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Full Length Research Paper

Evaluation of antioxidant activities of five selected brown seaweeds from China

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The antioxidant activities of Methanol/Chloroform (MC) extracts and fractions of five brown algae (*Sargassum fusiforme*, *Sargassum kjellmanianum*, *Sargassum. pallidum*, *Sargassum thunbergii* and *Sargassum horneri*) from China were evaluated through DPPH/hydroxyl radical-scavenging activity and reducing power. Total phenolic content was investigated using Folin–Ciocalteau reagent. The MC extract of *S. kjellmanianum* showed higher antioxidant activity than other seaweeds. However, the MC extracts of all the seaweeds showed lower DPPH radical-scavenging activity than Gallic Acid (GA) and ascorbic acid (AscA) at 50 µg/ml. The MC extracts were further purified by liquid–liquid partition to afford four fractions: Petroleum Ether (PE), Ethyl Acetate (EA), Butanol (BU) and Aqueous (AQ), among which the EA was found to be the most effective fraction and showed higher antioxidant activity than AscA, except that the EA fraction of *S. thunbergii* presented lower reducing power than GA. Antioxidant activities of MC extracts showed an increase with increasing concentration (between 15 and 45 µg/ml) indicating the dose dependency of these properties. The phenolic compounds with highest activities were concentrated in the EA fraction and were of medium polarity. The finding showed that the antioxidant-rich extracts or fractions had a significant potential to be use as a natural antioxidant agent.

Key words: Brown seaweeds, *Sargassum,* antioxidant activity, phenols content.

INTRODUCTION

Oxidation is an essential biological process for energy production in many living organisms. However, excessive reactive oxygen species, produced *in vivo* during some oxidative reactions, are not only strongly associated with lipid peroxidation but also involved in the development of some chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, and diabetes (Moskovitz et al., 2002). Over the last decade, considerable experimental evidence has confirmed the importance for health of following a diet rich in antioxidants, which can protect the organism against the damage caused by these radicals.

Since ancient times, fresh or dried seaweeds have China, particularly along the coastlines in Eastern and Southern China. Early records of herbal medicinal seaweeds (utilized by boiling in water and using in decoction as drugs) appeared in Chinese literature 'Pen Tsae Kan Mu' about 2000 years ago. Thus, Chinese cultures have used seaweeds since 300 BC to treat parasitic infections as well as other medical problems such as cancer, goiter and other glandular problems (Moo-Puca et al., 2008). Brown seaweeds that is, *Sargassum vulgare* from Cuba and *Sargassum thunbergii* from Japan have been or are used as anti-helmintics in Cuba and Japan (Kang et al., 2008). Recently, the seaweeds extracts and fractions have been considered to be a rich source of antioxidants and different types of antioxidants have been isolated from various species of seaweeds (Fujimoto and Kaneda, 1984; Cahyana et al., 1992; Nagai and Yukimoto, 2003; Huang and Wang, 2004; Wang et al., 2009; Mancini-Filho et al., 2009; Rioux et al., 2010; Hu et al., 2010). The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic

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Abbreviations: MC, methanol/chloroform; **DPPH, α,** α-Diphenyl-β-picrylhydrazyl; **GA,** gallic acid; **AscA,** ascorbic acid; **PE,** petroleum ether; **EA,** ethyl acetate; **BU,** butanol; **AQ,** aqueous; **GAE,** gallic acid equivalent.

acid, flavonoid, tannins e.g.), which are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation (Yan et al., 1999; Athukorala et al., 2003a; Athukorala et al., 2003b; Heo et al., 2003).

Zhoushan Archipelago is located in the East Sea of China and 163 seaweeds, including 32 brown algae, live in the region (Zhang et al., 2002). However, little attention has been given to the study of antioxidant compounds from marine algae. In the continuing research for the bioactive components of marine origin, the antioxidative activities of five brown seaweeds belonging to *Sargassum* genus were evaluated using DPPH free radical and hydroxyl radical scavenging assay systems. In addition, we assessed the phenolic content and the reducing power of the extracts and fractions obtained from those species, as well as the correlation between the antioxidant activity and phenolic content.

MATERIALS AND METHODS

Marine algal material

The marine algae *Sargassum fusiforme*, *Sargassum kjellmanianum*, *Sargassum pallidum*, *S. thunbergii* and *Sargassum horneri* used for this study were freshly collected from the Zhoushan Archipelago coastline of Zhejiang Province, People's Republic of China, in the summer of 2009. Samples collected were washed thoroughly with freshwater, transported to the laboratory immediately and dried (38 ± 2°C) in a drier for 24 h. The dried seaweeds were powdered and used for further experiments. The species were identified by Professor Sheng-long Zhao, School of Marine Science, Zhejiang Ocean University, where five voucher specimens were deposited (no. ZA0807007 - ZA0807011).

