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Chemical Composition, Iron Bioavailability, and Antioxidant Activity of *Kappaphycus alvarezzi* (Doty)

MOHAMED FAYAZ,[‡] K. K. NAMITHA,[†] K. N. CHIDAMBARA MURTHY,[†] M. MAHADEVA SWAMY,[†] R. SARADA,[†] SALMA KHANAM,[‡] P. V. SUBBARAO,[§] AND G. A. RAVISHANKAR*,[†]

Plant Cell Biotechnology Department, Central Food Technological Research Institute, Mysore − 570 020, India, Al-ameen College of Pharmacy, Bangalore − 560 027, India, and Central Salt and Marine Chemicals Research Institute, Bhavnagar − 364 002, India

Kappaphycus alvarezzi, an edible seaweed from the west coast of India, was analyzed for its chemical composition. It was found that *K. alvarezzi* is rich in protein (16.24% w/w) and contains a high amount of fiber (29.40% w/w) and carbohydrates (27.4% w/w). *K. alvarezzi* showed vitamin A activity of 865 μg retinal equivalents/100 g of sample. It contained a higher quantity of unsaturated fatty acids (44.50% of the total), in which relative percentage of oleic acid was 11%, *cis*-heptadecanoic acid 13.50%, and linoleic acid 2.3% and 37.0% of saturated fatty acids (mainly heptadecanoic acid). *K. alvarezzi* was also found to be good source of minerals, viz 0.16% of calcium, 0.033% of iron, and 0.016% of zinc, which are essential for various vital biological activities. Bioavailability of iron by in vitro methods showed a higher efficiency in intestinal conditions than in stomach conditions. Ascorbic acid influenced higher bioavailability of iron. Successive extracts of *n*-hexane, acetone, ethyl acetate, ethanol, and direct extractables of chloroform/methanol (1:1 and 2:1) were screened for antioxidant activity using a β-carotene linoleic acid model system (B-CLAMS), DPPH (α,α-diphenyl-β-picrylhydrazyl) model system and hydroxyl radical scavenging activity. The chloroform/methanol (2:1) extract has shown 82.5% scavenging activity at 1000 ppm. Acetone fraction extracts at the 1000 ppm level showed 63.31% antioxidant activity in β-carotene linoleic acid system. The acetone extract showed 46.04% scavenging activity at 1000 ppm concentration. In the case of hydroxyl radical scavenging activity, all the extracts showed better activity at the concentrations of 25 and 50 ppm, where at the 50 ppm level ethyl acetate extract showed 76.0%, acetone 75.12%, and hexane 71.15% activity, respectively. Results of this study suggest the utility of *K. alvarezzi* (Eucheuma) for various nutritional products, including antioxidant for use as health food or nutraceutical supplement.

KEYWORDS: Seaweed, *Kappaphycus alvarezzi,* Antioxidant activity, Nutraceutical, Eucheuma.

1. INTRODUCTION

Seaweeds have been used as a foodstuff in the Asian diet for centuries and are considered under-exploited resources (*1*). Some of the seaweeds such as *Porphyra*, *Gracilaria*, *Laminaria*, and Eucheuma are known for their food application (*2*). *Rhodymenia palmanta* and *Alaria esculenta* are known for the fodder application (*3*). Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products, and suspending agents in radiological preparations. Fresh and dried seaweeds are extensively consumed especially by people living in the coastal areas. Reports on the certain edible seaweeds show that they contain a significant amount of the protein, vitamins, and minerals essential for the human nutrition. The nutrient composition of seaweeds varies and is affected by the species, geographic areas, seasons of the year, and temperature of the water (*4*).

The consumption of food of vegetable origin has been associated with reduced risk of a range of chronic diseases(*5*, *6*). Owing to their antioxidant and free radical scavenging properties, constituents such as polyphenols and carotenoids may play a role in the etiology of chronic diseases through oxidative damage to body cells and molecules (*7*). Marine algae, like other photosynthesizing plants, are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents (*8*).

