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Free radical scavenging, reducing power, phenolic and biochemical composition of *Porphyra* species

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ABSTRACT

Inorganic elements, organic composition and antioxidant properties of *P. indica and P. veiatnamensis*, were investigated. Heavy metals were found below toxic level and protein and ash contents were found to be the most abundent components. Antioxidant potentials of algae was 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.

Practical Application

Overall data suggested that, theses seaweeds could be a potential rich source of natural antioxidants and a stuff of high nutritional values. These seaweeds were practically importance in the pharmaceuticals (medicine) and food industries for making candies and jelly's.

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

Introduction

Marine algae are looked upon as a valuable coastal bioresourse, particularly from nutraceutical, biofuel and biofertilizer, point of view. Inspite of being rich in vital chemical constituents and of commercial importance, utilization of seaweeds remains below their optimum level in India. Asian countries like Japan, South Korea and China have made seaweed farming for their nutraceutical and commercial chemicals. Recently, much research is focused to find naturally occurring antioxidant for use in food or pharmaceutical purposes, as synthetic antioxidants, which are being restricted due to their side effects of carcinogenicity (Zheng and Wang 2001).

Antioxidants scavenge the free radicals reducing oxidative damage, and thus protect living being. Phloroglucinol and phenolics in marine algae serve as ROS scavengers, metal chelators and enzyme modulators preventing lipid peroxidation (Rodrigo and Bosco, 2006). A number of marine algae have been reported to possess antioxidant properties (Nagai and Yukimoto, 2003), however, edible algal species from India remained to be explored for such kind of activities.

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.

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 *Porphyra* material was collected during November-December, 2008 from the central west coast of India at Malvan ($16^0$03'44.04"N, $73^0$27'17.28"E). Immediately after collection, fresh algal material was washed with distilled water to remove epiphytes and adhering debris, and then dried at a constant temperature (60°C) in oven. Dried material was ground to a fine powder and then extracted in methanol for 24 hrs at -20^{o}C. The extract was concentrated in a vacuum evaporator (BUCHI Rotavapor R-200) at 40 ^{o}C, and refrigerated at -20^{o}C. Dry powdered material was also used for estimating other chemical constituents described below.

Inorganic element analysis: Inorganic elements were analyzed using atomic absorption spectrophotometer (Perkin Elmer) from the extract prepared as per Toth *et al.,* 1948.

Biochemical analysis: Protein content was determined according to Lowry *et al*. (1951), using bovine serum albumin (BSA) as a standard. The carbohydrate contents were estimated by the method of Nelson (1944), utilizing glucose as a standard. Lipid content was measured following the process described by Folch and other (1957) and ash content by standard method of AOAC (1960).

Phenolic contents: Total Phenolic (TP) content was estimated by the method of (Slinkard and Singleton, 1977) using Folin-Ciocalteu reagent and Gallic acid standard.

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay: The free radical scavenging potential in methanol extract was measured as describe by Blois (1958) using DPPH. The scavenging effect was calculated in % after recording O.D. at 517 n.m.

Reducing power: Reducing power in methanol extracts was determined modifying the method of Oyaizu (1986). Ascorbic acid solution (ASA) was used as the standard.

Hydrogen peroxide scavenging assay: Hydrogen peroxide scavenging strength of extracts was determined by the method of Ruch *et al.* (1989). Ascorbic acid was used as the standard. The Scavenging effect was calculated in percentage.

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 $P < 0.05$.

Result and discussion

Inorganic elements: 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) than in *P. indica* (48.7-406 mg / 100g). Trace elements (Cu, Zn, Mn and Fe) varied from 6.8-659.8 mg / 100g in *P. vietnamensis* and 6.4-516 mg/100g in *P. indica*. The relatively higher concentrations of such essential elements in *Porphyra* spp. are indicative of their use as food supplements to provide required minerals to human beings. Seaweeds such as *Caulerpa lantillifra, Gracillaria parpispora,*

Monostroma oxysperum, and *Enteromorpha flexuosa* have been reported to contain relatively poor concentrations of these essential elements as compared to *Porphyra* spp (Subba Rao and others 2007). Variation in concentrations of such minerals may be attributed to different factors, such as 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 and other 1993).

Table 1: Inorganic Elements in *P. indica* and *P. vieatnamensis*.

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

Values were expressed in mg^{-1} 100gdry wt. tissues.

