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Free radical scavenging potential, reducing power, phenolic and biochemical constituents of *Porphyra* species from India

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Abstract

Inorganic elements, organic composition and antioxidant properties of *Porphyra indica* and *P. veiatnamensis*, were investigated. Heavy metals were found below toxic level, protein and ash contents were found to be the most abundant components. Antioxidant potentials of algae were assessed through phenolic content, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity, hydrogen peroxide (H_2O_2), scavenging power and reducing potential. A dose-dependent free radical scavenging action against DPPH and H_2O_2, and concentration dependent reducing potential were exhibited by the *Porphyra* spp. Overall data suggested, these species could be rich source of natural antioxidants as well as stuff of high nutritional values.

Keywords: Marine algae, *Porphyra*, India, Antioxidant, Nutraceuticals

Introduction

Marine algae are looked upon as a renewable energy source, particularly from neutraceutical, biofuel and biofertilizer, point of views. India's coastline of ~7,500 km, greatly differs in its geomorphological and hydrological characters at various latitudes. The mid, intertidal and shallow subtidal regions, (particularly sandy and rocky), favour the growth of marine algae. Seaweeds though rich in vital chemicals of

commercial importance, their utilization in India remain far below optimum level, compared to the same in other countries like Japan, South Korea and China. Antioxidants scavenge the free radical reducing oxidative damage, and thus protecting living being. Phloroglucinol and phenolics in marine algae serve as reactivescavenging species (ROS) scavengers, metal chelators and enzyme modulatorspreventin lipid peroxidation (Rodrigo and Bosco, 2006). A number of marine algae have been reported to posse's antioxidant properties (Nagai and Yukimoto, 2003), however, edible algal species from India remained to be explored for such kind of activities. Lately, global efforts are being made to discover naturally occurring antioxidant in human consumption or pharmaceutical purposes, as synthetic antioxidants, leads to side effects of carcinogenicity (Zheng and Wang, 2001). In view of the above, nutraceutical potential of edible *Porphyra* spp. were evaluated by analyzing their trace metals, biochemical constituents and antioxidant potentials.

Materials and Methods

Sample preparation

Fresh algal materials were collected during November-December, 2008 from central west coast of India at Malvan (16°03'44.04"N, 73°27'17.28"E). After collection, algal materials were immediately washed with distilled water to remove epiphytes and adhering debris, and then dried at a constant temperature (60°C) in oven. The dried tissues were ground to a fine powder, and then extracted with methanol for 24 hrs at -20°C. The extracts were concentrated in a vacuum evaporator (BUCHI Rota vapor R-200) at 40°C, and then stored at -20°C for estimating other chemical constituents.

Biochemical analysis

Protein contents were determined according to Lowry *et al*. (1951), using bovine serum albumin (BSA) as a standard, and were

expressed in mg/100g. The carbohydrate contents were estimated by the method of Nelson (1944), utilizing glucose as a standard and expressed as mg/100g. Lipid contents were measured following the process described by Folch *et al.* (1957) while for analyzing ash contents by standard method of AOAC (1960).

Inorganic element analysis

Inorganic elements were analyzed as described by Toth *et al.* (1948), using atomic absorption spectrophotometer (A Analyst 300, Perkin Elmer).

Phenolic contents

Total Phenolic (TP) contents were estimated by the method of Slinkard and Singleton (1977), using Folin-Ciocalteu reagent and Gallic acid standard. The absorbance was measured at 760nm.

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) assay

Free radical scavenging potential in methanol extract was measured (Blois, 1958) using DPPH. The reaction mixture containing 2.5 ml of DPPH solution (0.1 mM in methanol), plant extract (0.1-0.4ml) adjusted to 3 ml by adding methanol, and initial absorbance and absorbance after 30min. were measured at 517nm. Butylated hydroxytoluene (BHT) was used as the standard.