Eucheuma is a red algae belonging to family Solieraceae. *K. alvarezii* (*9*), grouped under Eucheuma contains approximately 45 species, among which only 8−9 are commercially found, such as *K. alvarezii*, *K. denticulatum*, *K. gelatinae*, and *K.*

* Author to whom correspondence should be addressed. Tel: + 91 821 2516501. Fax: +91 821 2517 233. E-mail: pcbt@cscftri.res.nic.in.
† Central Food Technological Research Institute.
‡ Al-ameen College of Pharmacy.
§ Central Salt and Marine Chemicals Research Institute.

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muricatum. The world production of Eucheuma is approximately 28 000 tons per annum (*10*). Indonesia and the Philippines are major producers of Eucheuma. In India, field cultivation of Eucheuma in the Mandapam region is successfully done (*11*).

The wide application of Eucheuma is seen in the areas of biopolymers and carrageenan production. Many studies on Eucheuma species have been reported, especially on its taxonomy and habitat characteristics. Studies on biochemical composition of other species of edible seaweed have been reported (*12*). One of the widespread nutritional problems in many parts of the developing world is iron deficiency-induced anemia. Low availability of dietary iron is one among the factors considered most important (*13*).

The present work aims at the determination of chemical composition of Eucheuma (*K. alvarezzi*), the bioavailability of iron, and the evaluation of the antioxidant activity.

2. MATERIALS AND METHODS

All the chemicals and solvents used for experiment were of analytical/HPLC grade obtained from Merck, Mumbai, India. Standard β-carotene (product no.10811BA, 98% all trans) was obtained from Sigma Chemicals, USA. Standard Ascorbic acid was obtained from Himedia Laboratories Pvt. Ltd, India. Standard fatty acid methyl esters were obtained from Sigma chemicals, USA. Pepsin used was from E. Merck (batch no. 6322704).

2.1. Collection of Samples. The authenticated samples of *K. alvarezzi* from the west coast of India were obtained from Central Salt and Marine Chemical Research Institute (CSMCRI), Bhavnagar, India. These samples were cleaned and dried in a tray drier for 6 h at 50 °C, the sample was pulverized using a multimill to 0.25 mm mesh size, and the powder was used for the studies reported here. This powder was stored in cold conditions in an airtight container, and analysis was done within three months of processing.

2.2. Analytical Methods. *2.2.1. Chemical Composition Analysis.* Total protein, fiber, ash, moisture, carbohydrate, and lipid contents in *K. alvarezzi* were determined by standard AOAC (*14*) methods.

2.2.2. HPLC of Ascorbic Acid. Ascorbic acid was extracted using the method described by Wimalasiri et al. (*15*). The ascorbic acid content in *K. alvarezzi* was determined using the HPLC method using a Shimadzu LC6A instrument (Shimadzu corporation, Kyoto, Japan). HPLC analyses were carried out on a Micro bondapack C_{18} column (30 cm × 4 mm i.d.) installed in a liquid chromatograph (Waters) equipped with a 4l-mpa pump and U6k injector. Column effluents were monitored by UV detectors set at 254 nm. The mobile phase was acetonitrile/water (70:30 v/v) with 0.01 M ammonium dihydrogen phosphate (pH 4.3 adjusted with orthophosphoric acid). The flow rate was maintained at 1.0 mL/min, and 10 μL was injected into HPLC. The ascorbic acid peak was identified by its retention time and was compared with that of ascorbic acid standard (1 mg/mL).

2.2.3. HPLC of β-Carotene. β-Carotene was extracted following the method described by Tee and Linn (*16*). The extractant obtained was passed through open column chromatogram containing a mixture of activated magnesia and diatomaceous earth in the ratio 1:1, using 10% v/v acetone in hexane as eluent for removal of chlorophyll pigments; the resulting solution was subjected to HPLC (*14*).

HPLC was done using 10 μm Water Bondapack C_{18} stainless steel 30 cm × 3.9 mm (i.d.) column with acetonitrile/methanol/ethyl acetate, (88:10:2 v/v) using the flow rate of 2.0 mL/min as mobile phase and 10 μL injected into HPLC. Peak responses were determined at 436 nm. β-Carotene was identified by its retention time and compared with that of β-carotene standard (0.1 mg/mL).