Data's are mean of triplicate determination ± sd (n = 3). ND - not detected

Biochemical composition: Total carbohydrate, content of *P. indica* was high (13.45%) than in and *P. vieatnamensis* (5.13%). Protein content was more less equal and so also was lipid (Table 2). Carbohydrate and proteins 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 was reported to be closer to cereals, eggs and some other food materials (Kathiresan 1992), which suggests its possible inclusion as supplementary food in human diet. A high percentage of ash was found (19.55 and 21.21%, respectively) in both the species under investigations (Table 2). Ash content in plants is 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 constituents in *P. Indica* and *P.vieatnamensis*.

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

Values were expressed in g^{-1} 100 g dry wt. Tissue.

Data's are mean of triplicate determination ± sd (n = 3).

Total phenolic compound: 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).

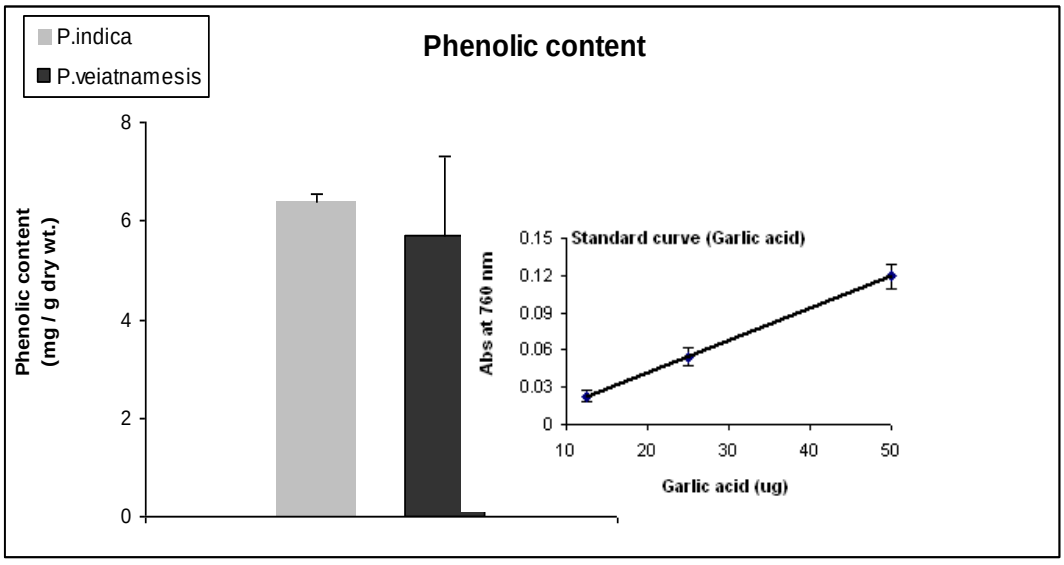

Fig. 1a: Total phenolic content in *Porphyra indica* and *P. veiatnamensis*. Values are mean of triplicate determination ± SD (N = 3).

Hydrogen peroxide scavenging assay: 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 is not very reactive, it generates highly reactive molecule such as $OH^{\bullet}$ by reacting with metals (Fe^{2+} or Cu^{2+}), and superoxide anions in the Haber-Weiss reaction. Therefore, removal 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. was observed during the present study ($P < 0.05$). Electronic donors might accelerate the conversion of H_2O_2 to H_2O (Ruch and other 1984), which could possible to scavenge H_2O_2 in the methanol extracts of *Porphyra* species.

