction	55.4 ± 8.6 [c]	42.8 ± 6.6 [a]	96.7 ± 3.5 [b]	60.6 ± 4.4 [a]	3.9 ± 0.9 [a]
S. boveanum-Alcalase					
PsR fraction	13.9 ± 3.6 [b]	40.5 ± 5.7 [a]	105.6 ± 45.0 [b]	40.9 ± 8.0 [a]	ND
PrR fraction	11.6 ± 2.4 [b]	154.9 ± 15.2 [b]	ND	ND	6.7 ± 0.4 [a]
PhR fraction	0.3 ± 0.08 [a]	342.3 ± 83.9 [c]	ND	ND	ND
LMW fraction	48.3 ± 5.8 [c]	29.5 ± 5.3 [a]	31.1 ± 3.1 [a]	58.1 ± 3.5 [a]	1.9 ± 0.5 [a]

Values are expressed as mean ± SD ($n = 3$)

PsR polysaccharide-rich, *PrR* protein-rich, *PhR* polyphenol-rich, *LMW* low molecular weight, *ND* not determined

high radical scavenging activities of polyphenolic compounds such as phlorotannins from brown algae are well known (Sathya et al. 2017). Similar to the present study, ethyl acetate fractions rich in phlorotannins from *Sargassum fusiformis* and *Sargassum hystrix* were reported to have high radical scavenging activity (Budhiyanti et al. 2011; Li et al. 2017).

Phenolics are electron-rich compounds, which can efficiently donate electrons and form phenoxyl radical species intermediates in the presence of oxidizing agents. Phenoxyl radicals in turn can be stabilized either by resonance delocalisation of the unpaired electron in the ortho/para positions or by hydrogen bonding with adjacent hydroxyl group (Leopoldini et al.

Fig. 2 The antioxidant activity of different fractions from *S. boveanum*-Viscozyme and *S. boveanum*-Alcalase extracts. **a** The antiradical power ($1/EC_{50}$), **b** iron chelating activity (EC_{50}), **c** reducing power (OD at 700 nm), and **d** inhibition of TBARS formation in liposome model system. PsR polysaccharide-rich, PrR protein-rich, PhR polyphenol-rich, LMW low molecular weight
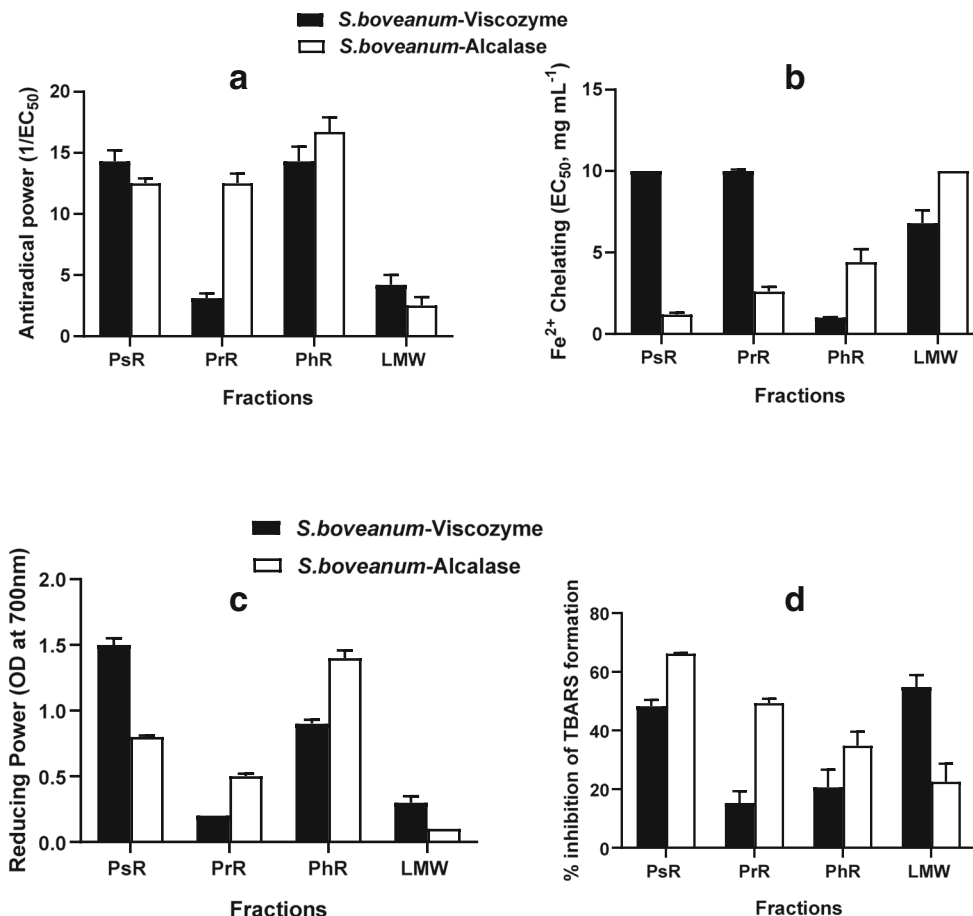
2004). In addition to the phenolic compounds, proteins, peptides, and sulphated polysaccharides have also been reported to have radical scavenging properties (Athukorala et al. 2006; Harnedy and FitzGerald 2011; Vijayabaskar et al. 2012).