The β-carotene content obtained was expressed as vitamin A equivalent (*16*).

2.2.4. Analysis of Fatty Acid Composition. A known quantity of lipid extract (extracted by Soxhlet extractor for 4 h at 35 ± 2 °C in petroleum ether 40−60 °C) was evaporated to dryness in a sidearm conical flask. Methanolic HCl (5 mL) (95 mL of chilled methanol + 5 mL of acetyl chloride) was added, shaken well, and refluxed for 3 h in a boiling water bath. After cooling, fatty acid methyl esters (FAME) were extracted with petroleum ether. The extract was evaporated in vacuo and FAME were dissolved in a known quantity of chloroform and analyzed by gas chromatography (*17*). FAME were stored under nitrogen until analysis in cold conditions. A stainless steel column (8 ft length and 1/8 in. diameter) 15% diethyl glycosuccinate coated on chromosorb w (100/20 mesh) was fitted to GC-15A Shimadzu (Shimadzu corporation, Japan). The FAME extract was analyzed at isothermal temperature with nitrogen as a carrier gas at the rate of 40 mL/min. The oven temperature was 180 °C, injector and detector (FID) temperature was 200 °C, and the total run time was 40 min. Fatty acids were identified by using authentic fatty acid methyl ester standards. The relative percentages of the fatty acids were determined using Shimadzu CR-4A connected to GC.

2.2.5. Mineral Elements. The ash of the sample was used for the estimation of mineral elements (calcium, iron, zinc), by AOAC procedure (*14*). The concentrations of the elements in *K. alvarezzi* were determined with atomic absorption spectrophotometry. Triplicate determinations for each element were carried out. The concentrations of the elements were determined from calibration.

3. ASSESSMENT OF BIOAVAILABILITY OF IRON BY IN VITRO METHOD

An in vitro assessment was done by simulating the conditions of gastric (pH 1.35) and intestinal fluids (pH 7.5) to determine the iron bioavailability.

3.1. Extraction of Ionizable and Soluble Iron. *K. alvarezzi* powder (2.0 g) was mixed with 50 mL of pepsin−HCl (Pepsin used was of concentration 10-FIP-U/mg). The pH of the mixture was adjusted to 1.35 with analytical grade HCl and incubated in a 100 mL conical flask at 37 °C in a metabolic shaker water bath for different time intervals of 15−60 min at an interval of 15 min each. At the end of incubation, the contents of the flask were centrifuged at 3000 rpm for 10 min and the supernatant filtered through Whatman no. 44 filter paper. Soluble and ionizable iron was determined in aliquots of filtrate at pH 1.35. In another aliquot, pH was adjusted to 7.5 with NaOH and incubated at 37 °C for different time intervals in a water bath as mentioned above for pH 1.35. At the end of incubation period, the content of the flask was filtered and the filtrate was used to determine ionizable and soluble iron (*18*).

3.2. Effect of Absorption Inhibitor and Promoters. The effect of known inhibitors on iron absorption like tannic acid and absorption promoter like ascorbic acid on bioavailability were determined. These compounds were included at different concentrations in the incubation mixture at the beginning before incubating the sample with pepsin−HCl.

3.3. Analytical Methods. *3.3.1. Total Iron.* The dry powder of *K. alvarezzi* was ashed as described by AOAC, and total iron in the mineral solution was estimated by the *o*-phenanthroline method (*14*).

3.3.2. Ionizable Iron. The free form of iron, which reacts with *o*-phenanthroline to yield color obtained after incubation at pH 1.35 and 7.5, was determined as described by AOAC (*14*). This form corresponds to the ionizable iron (*19*).

3.3.3. Soluble Iron. Soluble iron was determined by digesting the filtrate with potassium permanganate (*20*) until decolorization. It was again filtered, and iron in the filtrate was determined by the *o*-phenanthroline method (*14*). The concentration was calculated by comparing with standard curve prepared using ferrous ammonium sulfate (0.08−1.8 μg/mL).