Chemicals and reagents

DPPH (α, α-Diphenyl-β-picrylhydrazyl) and Folin-Ciocalteu reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid (GA), and ascorbic acid (AscA) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China) and were of the highest analytical grade. All other solvents and chemicals were of analytical grade.

Preparation of marine algal extracts, fractions

Each seaweed was prepared by pouring methanol/chloroform (2:1) into the bottle containing 100 g of seaweed powder at the ratio of 5:1 (v/w), and the mixture was ultrasonic at room temperature for 2 h under dark condition. The extraction was repeated three times. The total extracts were filtered and concentrated under reduced pressure to dryness, yielding the crude extract, which was then suspended in 90% aqueous methanol. The solution was partitioned with 3 × 100 ml of petroleum ether and the solution of aqueous methanol was evaporated under reduced pressure to a semisolid, dissolved in 200 ml distilled water and then successively partitioned with 3 × 100 ml of ethyl acetate and 3 × 100 ml of n-butanol, respectively. The resulting four extracts were evaporated to dryness in vacuum, to yield the petroleum ether (PE), ethyl acetate (EA), n-butanol-soluble (BU) fractions and aqueous residue (AQ), respectively. Yield of MC extracts was expressed as % w/w of seaweed on dry weight basis and fractions was indicated as percentage of MC extract.

Total phenolic content

Total phenols of the extracts and fractions were determined according to the Folin–Ciocalteu method (Duan et al., 2006). A 1.0 ml aliquot of sample was added to 1.5 ml of deionized water and 0.5 ml of 0.1 mol/l Folin–Ciocalteu reagent, and the contents were mixed thoroughly. After 1 min, 1.0 ml of 20% sodium carbonate solution was added, and the mixture was again mixed thoroughly. The controls contained all the reaction reagents except the sample. After 30 min of incubation at 37°C, the absorbance was measured at 750 nm, and compared to a gallic acid calibration curve. Total phenolics were estimated as gallic acid equivalent (GAE).

Antioxidant activities

Antioxidant assay for DPPH radical-scavenging activity

The DPPH radical-scavenging activity of samples was monitored according to the method of previous report (Yen and Chen, 1995). Briefly, a 2.0 ml aliquot of test sample (in methanol) was added 2.0 ml of 0.16×10^{-3} mol/l DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - (A_{sample} - A_{sample\ blank})/A_{control}] \times 100$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and the A_{sample} blank is the absorbance of the sample only (sample without DPPH solution). GA and AscA were used as positive controls.

Hydroxyl radical scavenging assay

The hydroxyl radical-scavenging activity of samples was measured according to the method of Jin et al. (1996) with some modifications. In this system, hydroxyl radicals were generated by the Fenton reaction. Hydroxyl radicals could oxidize Fe^{2+} into Fe^{3+}, and only Fe^{2+} could be combined with 1, 10-phenanthroline to form a red compound (1,10-phenanthroline-Fe^{2+}) with the maximum absorbance at 536 nm. The concentration of hydroxyl radical was reflected by the degree of decolourization of the reaction solution. Briefly, 1, 10-phenanthroline solution (1.0 ml, 1.865×10^{-3} mol/l), phosphate buffer saline (2.0 ml, 0.2 mol/l, pH 7.40), and samples (1.0 ml, 30 µg/ml) were added into a screw-capped tube orderly and mixed homogeneously. The FeSO4·7H2O solution (1.0 ml, 1.865×10^{-3} mol/l) was then pipetted into the mixture. The reaction was initiated by adding 1.0 ml H_2O_2 (0.03% v/v). After incubation at 37°C for 60 min in a water bath, the absorbance of reaction mixture was measured at 536 nm against reagent blank. The reaction mixture without any antioxidant was used as the negative control, and without H_2O_2 was used as the blank. The hydroxyl radical scavenging activity (HRSA) was calculated by the following formula:

$$\text{HRSA (\%)} = [(A_s - A_n)/(A_b - A_n)] \times 100$$

Where A_s, A_n, and A_b were the absorbance values determined at536 nm of the sample, the negative control, and the blank after reaction, respectively. AscA and GA were used as positive controls.