Interestingly, the iron chelating activity was significantly ($P < 0.001$) high for the PhR fractions in Viscozyme extracts while Alcalase extracts showed high iron chelating activity in the PsR fraction followed by PrR and PhR fractions. The metal chelating ability of polysaccharides, proteins, and peptides are well documented (Pavia and Toth 2000; Kuda et al. 2005; Wu et al. 2010). However, metal chelating ability of phenolics have a lot of controversial reports. Wang et al. (2009) and Farvin and Jacobsen (2015) reported that phlorotannins are not good metal chelators. However, Budhiyanti et al. (2011) have reported that phenolic compounds from membrane-bound fractions of *S. hystrix* extracts showed high ferrous ion chelating ability than cytoplasmic fractions. The multi-enzyme complex carbohydrase such as Viscozyme might have liberated phenolic compounds bound to cell walls which might have resulted in phenolic compounds with iron chelating ability. Moreover, the metal chelating potency of phenolic compounds depends on phenolic structure and the number and location of the hydroxyl groups (Santoso et al. 2004; Andjelkovic et al. 2006). The metal chelating ability of polyphenols were related to the presence of ortho-dihydroxy polyphenols and presence of 5-OH and/or 3-OH in conjunction with a C4 keto group as in quercetin and large number of OH groups as in tannins (Khokhar and Apenten 2003).

In the case of Viscozyme extract, the PsR fractions showed the highest reducing power which was followed by PhR fractions. In contrast, Alcalase extracts showed high reducing power in PhR fractions followed by PsR and PrR fractions. The high reducing power of phenolic compounds were reported by many studies (Wang et al. 2009; Farvin and Jacobsen 2015). As mentioned earlier, the high reducing sugar content of the PsR fraction of the Viscozyme extracts might be the reason for the high reducing power. The prevention of lipid oxidation in liposome showed a different trend. In the case of Viscozyme extracts, significantly ($P < 0.001$) high inhibition of lipid oxidation was shown by PsR and low molecular weight fraction, whereas in Alcalase extracts, the highest inhibition of lipid oxidation was shown by PsR fractions followed by PrR and PhR fractions. The high antioxidant activity of the PsR and LMW fractions in *S. boveanum*-Viscozyme extracts might be due to the release of fucoidans during the enzymatic hydrolysis. Fucoidans are fucus containing sulphated polysaccharides specific to brown algae and were reported to possess various bioactivities such as antioxidant activity, anti-inflammatory, and anticancer (Ale and Meyer 2013). The antioxidant activities of the phenolic compounds and proteins from seaweeds were also well documented (Khokhar and Apenten 2003; Farvin and Jacobsen 2015).