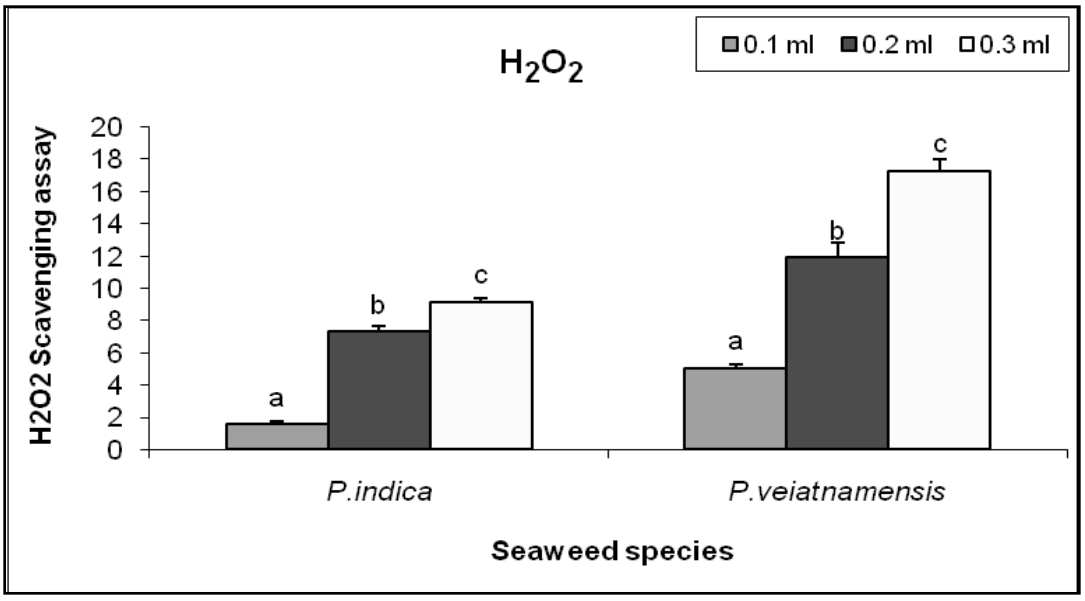

Fig.1b: Hydrogen peroxide scavenging assay in *Porphyra indica* and *P. veiatnamensis.* Values are mean of triplicate determination ± SD (N = 3). Superscripts of different letters are significantly different from each other at $P < 0.05$.

DPPH radical scavenging activity: The methanol extracts of both the *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 action, which might donate hydrogen to a free radical, reducing it to nonreactive species (Wang and other 2008). Higher DPPH scavenging potential of *P. indica* might be due to the higher reducing potential.

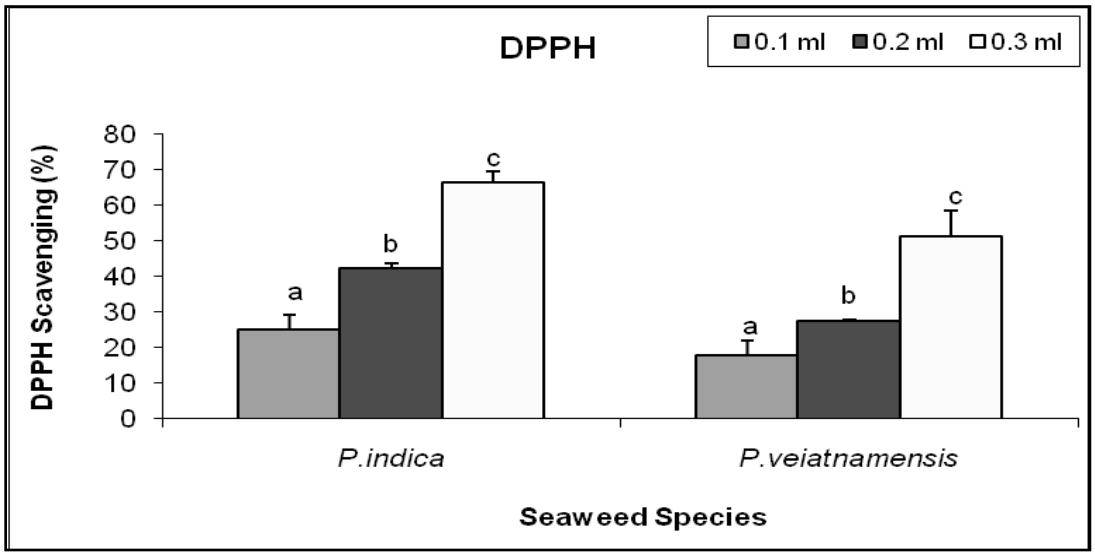

Fig.2a: DPPH radical scavenging activity in *Porphyra indica* and *P. veiatnamensis.* Values are mean of triplicate determination ± SD (N = 3). Superscripts of different letters are significantly different from each other at $P < 0.05$.

Reducing power: The reducing power of methanolic extracts of *Porphyra* spp. was found to be correlated with increasing concentration 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 & Mavi., 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).

