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Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*

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Abstract

Antioxidant activity (AA), total phenolic content, and reducing power of the crude extract, fractions, and subfractions derived from a red alga, *Polysiphonia urceolata*, were evaluated and determined. The antioxidative activity was measured using the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay and the β-carotene–linoleate assay systems, and compared with that of butylated hydroxytoluene (BHT), gallic acid (GA), and ascorbic acid (AscA). The results showed that the crude extract and the ethyl acetate-soluble fraction exhibited higher AA than BHT in the DPPH assay model, at all of four concentration levels tested (from 0.4 to 50 μg/ml), while, in the β-carotene–linoleate assay system, the crude extract and the ethyl acetate-soluble fraction exhibited similar or, in most cases, higher AA than GA and AscA at the same concentrations (from 10 to 200 μg/ml). The ethyl acetate-soluble fraction was further fractionated into seven subfractions F1–F7 by silica gel vacuum liquid chromatography. F1 was found to be the most effective subfraction in both assay systems. The total phenolic content and reducing power were determined using the Folin–Ciocalteu and the potassium ferricyanide reduction methods, respectively. Statistical analysis indicated a significant association between the antioxidant potency and total phenolic content as well as between the antioxidant potency and reducing power.

Keywords: *Polysiphonia urceolata*; Antioxidant; DPPH; β-Carotene–linoleate; Total phenolic content; Reducing power

1. Introduction

Reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), and nitric oxide radical ($NO^{\bullet}$), attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, and carcinogenesis (Fisch, Böhm, Wright, & König, 2003; Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003; Shon, Kim, & Sung, 2003; Valentão et al., 2002). Antioxidants are effective in protecting the body against damage by reactive oxygen species. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants, such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT), that are commonly used in lipid-containing food (Amarowicz, Naczk, & Shahidi, 2000; Howell, 1986; Ito et al., 1986). Many natural antioxidants have already been isolated from different kinds of plant, such as oilseeds, cereal crop, vegetables, leaves, roots, spices and herbs (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Shon et al., 2003; Wettasinghe & Shahidi, 1999). Among natural antioxidants, phenolic antioxidants are in the forefront as they are widely distributed in the plant kingdom. Plants contain a diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic classes have the structural requirements of free radical scavengers and have

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potential as food antioxidants (Bandoniene & Murkovic, 2002).

However, natural antioxidants are not limited to terrestrial sources. Some of the seaweeds are considered to be a rich source of antioxidants (Lim, Cheung, Ooi, & Ang, 2002). For example, chlorophylls, carotenoids, tocopherol derivatives such as vitamin E, and related isoprenoids, that are structurally related to plant-derived antioxidants, were found in some marine organisms (Takamatsu et al., 2003). During the course of antioxidant activity screening of seaweeds commonly found in the Qingdao coastline, we measured the total lipophilic content and antioxidant activity of lower polar (diethyl ether) extracts of 16 seaweed samples (Huang & Wang, 2004). Our present work on more polar (chloroform:methanol) extracts found that the crude extract of the red alga, *Polysiphonia urceolata*, revealed higher antioxidant activity in the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay and in the β-carotene–linoleate assay systems. It should be mentioned that the raw extracts derived from different solvent extractions of this alga have previously been screened for antioxidant activity (Yan, Nagata, & Fan, 1998). In contrast, the current communication focussed on the investigation and evaluation of the antioxidative capacity of extract, fractions, and semi-purified subfractions with different polarity that were derived from *P. urceolata*. Furthermore, the total phenolic content and the reducing power of all extract, fractions and subfractions were also determined. In addition, the relationships between the antioxidant activity and the phenolic content as well as between the antioxidant activity and the reducing power are also considered.

2. Material and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), α,α-diphenyl-β-picrylhydrazyl (DPPH), β-carotene and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Linoleic acid, Tween-40 (polyoxyethylene sorbitan monopalmitate), gallic acid (GA), and ascorbic acid (AscA) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). Other chemicals used were of analytical grade.

2.2. Plant material

The red alga *P. urceolata* used for this study was freshly collected from the Qingdao coastline of Shandong Province, People's Republic of China. Sample collected was transported to the laboratory immediately and was gently rinsed with filtered fresh seawater, dried at room temperature, and stored in the dark. The material was identified at the Institute of Oceanology, Chinese Academy of Sciences, where the voucher specimen was deposited.

