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In-Vitro Antioxidant Potential of Some Selected Seaweeds -Gulf of Mannar

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ABSTRACT
In recent years, there has been a growing interest in natural antioxidants as replacements of synthetic compounds because of increased safety concerns and worldwide trend toward the usage of natural additives in foods. One of the richest sources of natural antioxidants, now a days largely studied for their potential to decrease the risk of diseases and to improve oxidative stability of food products, is seaweeds. Portieria hornemannii, Padina gymnospora, Codium tomentosum of red, brown and green seaweeds respectively were collected from south east of Tamilnadu and solvents treated. The antioxidant compounds were extracted and analysed using DPPH, FRAP and Peroxide radical assays. Results revealed that among the three varieties, Portieria hornemannii showed better activity against the standard radical DPPH(79%), reducing power ability (65.12±1.31), Hydrogen peroxide scavenging assay (76.24±1.14) and Hydroxyl radical scavenging activity(82.16±0.29). Seaweeds are found to be potential antioxidant agents against free radicals.
Keywords: *Portieria hornemannii, Padina gymnospora, Codium tomentosum, Antioxidant Activity*

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INTRODUCTION

Algae generally has higher antioxidant activity due to a higher contents of non- enzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols and flavonoids [14]. As a result, many marine bio-sources in the last decades have attracted attention in the search for natural bioactive compounds to develop new drugs and healthy foods. Compounds with antioxidant, antiviral, antifungal, antimicrobial, antitumor and anti-inflammatory activities have been found in brown, red and green algae [15].

The antioxidant activity of several seaweeds has been reported [16, 17].The present study aimed to investigate the antioxidant properties of three species namely *Portieria hornemannii, Padina gymnospora, Codium tomentosum* of red, brown and green seaweeds from the Southern coasts for future applications in medicine, dietary supplements,or food industries.

MATERIAL AND METHODS

Collection

About 2 kg of fresh seaweed of *Portieria hornemannii, Padina gymnospora, Codium tomentosum* collected from the intertidal regions of Mandapam coast of Gulf of Mannar (Latitude9°17'N; Longitude79°08'E),TamilNadu, India and transported to the laboratory by plastic containers with seawater. The seaweeds belonging to the species were carefully examined, identified and authenticated by the Centre for Advanced Study in Marine Biology, Annamalai University, Parangipettai,Tamilnadu.

Preparation of seaweed powder

The preparation of seaweed extract procedure was adopted from Noorjahan *et al*., (2019). Fresh seaweed samples were handpicked during lowtide and manually cleaned from sand, epiphytes. Then the samples were rinsed with sea water to remove associated debris, planktons and loosely attached organisms. Morphologically distinct thallus of algae were placed separately in new polythene bags and kept in an ice box containing slush ice and transported to laboratory. Further, the material was thoroughly washed with tap water followed by distilled water to remove excess salt on the surface and

the water was drained off and spread on the blotting paper. The shade dried samples were ground in an electric mixer. Finally, quantity of 650g of powdered seaweed sample was obtained and stored in refrigerator (4°C) for further use.

Preparation of the Extract

From the sample (650 mg) 5mg of the sample was weighed and soaked in 10 mL of Ethanol and incubated in darkness for about 21 days occasionally shaken, the crude extracts were filtered by using muslin cloth and the filtrate extracts were concentrated by rotary vacuum evaporator (> 45°C) and then freeze dried (- 4°C) to obtain solid residue and were stored in individual sterile glass container for further use.

DPPH radical scavenging assay

The ethanolic extract was then subjected to four antioxidants. The free radical scavenging activity of the fractions was measured in vitro by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [2]. About 0.3 Mm solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations (25-400µg/ml).The mixture was shaken and allowed to stand at room temperature for 30min and the absorbance was measured at 517nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and the IC_{50} value of the fractions was compared with that of ascorbic acid, which was used as the standard.

Fe^{+} ion Reducing power ability

The reducing power was investigated by the Fe^{3+}-Fe^{2+} transformation in the presence of the fractions as described by [1]. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of the fraction (50- 800µg/ml),2.5ml of phosphate buffer (pH6.6)and 2.5ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000g. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Butylated hydroxy toluene (50-800 µg/ml) was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mm/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the fractions (25-400 µg/ml) in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and the IC50 values were compared with the standard, α-tocopherol [3].