4. ASSESSMENT OF ANTIOXIDANT ACTIVITY

4.1. Extraction. The dried powder (25 g) was subjected to successive extractions in Soxhlet extractor for 8−10 h for all the solvents (125 mL each). The solvents used were in the

increasing order of polarity i.e., *n*-hexane, acetone, ethyl acetate, ethanol, and a mixture of chloroform/methanol (1:1 and 2:1). Temperatures maintained for extraction were 30 ± 2 °C for *n*-hexane, 40 ± 2 °C for acetone and ethyl acetate, and 45 ± 2 °C for other solvents. The percent yield of extractables was expressed in a w/w basis.

4.2. Experimental Procedure. *4.2.1.* α,α*-Diphenyl-β-picrylhydrazyl (DPPH) Radical Scanning Assay.* Different concentrations (25−1000 ppm) of *K. alvarezzi* extractables and BHA were taken in test tubes, and the volume was adjusted to 500 mL using methanol. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 ± 1 °C for 20 min (*21*, *22*). The control was prepared without any extract, and methanol was used for baseline correction at 517 nm. Radical scavenging activity was expressed as the inhibition % and was calculated as follows. % Radical scavenging activity = (Control OD − Sample OD/Control OD) × 100

4.2.2. Antioxidant Assay Using β-Carotene Linoleate Model System (B-CLAMS). The antioxidant activities of *K. alvarezzi* extracts were evaluated by β-carotene linoleate system (*23*) with a slight modification. β-Carotene (0.2 mg), 20 mg of linoleic acid, and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed in 0.5 mL of chloroform. Chloroform was removed at 40 °C under vacuum using a rotary evaporator. The resulting mixture was immediately diluted with 10 mL of triply distilled water and was mixed well for 1−2 min. The emulsion was further made up to 50 mL with oxygenated water. Aliquots (4 mL) of this emulsion were transferred to different test tubes containing 0.2 mL of test samples in ethanol. Butylated hydroxy anisole (BHA) was used for comparative purposes. A control, containing 0.2 mL of ethanol without test sample and 4 mL of the above emulsion, was prepared. The tubes were placed in water bath maintained at 50 °C. Absorbance of all the samples at 470 nm were taken at zero time ($t = 0$). Measurement of absorbance was continued until the color of β-carotene disappeared in the control group ($t = 180$ min) at 15 min intervals. A mixture prepared as above without β-carotene served as blank. All determinations were carried out in triplicate. Dose−response relationships of antioxidant activity for *K. alvarezzi* extracts were determined at different concentrations. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula (*24*).

$$\mathrm{AA} = 100[1 - (A_0 - A_t)/(A^0{}_0 - A^0{}_t)]$$

Where A_0 and $A^0{}_0$ are the absorbance values measured at zero time of the incubation for test sample and in control, respectively, where A_t and $A^0{}_t$ after incubation for 180 min.

4.2.3. Hydroxyl Radical Scavenging Activity. This is based on the principle that hydroxyl radicals formed by the oxidation react with dimethyl sulfoxide (DMSO) to yield formaldehyde. The formaldehyde production provides a convenient method to detect hydroxyl radicals formed during the oxidation of DMSO by the Fe^{3+}/ascorbic acid system (*25*).

Aliquots of extractables prepared in 2% (v/v) alcohol were taken in different test tubes and evaporated on a water bath. To these, 1 mL of iron−EDTA solution, 0.5 mL of EDTA (0.018% w/v in water), and 1 mL of DMSO were added, and the reaction was initiated by adding 0.5 mL of ascorbic acid to each of the test tubes. Test tubes were capped tightly and heated in a water bath at 80−90 °C for 15 min. Then, the reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v) to all the test tubes, kept aside for 2 min, and the formaldehyde formed was determined by the adding 3 mL of Nash reagent, which was left for 10−15 min for color development. Intensity of yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. Percent scavenging of hydroxyl radicals was calculated by comparison of the results of the test compounds with that of the blank. The necessary corrections were made for the absorbance of the test compounds. The % hydroxyl radical scavenging is calculated by the following formula. % hydroxyl radical scavenging = (absorbance of sample/ absorbance of blank) × 100.