Table 1. Yield of MC extracts (as %w/w of seaweed on dry weight basis) and fractions (as % of MC extract) of five brown seaweeds (n = 3).

Alga	MC extracts	Fractions			
		PE	EA	BU	AQ
S. fusiforme	5.83 ± 0.26[a]	20.30 ± 0.09	14.68 ± 0.17	25.41 ± 0.16	33.58 ± 0.32
S. kjellmanianum	4.21 ± 0.26[b]	22.73 ± 0.21	15.74 ± 0.17	21.27 ± 0.09	44.35 ± 0.42
S. pallidum	6.54 ± 0.33[c]	21.25 ± 0.24	17.40 ± 0.25	24.90 ± 0.11	34.45 ± 0.28
S. thunbergii	3.43 ± 0.13[d]	16.67 ± 0.38	13.68 ± 0.28	18.72 ± 0.55	40.90 ± 0.2
S. horneri	5.42 ± 0.45[a]	19.21 ± 0.90	14.35 ± 0.32	23.02 ± 0.33	33.42 ± 1.49

All the values are mean ± SD; SD: standard deviation. [a–d]Column wise values with same superscripts of this type indicate no significant difference (P > 0.05).

Table 2. Total phenolic content (mg GAE/g extract) of MC extracts and fractions obtained from five brown seaweeds (n = 3).

Alga	MC extracts	Fractions			
		PE	EA	BU	AQ
S. fusiforme	9.00 ± 0.24[a]	2.80 ± 0.25[α]	18.56 ± 0.29[β]	3.52 ± 0.22[γ]	17.35 ± 0.47[δ]
S. kjellmanianum	16.27 ± 0.45[b]	6.47 ± 0.61[α]	33.40 ± 0.40[β]	5.82 ± 0.37[α]	8.39 ± 0.42[γ]
S. pallidum	10.29 ± 0.53[c]	25.41 ± 0.44[α]	15.56 ± 0.30[β]	4.36 ± 0.12[γ]	8.24 ± 0.18[δ]
S. thunbergii	11.45 ± 0.46[d]	4.44 ± 0.38[α]	29.35 ± 0.54[β]	6.91 ± 0.44[γ]	10.40 ± 0.47[δ]
S. horneri	12.25 ± 0.69[d]	6.92 ± 0.24[α]	27.66 ± 0.86[β]	18.55 ± 0.37[γ]	9.19 ± 0.38[δ]

All the values are mean ± SD; SD: standard deviation. [a–d] Column wise values with same superscripts of this type indicate no significant difference (P > 0.05). [α–δ] Row wise values with different superscripts of this type indicate significant difference (P < 0.05).

Determination of reducing power

The reducing power of all samples was determined as described by a literature report (Dorman et al., 2003). Generally, one milliliter of each sample dissolved in distilled water was mixed with 1.0 ml of phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of a 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. After 30 min incubation at 50°C, 1.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.0 ml of the upper layer were mixed with 2.0 ml of distilled water and 0.5 ml of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm. Increased absorbance indicates increased reducing power.

Statistical analysis

All experiments were performed in triplicate (n = 3), and an ANOVA test (using STATISTICA 8.0 software, StatSoft Inc., USA) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using Duncan's multiple range test (P < 0.05).

RESULTS AND DISCUSSION

Extracts and fractions yield

Yield of MC extracts and fractions of five brown seaweeds was shown in Table 1. Among the MC extracts of five seaweeds, *S. pallidum* exhibited higher yield of 6.54% followed by *S. Fusiforme* (5.83%), *S. horneri* (5.42%), *S. kjellmanianum* (4.21%) and *S. thunbergii* (3.43%). Among the fractions, AQ fraction had the highest yield in all the seaweeds. The result corroborates well with those studies by Duan et al. (2006) and Wang et al. (2009), who observed a higher yield of AQ fraction in the red alga, *Polysiphonia urceolata* (36.36%) and *Rhodomela confervoides* (72.30%), due to many polysaccharide was extracted from the algae. However, in contrast with results of Chandini et al. (2008), higher yield was observed in petroleum ether (28.40%), ethyl acetate (27.54%) and aqueous fraction (30.27%) of *T. conoides*, *P. tetrastomatica* and *S. marginatum*, respectively.