Conclusions

The enzymatic extraction was highly efficient than the conventional water extraction in releasing the bioactive components. Depending upon the species of seaweeds and the enzymes used for the extractions, the bioactivity differed. Hence, the selection and optimization of the extraction conditions for each species of seaweed is an important step to get the specific bioactivity. Among the seven species of brown seaweeds hydrolysed with eight enzymes, *S. boveanum*-Viscozymes and *S. boveanum*-Alcalase showed high antioxidant activity. Antimicrobial activity was very low and was species and enzyme specific. Flavourzyme resulted in a greater number of species with antimicrobial activity. The fractionation of each of these enzymatic extracts showed that the specific component responsible for this bioactivity varies with enzymes used of the hydrolysis. Hydrolysis with Alcalase resulted in high total phenolics in PhR and PrR fractions of *S. boveanum* whereas it does not produce the same effect when hydrolysed with Viscozyme. In general, active fractions are phenolic-rich fractions and protein-rich fractions. However, some seaweeds hydrolysed with specific enzymes give more bioactive polysaccharides (*S. boveanum*-Viscozymes). Thus, the results of the study showed that selective hydrolysis by specific enzymes could enrich the bioactivity of the different seaweeds. Tailor-made extracts with specific bioactivity can be produced from seaweeds with the help of enzymes. Further studies are needed to establish the bioactivity of these extracts in real food system and in animal models.

References

Ale MT, Meyer AS (2013) Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation. RSC Adv 3:8131–8141

Andjelkovic M, Camp JV, Meulenaer BD, Depaemelaere G, Socaciu C, Verloo M, Verhe R (2006) Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chem 98:23–31

Athukorala Y, Kim K-N, Jeon Y-J (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food Chem Toxicol 44:1065–1074

Beaulieu L, Bondu S, Doiron K, Rioux L-E, Turgeon S (2015) Characterization of antibacterial activity from protein hydrolysates of the macroalga *Saccharina longicruris* and identification of peptides implied in bioactivity. J Funct Foods 17:685–697

Berlowska J, Cieciura-Włoch W, Kalinowska H, Kregiel D, Borowski S, Pawlikowska E, Binczarski M, Witonska I (2018) Enzymatic conversion of sugar beet pulp: a comparison of simultaneous saccharification and fermentation and separate hydrolysis and fermentation for lactic acid production. Food Technol Biotechnol 56:188–196

Bradford MM (1976) A dye binding assay for protein. Anal Biochem 72: 248–254

Budhiyanti SA, Raharjo S, Marseno DW, Lelana IYB (2011) Free radical scavenging, metal chelating and singlet oxygen quenching activity of fractionated brown seaweed *Sargassum hystrix* extract. J Biol Sci 11:288–298

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. In: Fleisher S, Packer L (Eds.) Methods in enzymology, biomembranes. Part C: Biological oxidations. Academic Press, London, pp 302–310

Camargo AC, Regitano-d'Arce MAB, Biasoto ACT, Shahidi F (2016) Enzyme-assisted extraction of phenolics from winemaking by-products: antioxidant potential and inhibition of alpha-glucosidase and lipase activities. Food Chem 212:395–402

Chakraborty K, Joseph D, Praveen NK (2015) Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. J Food Sci Technol 52:1924–1935

Deniaud-Bouët E, Kervarec N, Michel G, Tonon T, Kloareg B, Herve C (2014) Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae. Ann Bot 114:1203–1216

Deniaud-Bouët E, Hardouin K, Potin P, Kloareg B, Hervé C (2017) A review about brown algal cell walls and fucose-containing sulfated polysaccharides: cell wall context, biomedical properties and key research challenges. Carbohydr Polym 175:395–408

Dittami SM, Gravot A, Goulitquer S, Rousvoal S, Peters AF, Bouchereau A, Boyen C, Tonon T (2012) Towards deciphering dynamic changes and evolutionary mechanisms involved in the adaptation to low salinities in Ectocarpus (brown algae). Plant J 71:366–377

Dudonné S, Vitrac X, Coutière P, Woillez M, Mérillon J (2009) Content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J Agric Food Chem 57:1768–1774

Eom S-H, Kim Y-M, Kim S-K (2012) Antimicrobial effect of phlorotannins from marine brown algae. Food Chem Toxicol 50:3251–3255

Farvin KHS, Jacobsen C (2013) Phenolic compounds and in vitro antioxidant activity of selected species of seaweeds from Danish coast. Food Chem 138:1670–1681