Table 1. Chemical Composition and Ascorbic Acid Content of *K. alvarezzi*

components	percentage (w/w)[a]
moisture	6.43 ± 0.05
fiber	29.4 ± 0.53
ash	19.7 ± 0.05
protein	16.24 ± 0.04
lipid	0.74 ± 0 0.03
carbohydrate	27.4 ± 0.11
ascorbic acid	0.107 ± 0.30
vitamin A (β-carotene)	8.7×10^{-4}

[a] Values are the mean of three replicates.

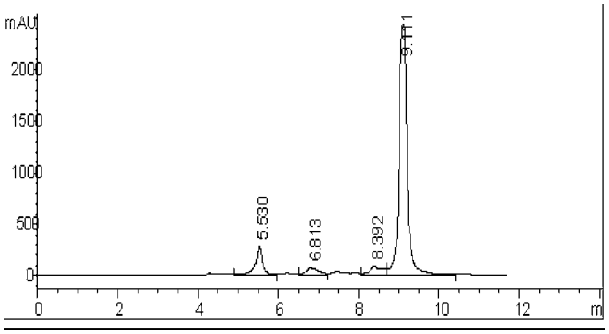

Figure 1. HPLC chromatogram of β-carotene in *K. alvarezzi* under conditions as given in the text (at RT = 9.111 min).

4.3. Determination of Total Polyphenol Content. Polyphenols were estimated by the *Folin* method. The concentration of polyphenols in extracts was calculated by using standard curve prepared with tannic acid (*26*).

5. STATISTICAL ANALYSIS

For all analysis, the mean and standard deviation of three replicate trials were calculated and values were reported as ±SEM.

6. RESULTS AND DISCUSSION

6.1. Chemical Composition. The chemical composition and ascorbic acid content of *K. alvarezzi* is shown in Table 1. The red algae *K. alvarezzi* was found to contain (dry weight basis) total protein, 16.24%; crude fiber, 29.4%; total lipid, 0.738%; ash, 19.7%; moisture, 6.43%; and carbohydrate, 27.4%. The content of ascorbic acid in *K. alvarezzi* was 107.1 mg/100 g sample (dry weight).

6.2. *β*-Carotene Content. Figure 1. Shows the HPLC chromatogram of β-carotene in the extracted sample of *K. alvarezzi*. It shows the β-carotene peak (RT = 9.111 min) along with other peaks which were not identified in our study. The concentration of β-carotene was 5.26 mg/100 g sample (dry weight). The vitamin A activity calculated on the basis of

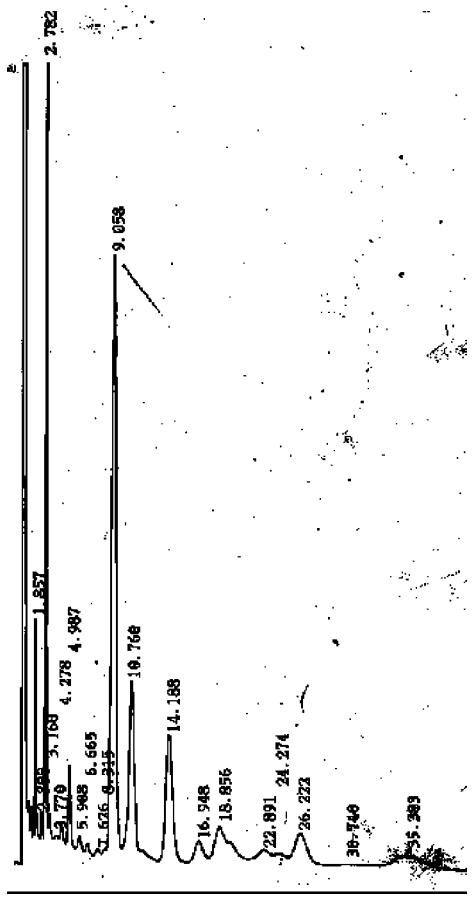

Figure 2. Fatty acid profile of *K. alvarezzi*.