Total phenolic content

Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially their polyphenols, have antioxidant activity (Yan et al., 1999; Duan et al., 2006; Chandini et al., 2008; Ganesan et al., 2008; Wang et al., 2009). The major active compounds in different seaweed extracts have been reported to be phlorotannins and fucoxanthin (Yan et al., 1996; Yan et al., 1999). The phenolic contents in the MC extract (Table 2) were significantly different between species (P < 0.05). EA fraction of *S. fusiforme*, *S. kjellmanianum*, *S. thunbergii* and *S. horneri* exhibited higher phenolic content of 18.56, 33.40, 29.35 and 27.66 mg GAE/g of seaweed extract, respectively (Table 2), as

compared with other fractions and MC extracts. But in the case of *S. pallidum*, PE fraction showed higher content of 25.41 mg GAE/g. Many researches stated that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yukimoto, 2003; Chandini et al., 2008). In our study, the MC extract of *S. kjellmanianum* had significantly higher phenol content (16.27 mg GAE/g) and *S. fusiforme* had significantly lower phenol content (9.00 mg GAE/g) as compared with other four seaweeds. However, as compared to results of Duan et al. (2006) and Wang et al., (2009), phenolic content of MC extracts in the five brown alga studied was obviously lower than the red algae, *R. confervoides* (24 mg GAE/g) and *P. urceolata* (71.6 mg GAE/g).

DPPH radical scavenging activity

Due to the presence of different antioxidant components in the crude extracts of biological tissue samples, it is relatively difficult to measure each antioxidant component separately. Therefore, several assay methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such samples (Prabhakar et al., 2006; Wangensteen et al., 2004). These methods target at different mechanisms of the oxidant defense system, that is, scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions.

In the present work, the DPPH and hydroxyl radical-scavenging assay systems were successfully used for the evaluation of the antioxidant activities of the MC extracts and fractions derived from five brown algae of China.

DPPH radical-scavenging activity has been extensively used for screening antioxidants, such as polyphenols and anthocyanins, from marine algae (Duan et al., 2006; Wang et al., 2009; Chandini et al., 2008). DPPH is scavenged by polyphenols and anthocyanins through the donation of hydrogen, forming the reduced DPPH-H·. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm (Bondet et al., 1997; Chang et al., 2007). Table 3 indicated the DPPH radical-scavenging activity of polyphenol in five brown algae, and the MC extract from *S. kjellmanianum* showed significantly higher scavenging activity ($P < 0.05$) of 58.25% followed by *S. horneri* (43.82%), *S. thunbergii* (38.55%), *S. pallidum* (29.42%) and *S. Fusiforme* (24.20%). Among the fractions of five algae, PE and EA fractions showed higher scavenging activity than BU and AQ fractions. Scavenging activity of 41.66% was observed in PE fraction of *S. pallidum*, 38.41, 82.18, 64.33 and 76.68% in EA fraction of *S. fusiforme*, *S. kjellmanianum*, *S. thunbergii* and *S. horneri*, respectively. The results are consistent with those reported by Chandini et al. (2008) and Wang et al. (2009) in cases of brown and red seaweeds.

Figure 1 suggested that percent DPPH scavenging activities of the MC extracts from five algae were dose-dependent. However, the MC extracts showed lower DPPH scavenging activity as compared to standard anti-oxidant (GA and AscA). The results corroborates well with earlier reports in other higher plants including brown/red seaweeds (Kuda et al., 2005; Kumaran and Karunakaran, 2007) and their enzymatic extracts (Park et al., 2004).

Hydroxyl radical scavenging activity

The hydroxyl radical is one of representative reactive oxygen species generated in the body. In this study, hydroxyl radical-scavenging activity was investigated on the MC extracts from the five brown algae using the Fenton reaction mechanism (Table 4). Scavenging activity of the MC extracts obtained from the five seaweeds were statistically significant ($P < 0.05$), and the inhibition rate of the MC extracts from *S. kjellmanianum* (67.65%), *S. thunbergii* (57.66%) and *S. horneri* (57.95%) were higher than the positive control, AscA (32.43%) and GA (53.52%). Among the fractions of the five brown algae, EA fraction showed higher inhibition rate than other fractions, the finding indicated that compounds with the strongest antioxidant activity in the Fenton reaction system were of medium polarity.

Figure 2 revealed that the scavenging activity of seaweed extracts and the standard antioxidant (AscA and GA) were concentration-dependent and in line with earlier reports (Chandini et al., 2008; Ganesan et al., 2008). Compared to the standard antioxidant of AscA, the MC extracts of five brown seaweeds showed a relatively higher hydroxyl scavenging activity at any given concentrations (Figure 2), but only the MC extract of *S. kjellmanianum* showed higher activity than the standard antioxidant of GA. The finding indicated that the seaweed extracts were effective in scavenging hydroxyl radicals in a dose-dependent manner. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membranes and bring about peroxidic reactions of lipids (Kitada et al., 1979). Based on the evidence, we hypothesized the extracts from brown algae would show antioxidant effects against lipid peroxidation on biomembranes and scavenge the hydroxyl radicals at the stage of initiation and termination of peroxy radicals.