2.3. Preparation of seaweed extract, fractions, and subfractions

Dried material of *P. urceolata* (100 g) was ground into powder and extracted with 500 ml methanol:chloroform (2:1) in a Soxhlet extractor for 6 h. The extraction was repeated twice. The combined organic solution was evaporated under vacuum to dryness, yielding 12.1 g crude extract and then the extract was dissolved in 90% aqueous methanol. The solution was partitioned with 3×100 ml petroleum ether and the aqueous methanol was evaporated under reduced pressure to give a semisolid, which was then dissolved in 200 ml distilled water and further extracted successively with 3×100 ml of ethyl acetate and 3×100 ml of *n*-butanol, respectively. The resulting extracts were evaporated to dryness in vacuum, to yield the petroleum ether- (1.4 g), ethyl acetate- (1.2 g), *n*-butanol-soluble (1.6 g) fractions, and aqueous residue (4.4 g), respectively.

The ethyl acetate-soluble fraction which showed the highest activity in the subsequent antioxidative activity assays was further fractionated by vacuum liquid chromatography over silica gel (200–300 mesh) using step-gradient elution of chloroform/methanol (99:1) to methanol (100%), each of 300 ml, to yield seven subfractions (F1–F7). Solvent in each subfraction was evaporated to dryness under vacuum.

2.4. Antioxidant assays

2.4.1. Antioxidant assay for DPPH radical-scavenging activity

The scavenging effects of samples for DPPH radical were monitored according to the method of the previous report (Yen & Chen, 1995). Briefly, a 2.0-ml aliquot of test sample (in methanol) was added 2.0 ml of 0.16 mM 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 the DPPH radical was calculated using the follow equation:

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

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 $A_{\text{sample blank}}$ is the absorbance of the sample only (sample without DPPH solution). Synthetic antioxidants, BHT, GA, and AscA were used as positive controls.

2.4.2. Antioxidant assay with β-carotene–linoleic acid system

The antioxidant activities of samples assayed using the β-carotene–linoleic acid system were measured as in the method of a literature report with some modification (Jayaprakasha, Singh, & Sakariah, 2001). Briefly, 4 ml of a solution of β-carotene in chloroform (1 mg/ml) were pipetted into a flask containing 40 mg of linoleic acid and 400 mg of Tween-40. The chloroform was removed by rotary evaporator under vacuum at 45 °C for 4 min, and 100 ml distilled water were added slowly to the semi-solid residue with vigorous agitation to form an emulsion. A 96-well microtitre plate (polystyrene) was charged with 50 μl of test sample and 200 μl of the emulsion, and the absorbance was measured at 450 nm, immediately, against a blank consisting of the emulsion without β-carotene. The plate was stood at room temperature (20–23 °C), and the absorbance measurements were conducted again at 30 min intervals up to 420 min. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula:

$$AA = [1 - (A_0 - A_t)/(A'_0 - A'_t)] \times 100,$$

where A_0 and A'_0 are the absorbance of values measured at zero time of the sample and the control, respectively, and A_t and A'_t are the absorbances measured in the test sample and the control, respectively, after 420 min.

2.5. Determination of total phenolic content

Total phenols of the extract, fractions, and subfractions were determined according to the Folin–Ciocalteu method (Velioglu, Mazza, Gao, & Oomah, 1998). A 1.0-ml aliquot of sample was added to 1.5 ml of deionized water and 0.5 ml of 0.1 M 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).

2.6. Determination of reducing power

The reducing power of all samples was determined as described by a literature report (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003). Generally, one millilitre of each sample dissolved in distilled water was mixed with 1.0 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. After a 30-min incubation at 50 °C, 1.5 ml of 10% trichloroacetic acid were 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. The data were presented as ascorbic acid equivalent (AscAE) in milligrammes of ascorbic acid per gramme of seaweed material. A greater value of the AscAE related to greater reducing power of the sample.