Table 2. Fatty Acid Composition of *K. alvarezzi*

composition	relative percent[a]
myristic acid, 14:0	0.46 + 0.11
myristoleic acid, 14:1	2.41 + 0.02
pentadecanoic acid, 15:1	0.48 + 0.05
palmitoleic acid, 16:1	0.31 + 0.01
heptadecanoic acid, 17:0	34.24 + 0.70
cis-heptadeconoic acid, 17:1	13.50 + 0.20
oleic acid, 18:1ω9	11.0 + 0.18
linoleic acid, 18:2 ω6	2.30 + 0.04
linolenic acid, 18:3 ω3	6.51 + 0.44
arachidic acid, 20:0	2.30 + 0.14
eicosaenoic acid, 20:1	1.33 + 0.10
eicosadienoic acid, 20:2	5.30 + 0.57
arachidonic acid, 20:4 ω6	1.40 + 0.50
unidentified	~18.00

*a*Values are the mean of three replicates.

β-carotene content was 865 μg RE (retinol equivalent)/100 g, using the classification of RE values adopted by Tee and Lim (*16*).

6.3. Fatty Acid Composition. A sample chromatogram of fatty acids composition in *K. alvarezzi* is as shown in Figure 2. Gas chromatographic analysis of sample extracts showed the presence of fatty acids (Table 2) mainly containing heptadecanoic acid, 17:0 (34.24%); *cis*-heptadeconoic acid, 17:1 (13.50%); oleic acid, 18:1ω9 (11.0%); linoleic acid, 18:2ω6 (2.30%); linolenic acid, 18:3 (6.513%); and arachidonic acid, 20:4ω6 (1.367%). *K. alvarezzi* has a higher composition of unsaturated fatty acids (44.5%) when compared to saturated fatty acids (37.0%). Some of the seaweeds are known to contain odd-number fatty acids (*27*). Both EPA and DHA could not be identified due to lack of standards. However, EPA is found in most of the seaweeds in the range of 3−25% of total FAME, which may be under unidentified portion (~18%).

Table 3. Content of Minerals in *K. alvarezzi*

elements	mg/100 g[a]
calcium	159.54 ± 0.41
iron	33.8 ± 0.65
zinc	1.58 ± 0.13

[a] Values are the mean of three replicates.

Table 4. Percent Soluble and Ionizable Iron from the Total Iron Content in *K. alvarezzi*[a]

total iron mg/ 100 g	pH 1.35		pH 7.5	
	soluble	ionizable	soluble	ionizable
33.8 ± 0.23	59.40 ± 0.4	53.77 ± 0.6	40.60 + 1.2	46.23 ± 0.86

[a] Values are the mean of three replicates.

Table 5. Effect of Ascorbic Acid on Available Ionizable Iron in *K. alvarezzi* at pH 7.5

molar ratio of ascorbic acid to iron	percent ionizable iron at pH 7.5[a]
0	46.23 ± 2.3
0.1	46.73 ± 0.96
0.5	47.10 ± 1.21
1.0	49.70 ± 2.3
2.0	53.52 ± 0.82

[a] Values are the mean of three replicates.

6.4. Mineral Contents. *K. alvarezzi* was found to contain calcium, 159.5; iron, 33.8; and zinc, 1.58 mg/100 g of the sample (w/w), (Table 3).The results show that *K. alvarezzi* is low in calcium and high in iron when compared to other edible red seaweeds, such as nori and *Chondrus* (*28*), whereas zinc is present in low quantity. The presence of significant amounts of calcium and iron in *K. alvarezzi* may be due to its metabolic system in which it is capable of directly absorbing elements from the seawater.

6.5. Iron Content and Its Bioavailability. The total soluble and ionizable iron at pH 1.35 and 7.5 in the *K. alvarezzi* studied are given in Table 4. At pH 1.35, the soluble iron was slightly higher than ionizable iron in Eucheuma. When this was increased from pH 1.35 to 7.5, both ionizable and soluble iron decreased in the *K. alvarezzi;* the decrease in the ionizable was of a greater magnitude.