Reducing power

In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound (Barros et al., 2007). The presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex into the ferrous form. By measuring the formation of Perl's Prussian blue at 700

Table 3. DPPH radical scavenging activity (%) of MC extracts and fractions obtained from five brown seaweeds (concentration of extracts used = 50 µg/ml) (n = 3).

Alga	MC extracts	Fractions				GA	AscA
		PE	EA	BU	AQ		
S. fusiforme	24.20 ± 0.56^{a}	$10.14 \pm 0.43^{\alpha}$	$38.41 \pm 0.55^{\beta}$	$12.44 \pm 0.48^{\gamma}$	$6.08 \pm 0.32^{\delta}$	$92.43 \pm 0.21^{\varepsilon}$	$93.71 \pm 0.59^{\zeta}$
S. kjellmanianum	58.25 ± 1.36^{b}	$35.86 \pm 0.43^{\alpha}$	$82.18 \pm 0.44^{\beta}$	$42.86 \pm 0.45^{\gamma}$	$12.03 \pm 0.54^{\delta}$	$92.43 \pm 0.21^{\varepsilon}$	$93.71 \pm 0.59^{\zeta}$
S. pallidum	29.42 ± 0.66^{c}	$34.36 \pm 0.46^{\alpha}$	$41.66 \pm 0.43^{\beta}$	$27.40 \pm 0.26^{\gamma}$	$12.49 \pm 0.14^{\delta}$	$92.43 \pm 0.21^{\varepsilon}$	$93.71 \pm 0.59^{\zeta}$
S. thunbergii	38.55 ± 1.08^{d}	$39.13 \pm 0.37^{\alpha}$	$64.33 \pm 0.22^{\beta}$	$38.48 \pm 0.22^{\gamma}$	$24.55 \pm 0.29^{\delta}$	$92.43 \pm 0.21^{\varepsilon}$	$93.71 \pm 0.59^{\zeta}$
S. horneri	43.82 ± 0.65^{e}	$45.29 \pm 0.38^{\alpha}$	$76.68 \pm 0.13^{\beta}$	$14.57 \pm 0.30^{\gamma}$	$27.45 \pm 0.09^{\delta}$	$92.43 \pm 0.21^{\varepsilon}$	$93.71 \pm 0.59^{\zeta}$
GA	92.43 ± 0.21^{f}	NA	NA	NA	NA	NA	NA
AscA	93.71 ± 0.59^{f}	NA	NA	NA	NA	NA	NA

All the values are mean ± SD; SD: standard deviation; NA: not analysed. $^{a-f}$ Column wise values with same superscripts of this type indicate no significant difference ($P > 0.05$). $^{\alpha-\zeta}$ Row wise values with different superscripts of this type indicate significant difference ($P < 0.05$).

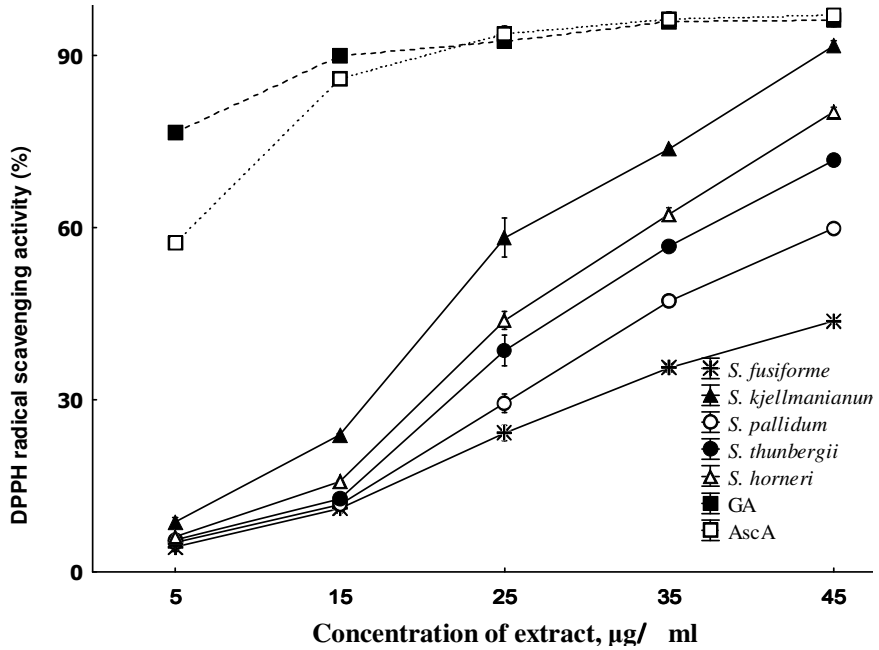

Figure 1. DPPH radical scavenging activity (%) of MC extracts from five brown seaweeds of China (n = 3).