2.7. Statistical analysis

Values expressed are means of three replicate determinations ± standard deviation. All statistical analyses were carried out using SPSS 11.01 for Windows. To determine whether there were any differences between activities of samples, variance analysis was applied to the result. Values of $p < 0.05$ were considered as significant different ($\alpha = 0.05$).

3. Results and discussion

3.1. Antioxidant assays

3.1.1. DPPH radical-scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH$^{\cdot}$ to the yellow-coloured diphenylpricrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (Shon et al., 2003). As can be seen in Table1, the crude extract of *P. urceolata* exhibited a concentration-dependent DPPH radical scavenging activity, which was higher than that of the positive control, BHT, in all of four tested concentrations from 0.4 to 50 μg/ml, but lower than those of GA and AscA at the same concentration. Among the four different polarity fractions isolated from the crude extract by solvent partition, the petroleum ether- and *n*-butanol-soluble fractions, as well as the aqueous residue, revealed no or only moderate activities (Table 1) in this assay system, while the ethyl acetate-soluble fraction showed the most potent activity and indicated that compounds with strongest radical-scavenging activity in *P. urceolata* are of medium polarity. The activity of this fraction was observed to be significantly higher than that of crude extract and the positive control, BHT, at the same concentration, at four levels. None of the fractions were more effective at scavenging DPPH radical in this assay than the positive controls GA and AscA, at the same concentrations.

Table 2 shows the DPPH radical-scavenging activities of subfractions F1–F7 isolated from the ethyl acetate-soluble fraction by vacuum liquid chromatography.

Table 1
DPPH radical-scavenging activities of different solvent fractions obtained from the *P. urceolata* crude extract

Fraction	Antioxidant activity ± SD (%)			
	0.4 (μg/ml)	2 (μg/ml)	10 (μg/ml)	50 (μg/ml)
Crude extract	3.46 ± 1.96a	7.30 ± 0.14b	20.9 ± 0.73c	67.9 ± 0.61e
Petroleum ether fraction	4.44 ± 0.65ab	3.61 ± 0.14a	5.89 ± 0.39a	11.8 ± 2.12b
Ethyl acetate fraction	4.77 ± 0.14b	13.6 ± 0.31c	61.8 ± 2.68d	93.9 ± 0.12f
n-Butanol fraction	1.73 ± 0.81a	3.90 ± 0.38ab	9.57 ± 0.32b	28.2 ± 0.46c
Aqueous residue	2.35 ± 1.19ab	2.83 ± 0.64a	3.85 ± 0.15a	5.92 ± 0.13a
BHT	2.18 ± 1.37ab	5.56 ± 0.48ab	18.1 ± 1.54c	63.5 ± 1.37d
GA	19.2 ± 0.72c	79.1 ± 3.15e	96.3 ± 0.15e	96.1 ± 0.18g
AscA	4.38 ± 0.25ab	31.0 ± 0.06d	96.0 ± 0.07e	96.8 ± 0.07g

Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–g) differ significantly ($p < 0.05$).

Table 2
DPPH radical-scavenging activities of subfractions F1–F7 derived from the ethyl acetate-soluble fraction of *P. urceolata*

Fraction	Antioxidant activity ± SD (%)			
	0.4 (μg/ml)	2 (μg/ml)	10 (μg/ml)	50 (μg/ml)
Ethyl acetate fraction	4.77 ± 0.14a	13.6 ± 0.31c	61.8 ± 2.68e	93.9 ± 0.12de
F1	11.7 ± 1.45b	28.3 ± 0.37e	94.0 ± 0.19g	96.0 ± 0.07f
F2	11.7 ± 1.28b	19.8 ± 0.14d	79.2 ± 3.04f	95.1 ± 0.00ef
F3	11.9 ± 0.57b	12.3 ± 0.30c	45.0 ± 0.87d	90.8 ± 0.73d
F4	8.76 ± 0.91b	7.67 ± 0.56ab	33.7 ± 0.62c	93.0 ± 0.44de
F5	10.4 ± 0.77b	9.84 ± 0.37bc	42.1 ± 1.14d	91.5 ± 0.22d
F6	9.04 ± 2.82b	6.75 ± 0.48ab	17.9 ± 1.45b	77.5 ± 2.42c
F7	9.96 ± 0.61b	4.82 ± 0.55a	9.74 ± 0.62a	34.1 ± 0.66a
BHT	2.18 ± 1.37a	5.56 ± 0.48a	18.1 ± 1.54b	63.5 ± 1.37b
GA	19.2 ± 0.72c	79.1 ± 3.15f	96.3 ± 0.15g	96.1 ± 0.18f
AscA	4.38 ± 0.25a	31.0 ± 0.06e	96.0 ± 0.07g	96.8 ± 0.07f

Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–g) differ significantly ($p < 0.05$).