The effect of inclusion of ascorbic acid on ionizable iron at pH 7.5 is shown in Table 5. The ionizable iron progressively increased with an increase in the level of added ascorbic acid and reached a maximum when the molar ratio of iron to ascorbic acid was 1:10.

With an increase in the tannic acid concentration in the incubation mixture, the ionizable iron at pH 7.5 decreased. The same was true with the addition of tannins at different concentrations (Table 6). Several workers demonstrated that nonheme iron absorption from vegetables and fruits can be significantly increased by the inclusion of ascorbic acid (*29*, *30*). The available iron as measured by this in vitro procedure was also shown to decrease considerably when inhibitors such as tannins were included. Studies in humans have shown that phytates (*31*, *32*) and tannins (*33*) considerably decrease the absorption of nonheme iron from diets. Thus, ionizable iron at pH 7.5 can be used as a valid measure of bioavailability of iron from foods in human diets.

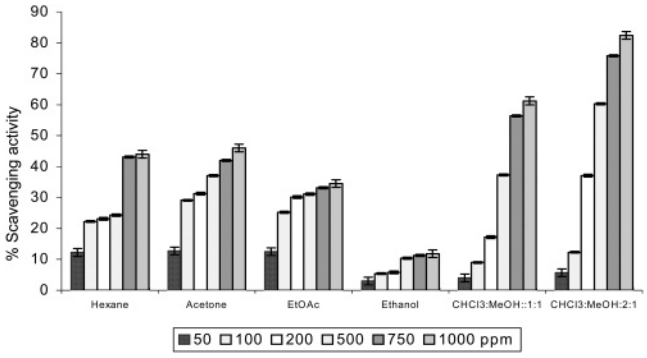

Figure 3. Antioxidant activity of various extracts of *K. alvarezzi* by the DPPH method.

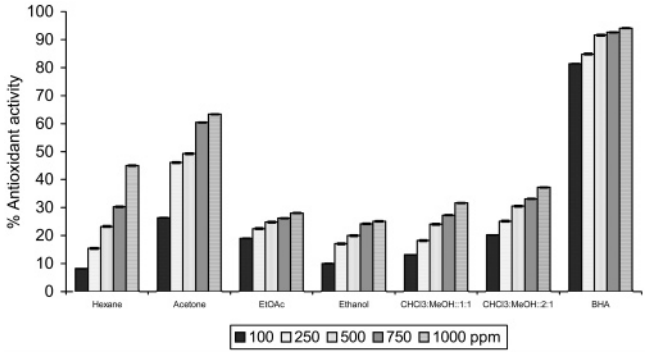

Figure 4. Antioxidant activity of various extracts of *K. alvarezzi* by b-CLAMS method.

Table 6. Effect of Tannic Acid on Available Ionizable Iron in *K. alvarezzi* at pH 7.5

tannic acid mg/100 g	percent ionizable iron at pH 7.5[a]
0	46.23 ± 2.3
1	39.5 ± 1.34
2	35.13 ± 0.89
4	28.80 ± 0.28

[a] Values are the mean of three replicates.

Promoting effect of ascorbic acid on iron absorption has attributed to its chelating property, which keeps iron soluble even at alkaline pH (*34*). Thus, conditions chosen for determining ionizable iron in the present study represent the conditions somewhat similar to those in the duodenum or small intestine, where most of the dietary iron is absorbed. This measures the quantity of ionizable iron that may be available for actual absorption.

Table 7. Percent Yield and Polyphenol Content of Extractables for *K. alvarezzi* in Various Solvents

solvents	yield of extractables (% w/w)[a]	% polyphenol[a]
n-hexane	0.25 ± 1.23	1.34 ± 1.22
acetone	0.50 ± 1.46	0.7 ± 1.11
ethyl acetate	0.43 ± 0.97	1.52 ± 0.98
ethanol	2.23 ± 0.66	4.83 ± 1.45
chloroform:methanol (1:1)	9.48 ± 1.24	2.55 ± 1.37
chloroform:methanol (2:1)	7.8 ± 0.60	3.32 ± 0.89

[a] Values are the mean of three replicates.