Table 4. Hydroxyl radical scavenging activity (%) of MC extracts and fractions obtained from five brown seaweeds (concentration of extracts used = 50 µg/ml) (n = 3).

Alga	MC extracts	Fractions				GA	AscA
		PE	EA	BU	AQ		
S. fusiforme	30.84 ± 0.71^{a}	$13.65 \pm 0.12^{\alpha}$	$58.07 \pm 0.27^{\beta}$	$30.73 \pm 0.09^{\gamma}$	$9.39 \pm 0.08^{\delta}$	$53.52 \pm 0.18^{\varepsilon}$	$32.43 \pm 0.32^{\zeta}$
S. kjellmanianum	67.65 ± 0.19^{b}	$34.82 \pm 0.12^{\alpha}$	$82.39 \pm 0.36^{\beta}$	$24.66 \pm 0.31^{\gamma}$	$24.48 \pm 0.15^{\gamma}$	$53.52 \pm 0.18^{\delta}$	$32.43 \pm 0.32^{\varepsilon}$
S. pallidum	42.62 ± 0.26^{c}	$23.82 \pm 0.15^{\alpha}$	$64.53 \pm 0.10^{\beta}$	$30.13 \pm 0.66^{\gamma}$	$24.40 \pm 0.51^{\alpha}$	$53.52 \pm 0.18^{\delta}$	$32.43 \pm 0.32^{\varepsilon}$
S. thunbergii	57.66 ± 0.44^{d}	$27.34 \pm 0.22^{\alpha}$	$71.55 \pm 0.20^{\beta}$	$17.54 \pm 0.36^{\gamma}$	$25.61 \pm 0.18^{\delta}$	$53.52 \pm 0.18^{\varepsilon}$	$32.43 \pm 0.32^{\zeta}$
S. horneri	57.95 ± 0.43^{d}	$31.26 \pm 0.28^{\alpha}$	$69.52 \pm 0.23^{\beta}$	$13.43 \pm 0.09^{\gamma}$	$15.58 \pm 0.34^{\delta}$	$53.52 \pm 0.18^{\varepsilon}$	$32.43 \pm 0.32^{\zeta}$
GA	53.52 ± 0.18^{e}	NA	NA	NA	NA	NA	NA
AscA	32.43 ± 0.32^{f}	NA	NA	NA	NA	NA	NA

All the values are mean ± SD; SD: standard deviation; NA: not analysed. $^{a-f}$ Column wise values with same superscripts of this type indicate no significant difference ($P > 0.05$). $^{\alpha-\zeta}$ Row wise values with different superscripts of this type indicate significant difference ($P < 0.05$).