The subfractions F1 and F2 were more effective scavengers than the ethyl acetate-soluble fraction itself and positive control, BHT, at all concentration levels. As indicated, the DPPH radical-scavenging activities of all subfractions, F1–F7, were similar or, in most cases, higher than those of the positive control, BHT, at each concentration tested. At concentrations of 2 and 10 μg/ml, F1 exhibited the highest DPPH radical-scavenging activity, followed by F2, F3, F5, F4, F6, and F7. At the higher concentrations (10 and 50 μg/ml), the DPPH radical scavenging ability of F1 was comparable to the positive controls GA and AscA, respectively.

3.1.2. The antioxidant activity with β-carotene–linoleate assay system

The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radical from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate- and other free radicals formed in the system (Wettasinghe & Shahidi, 1999; Shon et al., 2003). The antioxidant activities of the crude extract and four fractions derived from *P. urceolata*, as well as the positive controls, BHT, GA, and AscA, as measured by the bleaching of β-carotene, are presented in Table 3. In this assay, the crude extract exhibited stronger AA than the positive controls GA and AscA, at concentrations of 50, 100, and 200 μg/ml. In addition, at the higher concentration (200 μg/ml), the AA value of the crude extract was slightly higher than that of BHT (93.7 vs. 92.8%). Among the four different fractions, the ethyl acetate-soluble fraction again presented the highest activity (higher than GA and AscA at all concentrations tested), which indicated that compounds with strongest antioxidant activity in the β-carotene–linoleate assay system were also of medium polarity. The antioxidant activities of subfractions F1–F7 derived from the ethyl acetate-soluble fraction are shown in Table 4. Two of the most active subfractions belonged to the statistically indistinct group comprising F1 and F2, which showed similar or higher antioxidant activities than that of the ethyl acetate-soluble fraction itself and the positive controls, BHT, GA, and AscA, in the β-carotene–linoleate assay system.

It should be noted that, as statistical analyses indicated, there was no significant association between the DPPH radical-scavenging ability and antioxidant activity with the β-carotene linoleate assay in the different polarity fractions derived from *P. urceolata*. Also, there was no significant association between the two assay systems for the subfractions F1–F7. These results may sug-

Table 3
Antioxidant activities of different solvent factions obtained from the *P. urceolata* crude extract in the β-carotene–linoleate assay system

Fraction	Antioxidant activity ± SD (%)			
	10 (μg/ml)	50 (μg/ml)	100 (μg/ml)	200 (μg/ml)
Crude extract	20.3 ± 2.05ab	61.7 ± 2.05cd	81.5 ± 2.01f	93.7 ± 2.26e
Petroleum ether fraction	8.45 ± 1.40a	11.9 ± 0.51a	22.0 ± 3.01c	53.8 ± 2.48b
Ethyl acetate fraction	36.8 ± 0.27bc	74.9 ± 0.38cd	85.6 ± 1.19fg	92.1 ± 1.02e
n-Butanol fraction	10.2 ± 1.64a	52.5 ± 2.08bc	56.9 ± 2.03d	80.4 ± 2.96d
Aqueous residue	13.9 ± 0.14a	11.7 ± 1.62a	2.99 ± 0.30a	4.01 ± 1.08a
BHT	46.6 ± 5.19c	81.3 ± 0.24d	90.8 ± 0.83g	92.8 ± 0.15e
GA	24.6 ± 3.75ab	46.7 ± 2.63b	63.0 ± 2.42e	73.5 ± 0.91c
AscA	16.9 ± 1.74ab	15.7 ± 3.10a	14.0 ± 1.81b	48.2 ± 1.37d

Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–g) differ significantly ($p < 0.05$).