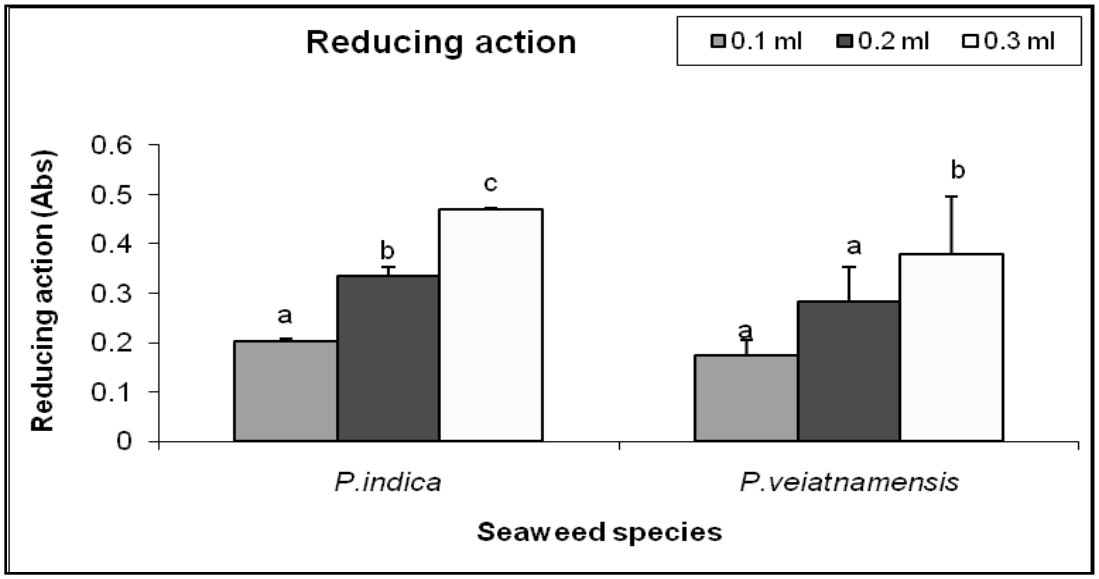

Fig.2b: Reducing power activity in *Porphyra indica* and *P. veiatnamensis.* Values are mean of triplicate determination ± SD (N = 3). Superscripts of different letters are significantly different from each other at $P < 0.05$.

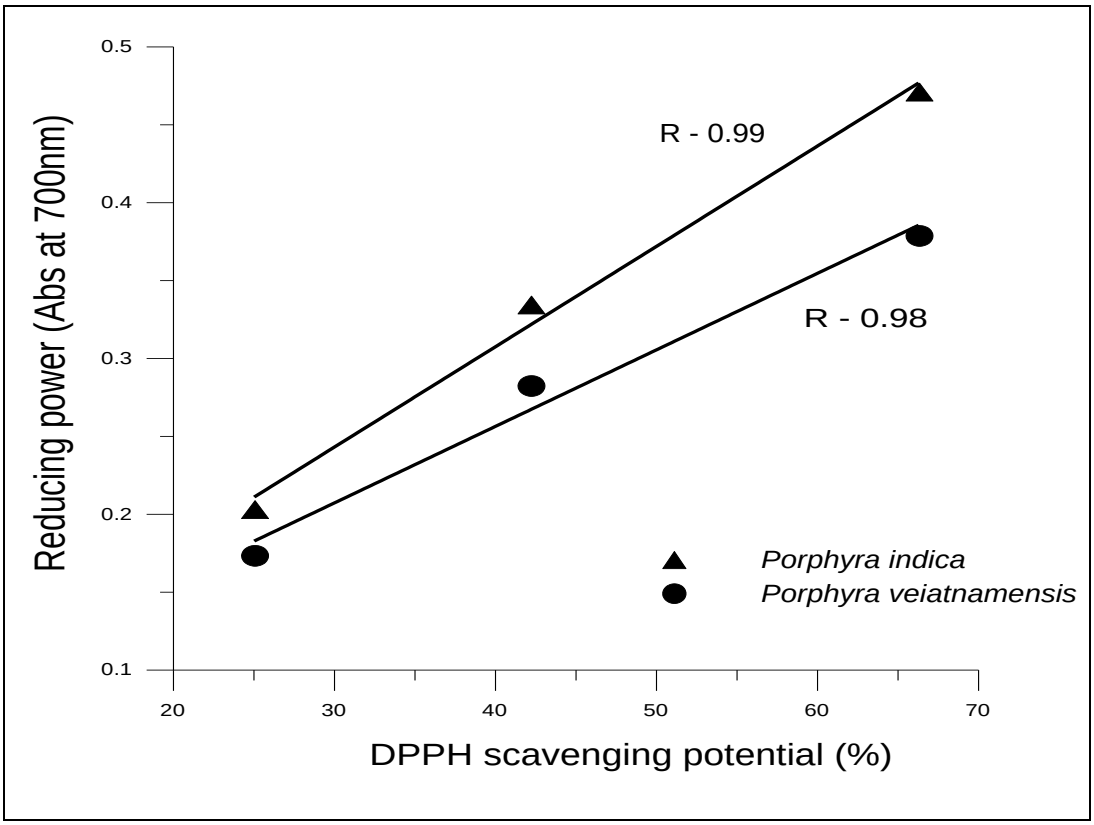

Fig.3: Correlation between DPPH and reducing power in *Porphyra indica* and *P. veiatnamensis.*

Conclusion

To conclude, the results confirmed that *Porphyra* spp. represent to be a potential source of nutraceuticals, as well as antioxidants and provide dietary alternatives due to their nutritional values. Both samples showed better radical scavenging and reducing power ability, and higher phenolic contents. 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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