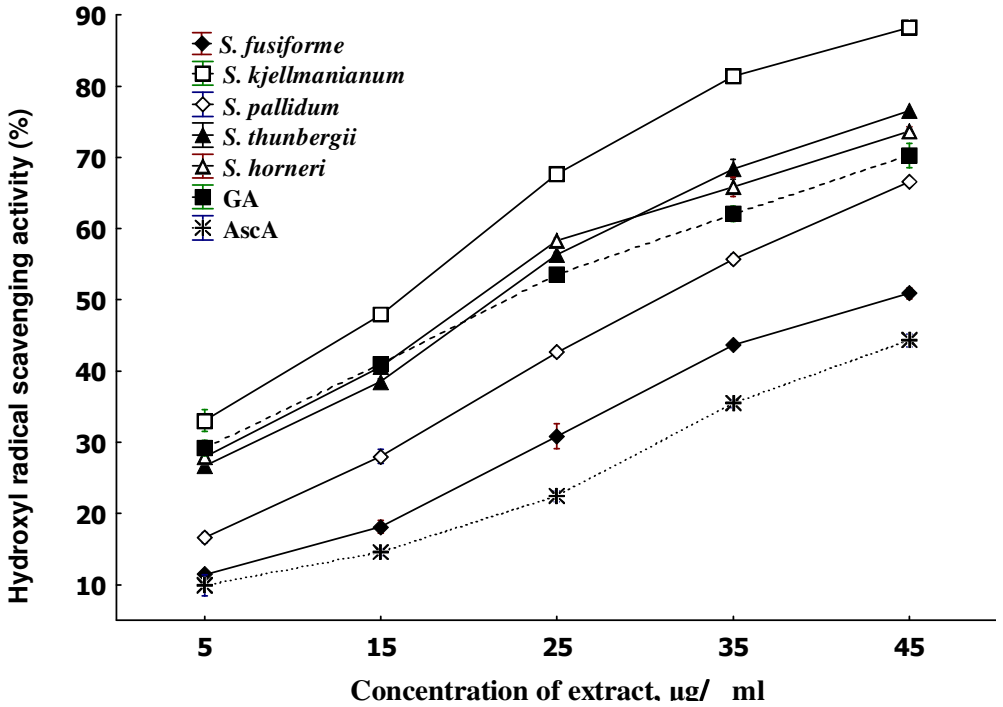

Figure 2. Hydroxyl radical scavenging activity (%) of MC extracts from five brown seaweeds of China (n = 3).

Table 5. Reducing power of MC extracts and fractions obtained from of five brown seaweeds (concentration of extracts used = 50 µg/ml) (n = 3).

Alga	MC extracts	Fractions				GA	AscA
		PE	EA	BU	AQ		
S. fusiforme	0.706 ± 0.074^{a}	$0.617 \pm 0.032^{\alpha}$	$0.843 \pm 0.015^{\beta}$	$0.550 \pm 0.020^{\gamma}$	$0.310 \pm 0.036^{\delta}$	$0.791 \pm 0.028^{\varepsilon}$	$0.706 \pm 0.019^{\zeta}$
S. kjellmanianum	0.982 ± 0.081^{b}	$1.085 \pm 0.022^{\alpha}$	$1.247 \pm 0.038^{\beta}$	$0.607 \pm 0.038^{\gamma}$	$0.314 \pm 0.020^{\delta}$	$0.791 \pm 0.028^{\varepsilon}$	$0.706 \pm 0.019^{\zeta}$
S. pallidum	0.744 ± 0.064^{c}	$0.687 \pm 0.015^{\alpha}$	$0.990 \pm 0.057^{\beta}$	$0.587 \pm 0.040^{\gamma}$	$0.375 \pm 0.037^{\delta}$	$0.791 \pm 0.028^{\varepsilon}$	$0.706 \pm 0.019^{\alpha}$
S. thunbergii	0.607 ± 0.011^{d}	$0.613 \pm 0.021^{\alpha}$	$0.763 \pm 0.025^{\beta}$	$0.357 \pm 0.035^{\gamma}$	$0.397 \pm 0.021^{\gamma}$	$0.791 \pm 0.028^{\beta}$	$0.706 \pm 0.019^{\delta}$
S. horneri	0.748 ± 0.071^{c}	$0.723 \pm 0.031^{\alpha}$	$0.880 \pm 0.017^{\beta}$	$0.500 \pm 0.030^{\gamma}$	$0.357 \pm 0.025^{\delta}$	$0.791 \pm 0.028^{\varepsilon}$	$0.706 \pm 0.019^{\alpha}$
GA	0.791 ± 0.011^{e}	NA	NA	NA	NA	NA	NA
AscA	0.706 ± 0.091^{a}	NA	NA	NA	NA	NA	NA

Reducing power is expressed as OD value at 700 nm. All the values are mean ± SD; SD: standard deviation. $^{a-e}$ Column wise values with same superscripts of this type indicate no significant difference ($P > 0.05$). $^{\alpha-\zeta}$ Row wise values with different superscripts of this type indicate significant difference ($P < 0.05$).