Table 4
Antioxidant activities of subfractions F1–F7 derived from the ethyl acetate-soluble fraction of *P. urceolata* in the β-carotene–linoleate assay system

Fraction	Antioxidant activity ± SD (%)			
	10 (μg/ml)	50 (μg/ml)	100 (μg/ml)	200 (μg/ml)
Ethyl acetate fraction	36.8 ± 0.27c	74.9 ± 0.38de	85.6 ± 1.19e	92.1 ± 1.02f
F1	52.5 ± 1.93f	75.2 ± 0.88de	89.5 ± 1.12e	92.6 ± 0.67f
F2	34.5 ± 1.75c	73.6 ± 0.45de	87.7 ± 0.68e	91.4 ± 1.46ef
F3	15.7 ± 2.54ab	68.6 ± 1.86d	84.9 ± 1.51de	92.2 ± 0.38f
F4	16.9 ± 2.25ab	67.9 ± 1.43d	76.9 ± 0.59de	87.7 ± 0.72de
F5	4.96 ± 0.48a	49.3 ± 0.29c	54.2 ± 3.49bc	85.2 ± 1.07d
F6	9.27 ± 1.24a	46.8 ± 4.47c	67.1 ± 0.59bc	85.1 ± 3.13d
F7	10.6 ± 0.60a	36.0 ± 4.49b	44.0 ± 1.69ab	77.3 ± 1.49c
BHT	46.6 ± 5.19d	81.3 ± 0.24e	90.8 ± 0.83e	92.8 ± 0.15f
GA	24.6 ± 3.75bc	46.7 ± 2.63bc	63.0 ± 2.42bc	73.5 ± 0.91b
AscA	16.9 ± 1.74ab	15.7 ± 3.10a	14.0 ± 1.81a	48.2 ± 1.37a

Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–f) differ significantly ($p < 0.05$).

gest that although DPPH radical-scavenging activity might be an indication of potential antioxidant activity, there may not be a linear correlation between these two activities. The antioxidant activities of putative antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical-scavenging (Cakir et al., 2003; Heinonen et al., 1998).

3.2. Identification of phenolic components

The presence of phenolic compounds in the subfractions F1–F7 was tentatively detected by thin-layer chromatography (TLC) and by infrared (IR) spectral analyses. After spraying with spray solution composed of 1% potassium ferricyanide solution and 1% ferric chloride solution, the TLC chromatograms indicated that F1–F7 contained phenolic compounds (data not shown). The absorption peaks observed for hydroxyl group (around 3337–3427 cm^{-1}) and aromatic ring (around 1450–1470, 1582–1610, and 2854–2958 cm^{-1}) in the IR spectra (figure not shown) of F1–F7 also suggested the presence of phenolic compounds. However, it was difficult to use the current data to elucidate the chemical structure of the compounds in the subfractions unless they were compared with the respective authentic standards.

3.3. Total phenolic content

As can be seen in Table 5, the distribution of phenolic compounds in *P. urceolata* demonstrated that the ethyl acetate-soluble fraction contained the highest amount, 73.7 mg GAE per gramme of extract. Among the subfractions F1–F7, F1 presented the highest total phenol content (222 mg, GAE), followed by F2 (205 mg, GAE), the same order as the antioxidant activities revealed in both assay systems. The FC method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substance, i.e. phenolic compounds (Wangensteen, Samuelsen, & Malterud, 2004). Correlation between the content of phenolic compounds and antioxidant activity has been described (Wangensteen et al., 2004). A high correlation between the total phenolic content and DPPH radical-scavenging ($r^2 = 0.835$, $p < 0.001$) and inhibiting of the bleaching of carotene ($r^2 = 0.899$, $p < 0.001$) was found in different polarity fractions from *P. urceolata* at the concentration of 50 μg/ml. In the subfractions F1–F7, a positive relationship was also observed between the phenolic content

Table 5
Total phenolic content of the crude extract, fractions, and subfractions F1–F7 derived from *P. urceolata*

Fraction	GAE ± SD
Crude extract	71.6 ± 0.00e
Petroleum ether fraction	11.3 ± 0.11b
Ethyl acetate fraction	73.7 ± 0.00f
n-Butanol fraction	52.1 ± 0.36d
Aqueous residue	8.16 ± 0.07a
F1	222 ± 1.05l
F2	205 ± 0.82k
F3	115 ± 0.49h
F4	152 ± 1.41i
F5	196 ± 2.25j
F6	88.4 ± 0.13g
F7	48.4 ± 0.62c

Total phenolic content is expressed as gallic acid equivalents (GAE; mg/g seaweed dry weight of GAE). Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–l) differ significantly ($p < 0.05$).

and DPPH radical-scavenging activity ($r^2 = 0.649$, $p < 0.001$) and antioxidant activity in the β-carotene–linoleate system ($r^2 = 0.491$, $p < 0.001$).