Reducing power

Methanol extracts were determined for their reducing power modifying the method of Oyaizu (1986). Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml Potassium Ferricyanide (1%) and varying concentrations of extracts (40-200mg). After, the reaction mixtures were incubated at 50°C in water bath for 30 min, allowed to cool at room temperature (28°C), and 2.5 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture, and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the

test tube and added with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%), and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution (ASA) was used as standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging strengths of extracts were determined the method described by Ruch *et al.* (1989). A solution of H_2O_2 (10mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures contained 10mM of H_2O_2 and different concentrations of test samples, and absorbance values were measured at 0 min and after 60 min using wavelength of 240nm. Ascorbic acid was used as the standard.

Stastical analysis

Data, thus obtained in all experiments were expressed as means ± standard deviation, and were analyzed using one way ANOVA. A significant difference was considered at the level of $P < 0.05$.

Results and Discussion

Inorganic elements

Concentrations of inorganic elements of *P. indica* and *P. vieatnamensis* are presented in Table 1. Heavy metals such as Ni and Cr were not detected in both the species, which is good indication from the nutraceutical point of view. In *P. vieatnamensis*, macromolecules such as Na, Ca, K and Mg were higher (98.2- 501 mg / 100g) comparing theses values in *P. indica* (48.7-406 mg /100g). Trace elements (Cu, Zn, Mn and Fe) were also observed to be higher (6.8-659.8 mg /100g) in *P. indica* compared to those in *P. vietnamensis* (6.4-516 mg/100g). The relatively higher concentrations of such essential elements in *Porphyra* spp. are indicative to their use as food supplements to the requirement of minerals in human. Other seaweeds such as *Caulerpa lantillifra, Gracillaria*

parpispora, *Monostroma oxysperum*, and *Enteromorpha flexuosa* were reported to contain relatively poor concentrations of these essential compared is the same in present *Porphyra* spp. elements in *Porphyra* spp. (Subba Rao *et al*., 2006). Variation in concentrations of such minerals may be attributed to different factors, such type of seaweed spp., oceanic residence time, geographical place of harvest, wave exposure, seasonal, environmental and physiological factors, as well as method of processing and mineralization (Honya *et al*., 1993).

Table 1

Inorganic elements	Seaweed species	
	P. indica	*P .vieatnamensis*
Na	48.7 ± 0.06	54.3 ± 0.07
K	310± 0.05	318 ± 0.6
Ca	77.8 ± 0.05	98.2± 0.2
Mg	406± 0.5	501 ± 0.1
Co	200.2 ± 0.06	201. 8 ± 0.02
Cu	6.4 ± 0.06	6.8 ± 0.05
Zn	32.6 ± 0.4	50.4 ± 0.08
Fe	516.0 ± 0.4	659.8 ± 0.5
Mn	30.6 ± 0.04	67.6± 0.4
Ni	ND	ND
Cr	ND	ND

(Inorganic elements in *P. indica* and *P. vieatnamensis*, values were expressed in mg^{-1}100g dry wt. tissue)

Biochemical composition

Total carbohydrate, protein and lipid contents of *P. indica* and *P. vieatnamensis* were 13.45 and

5.13%, 12.40 and 11.867 %, and 04.03 and 03.88%, respectively (Table 2), and were more or less in the same range as reported in other seaweeds (Ratnan-arporn and Chirapart, 2006). The amount of protein contents in seaweeds were reported to be closer to cereals, eggs and some other food materials (Katheresan, 1992), suggesting its importance of algae as supplementary food in human diet. Ash contents were also found to be 19.55 and 21.21%, respectively (Table 2) in *Porphyra* spp. Ash contents in plants were associated with mineral contents (Ratnan-arporn and Chirapart, 2006), hence the higher contents of ash in *Porphyra* spp. could be attributed to higher concentrations of minerals. The rich biochemical constituents in *Porphyra* spp. Compared with the other marine algae also indicate

their better nutritive values. Consumption of marine algae for various nutrients has been reported to be safe for human health (Katheresan, 1992).

Table 2.