Farvin KHS, Jacobsen C (2015) Antioxidant activity of seaweed extracts: in vitro assays, evaluation in 5% fish oil-in-water emulsions and characterization. J Am Oil Chem Soc 92:571–587

Farvin KHS, Andersen LL, Nielsen HH, Jacobsen C, Jakobsen G, Johansson I, Jessen F (2014) Antioxidant activity of Cod (*Gadus morhua*) protein hydrolysates: part 1—*in vitro* assays and evaluation in 5% fish oil- in-water emulsion. Food Chem 149:326–334

Farvin KHS, Surendraraj A, Al-Ghunaim A, Al-Yamani F (2019) Chemical profile and antioxidant activities of 26 selected species of seaweeds from Kuwait coast. J Appl Phycol 31:2653–2668

Fleurence J, Massiani L, Guyader O, Mabeau S (1995) Use of enzymatic cell wall degradation for improvement of protein extraction from *Chondrus crispus*, *Gracilaria verrucosa* and *Palmaria palmata*. J Appl Phycol 7:393–397

Habeebullah SFK, Nielsen NS, Jacobsen C (2010) Antioxidant activity of potato peel extracts in a fish-rapeseed oil mixture and in oil-in-water emulsions. J Am Oil Chem Soc 87:1319–1332

Hardouin K, Bedoux G, Burlot A-S, Collén PN, Bourgougnon N (2014a) Enzymatic recovery of metabolites from seaweeds: potential applications. Adv Bot Res 71:279–320

Hardouin K, Burlot A-S, Umami A, Tanniou A, Stiger-Pouvreau V, Widowati I, Bedoux G, Bourgougnon N (2014b) Biochemical and antiviral activities of enzymatic hydrolysates from different invasive French seaweeds. J Appl Phycol 26:1029–1042

Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides, and amino acids from macroalgae. J Phycol 47:218–232

Heo S, Lee K, Song CB, Jeon Y-J (2003) Antioxidant activities of enzymatic extracts from brown seaweeds. Algae 18:71–81

Heo S, Park E, Lee K, Jeon Y (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 96:1613–1623

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Huang C-Y, Kuo C-H, Lee C-H (2018) Antibacterial and antioxidant capacities and attenuation of lipid accumulation in 3T3-L1 adipocytes by low-molecular-weight fucoidans prepared from compressional-puffing-pretreated *Sargassum crassifolium*. Mar Drugs 16:24

Jeon YJ, Wijesinghe WAJP, Kim SK (2011) Enzyme-assisted extraction and recovery of bioactive components from seaweeds. In: Kim SK (ed) Handbook of marine macroalgae. John Wiley & Sons, Ltd., London, pp 221–228

Jiao G, Yu G, Zhang J, Ewart HS (2011) Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar Drugs 9:196–223

John DM, Al-Thani RF (2014) Benthic marine algae of the Arabian Gulf: a critical review and analysis of distribution and diversity patterns. Nova Hedwigia 98:341–392

Jun J-Y, Jung M-J, Jeong K, Yamazaki Y, Kawai I-H, Kim B-M (2018) Antimicrobial and antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque bacteria. Mar Drugs 16:301

Khokhar S, Apenten RKO (2003) Iron binding characteristics of phenolic compounds: some tentative structure–activity relations. Food Chem 81:133–140

Kuda T, Tsunekawa M, Hishi T, Araki Y (2005) Antioxidant properties of dried, kayamo-nori', a brown alga *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). Food Chem 89:617–622

Leopoldini M, Marino T, Russo N, Toscano M (2004) Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. J Phys Chem A 108:4916–4922

Li B, Lu F, Wei X, Zhao R (2008) Fucoidan: structure and bioactivity. Molecules 13:1671–1695

Li Y-X, Wijesekara I, Li Y, Kim S-K (2011) Phlorotannins as bioactive agents from brown algae. Process Biochem 46:2219–2224

Li Y, Fu X, Duan D, Liu X, Xu J, Gao X (2017) Extraction and identification of phlorotannins from the brown alga, *Sargassum fusiforme* (Harvey) Setchell. Mar Drugs 15:49

Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S, Lee YC (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal Biochem 339:69–72