3.4. Iron (III) to iron (II) reducing activity

Different studies have indicated that the antioxidant effect is related to the development of reductones (Dorman et al., 2003; Shon et al., 2003). Reductones were reported to be terminators of free radical chain reactions. Thus the antioxidant activity of *P. urceolata* extract and fractions, as well as subfractions, may relate to their reductive activity. Table 6 shows the reducing power of the various fractions and subfractions isolated from *P. urceolata*. All of the extracts possessed the ability to reduce iron (III) and also in a linear concentration-dependent fashion. In the four fractions extracted by different solvent partitions, the ethyl acetate-soluble fraction was a significantly ($p < 0.05$) better iron (III) reducer than the other fractions. Among the seven subfractions F1–F7, F1 appeared to possess the highest reducing activity, followed by F2, the same order as the antioxidant activities in both assay systems. Statistical analyses indicated that, at a concentration of 10 μg/ml, there was a strong correlation between the reducing activity and DPPH radical-scavenging activities in both of the fractions ($r^2 = 0.948$, $p < 0.001$) and subfractions ($r^2 = 0.902$, $p < 0.001$). This may be due to a common underpinning mechanism, i.e. electron/hydrogen donation. A positive correlation was also observed between the reducing power and the antioxidant activity in the β-carotene–linoleate assay system in both of the fractions ($r^2 = 0.633$, $p < 0.001$) and subfractions ($r^2 = 0.687$, $p < 0.001$).

Table 6
Reducing power of the crude extract, fractions, and subfractions F1–F7 derived from *P. urceolata*

Fraction	AscAE ± SD
Crude extract	226 ± 2.49e
Petroleum ether fraction	96.6 ± 1.51b
Ethyl acetate fraction	383 ± 6.67g
n-Butanol fraction	138 ± 1.51c
Aqueous residue	63.6 ± 0.33a
F1	606 ± 10.9i
F2	517 ± 2.01h
F3	374 ± 2.49g
F4	287 ± 5.31f
F5	218 ± 1.44e
F6	226 ± 1.98e
F7	198 ± 2.01d

Reducing power is expressed as ascorbic acid equivalents (AscAE; mg/g seaweed dry weight of AscAE). Each value is presented as mean ± SD ($n = 3$). Means within each column with different letters (a–i) differ significantly ($p < 0.05$).

4. Conclusion

In summary, the results of the present work indicated that, among the crude extract and different polarity fractions derived from *P. urceolata*, the ethyl acetate-soluble fraction possessed the highest antioxidant activity and free radical-scavenging activity. Seven subfractions, F1–F7, which were isolated from the ethyl acetate-soluble fraction by vacuum liquid chromatography, exhibited potent antioxidant activities. They all showed excellent activity by the DPPH radical-scavenging assay and in inhibiting the bleaching of carotene in the β-carotene–linoleate assay systems, with F1 being the most effective subfraction. The total phenolic content and reducing power showed positive correlations with antioxidant activity and DPPH radical-scavenging activity. The activity of F1 was comparable to that of the positive controls, GA and AscA, and was significantly higher than that of BHT in the DPPH radical-scavenging assay. In the β-carotene–linoleate assay system, F1 and F2 were as effective as BHT, and exhibited similar or higher activities than GA and AscA.

The present findings appear useful in leading to further experiments on the identification and characterization specific compounds that are responsible for the relatively high antioxidant activities in fractions and subfractions of *P. urceolata*. These experiments are now in progress. Our results also indicated that inclusion of the antioxidant-rich extract or fractions/subfractions of *P. urceolata* will most probably prevent the oxidative deterioration of food.

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