6.6. Antioxidant Activity. The yields of extractables obtained from *K. alvarezzi* using various solvents were analyzed (Table 7). Among the different individual solvents used, ethanol extracted the most constituents in successive extractions. In the case of chloroform:methanol, the yield of extractables was comparatively high, 9.48% (1:1) and 7.8% (2:1). The polyphenol content in the ethanol extract was maximum (4.83%) followed by the chloroform:methanol extract (3.32%), whereas individual solvent extracts using ethyl acetate, *n*-hexane, and acetone showed less than 1.5% polyphenol. Free radical scavenging potentials of *K. alvarezzi* extractables at different concentrations were tested by the DPPH method, (Figure 3). Acetone and hexane extracts showed moderate activity at all the concentrations. The extracts of chloroform:methanol mixture showed comparatively higher activities 56.4%, 61.24% and 75.81%, and 82.5% at 1:1 and 2:1 concentration, respectively, at 750 and 1000 ppm levels. This mainly due to the presence of ascorbic acid (Table 1) and polyphenols (Table 7), which are hydrophilic antioxidants.

The antioxidant activity of *K. alvarezzi* extracts were also measured at different concentrations by bleaching β-carotene and compared with BHA (Figure 4). It can be seen that *K. alvarezzi* extracts prepared by different solvents exhibited varying degree of antioxidant activity. The results indicate that activity of acetone extract was highest followed by *n*-hexane and a mixture of chloroform:methanol; this is due to β-carotene (Table 1, Figure 1) content of the extract, which shows additional protection against oxygen-mediated lipid peroxidation.

Hydroxyl radicals are one of the quick initiators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids (*35*, *36*). Among the extracts tested,

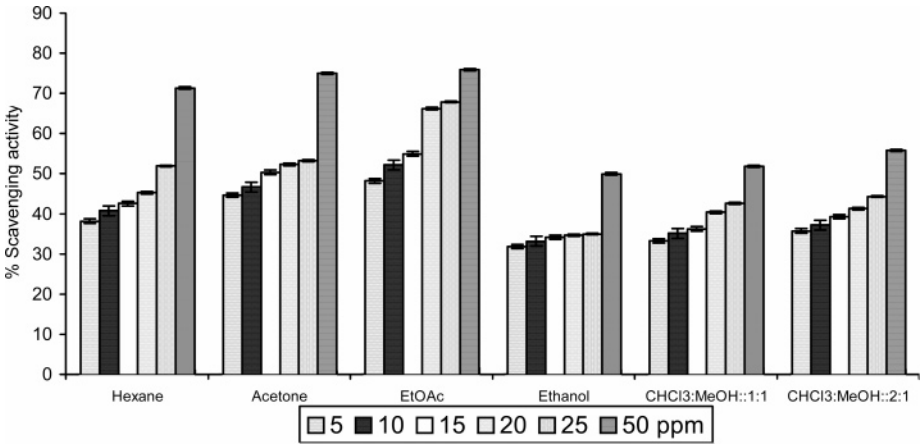

Figure 5. Hydroxyl radical scavenging activity of *K. alvarezzi* in various extracts.

acetone and ethyl acetate extracts showed maximum activity, ethyl acetate has shown 68.04%, 69.42%, and 76.0% activity at 20, 25, and 50 ppm (Figure 5). Other extracts have shown an almost similar trend of results as both hydrophilic and hydrophobic components are likely to quench hydroxyl radicals.

The results of the study shows that Eucheuma is rich in protein, crude fiber, essential fatty acids, minerals, and vitamins A (precursor) and C; hence, it can serve as functional food with vital nutritional and biological values. This also has implications in utilizing Eucheuma (*K. alvarezzi*) as a source of antioxidants in several applications requiring these properties. This can serve as authentication for better utilization of the seaweed as nutraceutical.

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