Megías C, Pedroche J, Yust MM, Girón-Calle J, Alaiz M, Millan F, Vioque J (2007) Affinity purification of copper chelating peptides from sunflower protein hydrolysates. J Agric Food Chem 55:6509–6514

Michel G, Tonon T, Scornet D, Cock JM, Kloareg B (2010) The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in eukaryotes. New Phytol 188:82–97

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH (1995) Manual of clinical microbiology, 6th edn. Mosby Year Book, London

Nagaoka M, Shibata H, Kimura-Takagi I, Hashimoto S, Kimura K, Makino T, Aiyama R, Ueyama S, Yokokura T (1999) Structural study of fucoidan from *Cladosiphon okamuranus* Tokida. Glycoconj J 16:19–26

Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002) Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. J Antimicrob Chemother 50:889–893

Paixão N, Perestrelo R, Marques JC, Câmara JS (2007) Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. Food Chem 105:204–214

Park P-J, Fereidoon S, Jeon Y-J (2004) Antioxidant activities of extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry J. Food Lipids 11:15–27

Pavia H, Toth G (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. Ecology 81:3212–3225

Peng J, Yuan J-P, Wu C-F, Wang J-H (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. Mar Drugs 9:1806–1828

Puspita M, Deniel M, Widowati I, Radjasa OK, Douzenel P, Bedoux G, Bourgougnon N (2017a) Antioxidant and antibacterial activity of solid-liquid and enzyme-assisted extraction of phenolic compound from three species of tropical *Sargassum*. IOP Conf. Ser.: Earth Environ Sci 55:012057

Puspita M, Déniel M, Widowati I, Radjasa OK, Douzenel P, Marty C, Vandanjon L, Bedoux G, Bourgougnon N (2017b) Total phenolic content and biological activities of enzymatic extracts from *Sargassum muticum* (Yendo) Fensholt. J Appl Phycol 29:2521–2537

Sánchez-Camargo AP, Montero L, Stiger-Pouvreau V, Tanniou A, Cifuentes A, Herrero M, Ibáñez E (2016) Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae. Food Chem 192:67–74

Santoso J, Yoshie-Stark Y, Suzuki T (2004) Antioxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. Fish Sci 70:183–188

Sathya R, Kanaga N, Sankar P, Jeeva S (2017) Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forsskål) Arab. J Chem 10:S2608–S2614

Silberfeld T, Leigh JW, Cruaud C, Verbruggen H, Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the "brown algal crown radiation". Mol Phylogenet Evol 56:659–674

Siriwardhana N, Kim K-N, Lee K-W, Kim S-H, Ha J-H, Song CB, Lee J-B, Jeon Y-J (2008) Optimisation of hydrophilic antioxidant extraction from *Hizikia fusiformis* by integrating treatments of enzymes, heat and pH control. Int J Food Sci Technol 43:587–596

Staub AM (1965) Removal of proteins: Sevag method. Meth Carbohydr Chem 5:5–6

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phlorotannin–protein interactions. J Chem Ecol 22:1877–1899

Taha FS, Mohame SS, Wagdy M, Mohamed GF (2013) Antioxidant and antimicrobial activities of enzymatic hydrolysis products from sunflower protein isolate. World Appl Sci J 21:651–658

Vásquez V, Martínez R, Bernal C (2019) Enzyme-assisted extraction of proteins from the seaweeds *Macrocystis pyrifera* and *Chondracanthus chamissoi*: characterization of the extracts and their bioactive potential. J Appl Phycol 31:1999–2010

Vijayabaskar P, Vaseela N, Thirumaran G (2012) Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae *Sargassum swartzii*. Chin J Nat Med 10:421–428

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

Wehr JD (2002) Brown algae. In: Wehr JD, Sheath RG (eds) Freshwater algae of North America—ecology and classification. Academic Press, New York, pp 757–773

Wijesinghe WAJP, Jeon YJ (2012) Biological activities and potential industrial application of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweed: a review. Carbohydr Polym 88:13–20

Wu S-C, Wang F-J, Pan C-L (2010) The comparison of antioxidative properties of seaweed oligosaccharides fermented by two lactic acid bacteria. J Mar Sci Technol 18:P537–P545