Biochemical parameters	Seaweed species	
	P. indica	*P. vieatnamensis*
Total carbohydrate	13.45 ± 0.03	5.13 ± 0.03
Total Protein	12.404 ± 0.92	11.867 ± 0.56
Total Lipid	04.03 ± 0.04	03.88 ± 0.05
Total Ash	19.55 ± 0.02	21.215 ± 0.02

(Data's are mean of triplicate determination ± SD (n = 3), values were expressed in g^{-1} 100g dry wt. tissue)

Total phenolic compounds and H_2O_2 scavenging assay

Phenolic compounds commonly found in all sort of plants and are responsible for multiple biological effects, including antioxidant properties. The total phenolic contents in *P. indica* and *P. vietnamensis* were estimated 6.40 mg/g and 5.68 mg/g, respectively (Fig.1A). The antioxidant activity of phenolic compounds is mainly attributed for their redox actions, neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Presently spice yielding varieties rich in phenolics are in a great demand in the food industries (Nakano, 1997) as natural antioxidants. However, such varieties of spices are very limited and not feasible to exploit due to their higher coast. Therefore, alternative natural source of antioxidants need to be explored. Antioxidants from bioresources have created deep interest among researchers, food manufacturers, and consumers due to their protective role against dreadful diseases such as coronary heart disease and cancer (Loliger, 1991). The search for novel antioxidant biomolecule with high phenolic contents has become an important issue, because of their inhibitor role in on mutagenesis and carcinogenesis in human beings.

The scavenging ability of species of *Porphyra* extracts with H_2O_2 is compared with the ascorbic acid and is depicted in Fig.1B. Though H_2O_2 itself not very

reactive, it generates highly reactive molecule such as OH● by reacting with metals (Fe^{2+} or Cu^{2+}), and superoxide anions in the Haber-Weiss reaction. Therefore, removing of H_2O_2 is very essential from the cell or food systems. A significant dose dependent H_2O_2 scavenging potential of *Porphyra* spp. Were observed during the present study ($P < 0.05$). Electronic donors might accelerate the conversion of H_2O_2 to H_2O (Ruch *et al*, 1984), which could possible to scavenge H_2O_2 in the methanol extracts of *Porphyra* species.

Fig.1A

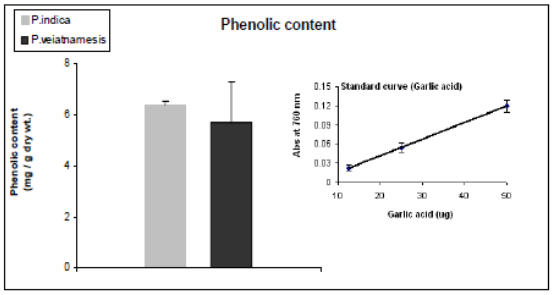


Fig.1B:

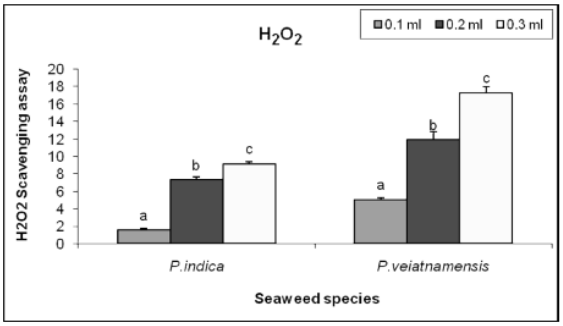


Fig. 1A. Total phenolic content in *Porphyra indica* and *P. veiatnamensis* and **Fig.1B.** Hydrogen peroxide scavenging assay, where values are mean of triplicate determination ± SD (n = 3). Superscripts of different letters are significantly different from each other at $P < 0.05$.