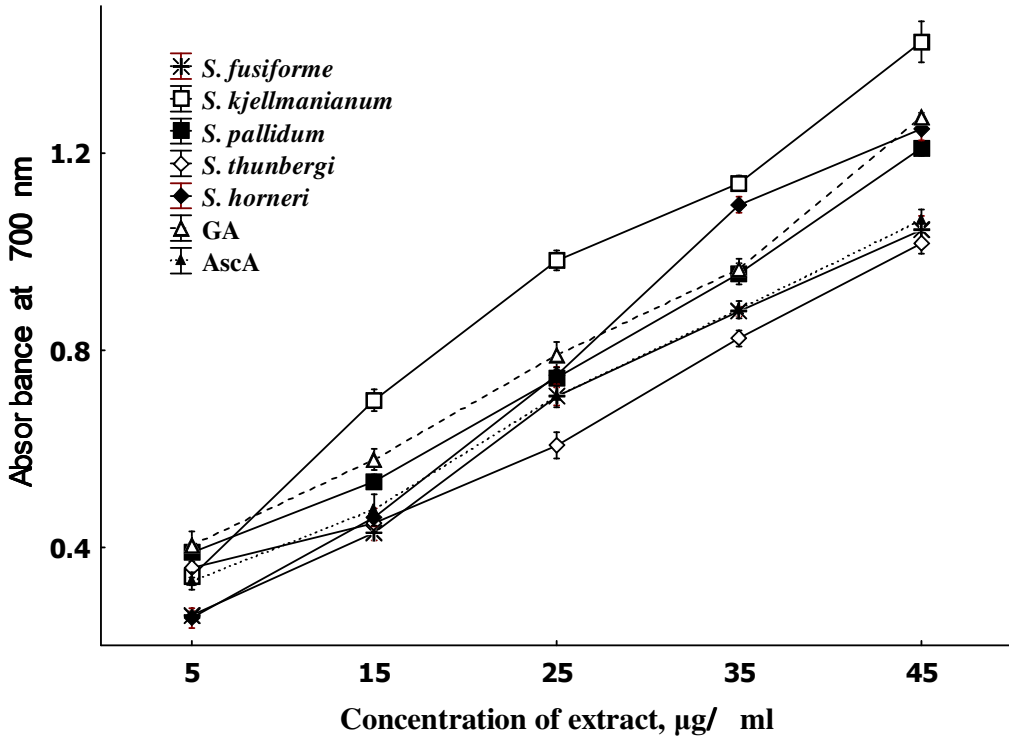

Figure 3. Reducing power of MC extracts from five brown seaweeds of China (n = 3).

nm, it is possible to determine the Fe^{2+} concentration. The reducing power of different extracts and fractions from five algae (to reduce ferric ions) was determined and expressed as OD value in this study. As listed in Table 5, amongst the MC extracts, the highest amount of reducing power was observed in the extract of *S. kjellmanianum* (0.982), followed by *S. horneri* (0.748), *S. pallidum* (0.744), *S. fusiforme* (0.706) and *S. thunbergii* (0.607). Compared with the PE, BU and AQ fractions, the EA fraction presented higher reducing power. Certainly, all of the EA fractions obtained the higher activity than the positive control (AscA), only the EA fraction of *S. thunbergii* showed lower reducing power than the positive control (GA). This result indicated that compounds with strongest reducing power were concentrated in the EA fraction and were of medium polarity. Interestingly, the EA fraction also exhibited the highest antioxidant activity in both the DPPH radical-scavenging assay and the Hydroxyl radical scavenging assay, indicating that there may be relationship between the antioxidant activities and reducing power.

Figure 3 presented the reducing power increased with increasing concentration in all samples. Similar trend was also achieved by Chandini et al. (2008) and Ganesan et al. (2008) in methanol extracts of Indian red and brown seaweeds. All concentrations exhibited the OD value <1.5. This property is associated with the presence of reductones that are reported to be terminators of free radical chain reaction (Duh, 1998). Moreover, it was observed that the MC extracts of *S. kjellmanianum* had higher reducing power than other four brown algae and the positive controls at given concentration (between 15 and 45 µg/ml). *S. pallidum* had higher reducing power than the positive control of AsaA at any give concentrations, but lower than the positive control of GA. *S. thunbergii* showed higher reducing power (from 25 to 45 µg/ml concentrations) as compared with AscA.

Conclusion

Our data of the present work indicated the EA fraction, derived from the MC extracts of five *Sargassum* genus species, was a fairly active fraction for *in vitro* DPPH radical-scavenging activities and hydroxyl radical-scavenging assay systems. The inhibitory characteristics of the extracts and fractions were determined in multiple ways, including the measurement of reducing power and phenolic content. In addition, the results suggested that phenolic compounds with medium polarity might be major contributors to the antioxidant activities of five brown seaweeds. The present findings seem promising to facilitate further experiments on the identification and characterization specific compounds which are responsible for the relatively high antioxidant activities.

Importantly, this research may contribute to a rational basis for the application of marine algal extract in possible therapy of diseases associated with oxidative stress and further supported that the antioxidant-rich extracts or fractions may be used as a dietary supplementary promoting good health.

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