DPPH radical scavenging activity and reducing power

Methanol extracts of *Porphyra* spp. showed a significant dose-dependent reduction of DPPH radicals ($P < 0.05$). The scavenging action was higher in *P. indica* in comparison

with *P. vieatnamensis* (Fig. 2A). Free radical accepts an electron or hydrogen radical as DPPH being a stable, forming a stable diamagnetic molecule. The DPPH scavenging potential of *Porphyra* spp. may be due to their reducing actions, which might donate hydrogen to a free radical, reducing it to nonreactive species (Wang *et al*., 2008). Higher DPPH scavenging potential of *P. indica* might be due to the higher reducing potential.

The reducing power of methanolic extracts of *Porphyra* spp. were found to be correlated with increasing absorbance (at 700 nm) as compared with ASA, a known antioxidant (Fig. 2B). Similar observations were also reported earlier (Duh, 1998). The reducing power of *P. indica* was relatively more prominent than *P. vieatnamensis*. The presence of reductones are responsible for reducing capacity, which involved in prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging (Yildirm *et al*., 2001). The present data also revealed a significant antioxidant power potential of *Porphyra* species. A significant correlation between DPPH scavenging potential vs reducing power was observed in *P. indica* ($P < 0.01$) and *P. vieatnamensis* ($P < 0.05$) to support the above statement (Fig. 3).

Fig.2A

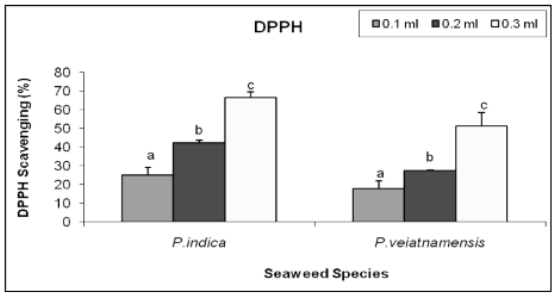


Fig.2B:

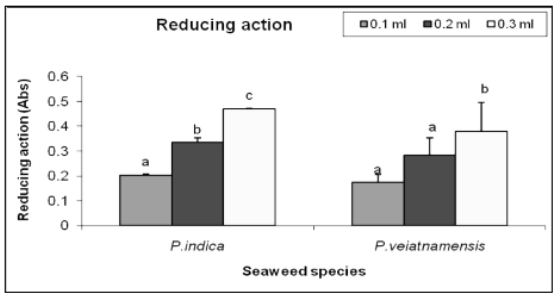
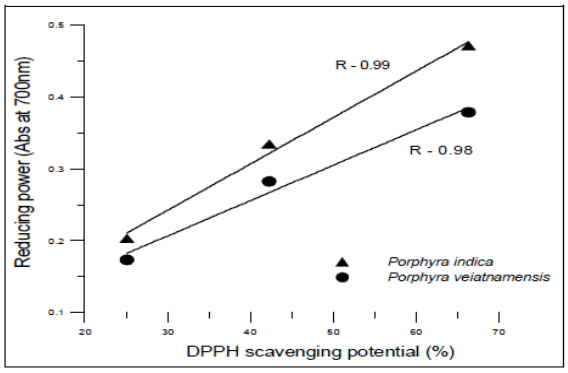


Fig. 2A. DPPH radical scavenging activity in *Porphyra indica* and *P. veiatnamensis* and **Fig. 2B.** Reducing power ability, where values are mean of triplicate determination ± SD (n = 3). Superscripts of

different letters are significantly different from each other at $P < 0.05$

Fig.3



(**Fig. 3.** Correlation between DPPH and reducing power in *Porphyra indica* and *P. veiatnamensis*)

Conclusion

Porphyra species showed better radical scavenging and reducing power ability, and higher phenolic contents. The present investigations reveled that *Porphyra* spp. were observed to be a great potential sources of nutraceuticals, as well as antioxidants. It is therefore, *Porphyra* spp. from alternative renewable sources for nutraceutical and antioxidants. Present findings encourage further evaluation by isolation, characterization and identifications of antioxidant molecules in *Porphyra* spp. and also *in vivo* studies for their mechanism of action as effective antioxidants.

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