Hydroxyl radical-scavenging activity:

One milliliter of the final reaction solution consisted of aliquots (500 µL) of various concentrations of the extract, 1 mM $FeCl_3$, 1 mM EDTA, 20 mM H_2O_2,1 mML-ascorbic acid, and 30mM phosphate buffer (pH7.4). The reaction mixture was incubated for 1 h at 37 °C, and further heated in a boiling water bath for 15 min afteradditionof1mLof 2.8%(w/v) trichloroacetic acid and 1mLof1%(w/w)2-thiobarbituric acid. The color development was measured of 532 nm against a blank containing phosphate buffer [4].

Calculation of 50% Inhibitory Concentration (IC50)

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhibition (I%) was calculated using the formula,

I % = (Ac-As) x 100 Ac

Where Ac is the absorbance of the control and as is the absorbance of the sample.

RESULTS AND DISCUSSION

During the study, three seaweeds belonging to red, brown and green such as *Portieria hornemannii, Padina gymnospora, Codium tomentosum* species were collected from southern coasts of Tamilnadu, India. The Extraction yields of samples (S1-S3) were 10.60,28.43 and 20.42 %, respectively.

The scavenging effect of the tested extracts at concentration of 2mgmL^{-1}on the DPPH radical decreased in the order of: S1 > S2 > S3, and were 90.3, 49.19 and 52.15 % , respectively. The inhibitory effect of all extracts were dose dependent in the range of the tested concentrations [Table1]. The inhibitory effect increased with increasing concentration. However, the extract of *Portieria hornemannii* was found to be the most potent scavenger in these tested algae. The activity of the *Padina gymnospora* extract (2 mg mL^{-1}) was comparable to that of the positive control, ascorbic acid (at concentration of0.1 mg mL-1) ($p < 0.05$). Many studies have been done to determine antioxidant capacity of seaweeds. For instance, 48 marine algae were tested for their antioxidant activity and a low antioxidant activity with a relatively

high IC50 (43.23±0.28mgmL^{-1}) were reported for green seaweeds *Ulva intestinalis* among the all tested seaweeds [5-7]. However, some researchers have stated high scavenging activity for red seaweed species. For example, three edible species including *Portieria hornemannii, Padina gymnospora, Codium tomentosum* exhibited high antioxidant activity in linoleic acid system and the best DPPH radical scavenging was observed in methanolic extract of *Padina gymnospora* (IC50 = 1.89 mg mL^{-1}) [8].

In modern times, seaweeds are broadly utilized in manifold pharmaceutical applications, such as antimicrobial, antiviral, antibacterial, and antifungal, anti-allergic, anticoagulant, anticancer, antifouling, and anti-oxidant. The functions of these secondary metabolites are defence mechanism against herbivores, fouling organisms and pathogens [5]. Most of the secondary metabolites produced by seaweeds have bacterial or the antimicrobial compounds,viz., phenols, oxygen heterocyclic, terpenols, sterols, polysaccharides, dibutenolides peptides and proteins [5].

Hydroxyl radical scavenging activity was employed to understand the potential of different seaweeds against short-lived radicals, namely, the HO. radical. The EtOAc fraction of *Portieria hornemannii* exhibited a significantly greater ($p<0.05$)HO. radical scavenging activity (IC500.19mg/mL) than (Table 2) the EtOAc of *Padina gymnospora* contributed significantly ($p < 0.05$) toward HO. scavenging activity (IC50 0.39 and 0.41 mg/mL, respectively) than the *Codium tomentosum* fractions (IC50 2.67 and 50.7 mg/ mL, respectively). There are earlier reports showing that the antioxidants from *Sargassum spp* exhibited higher hydroxyl radical scavenging activity (Heo et al., 2005). Likewise, the hydroxyl radical scavenging activity of the EtOAc fraction derived from *Portieria hornemannii* (IC500.19mg/mL) showed no significant difference in activity with the synthetic antioxidants BHA (IC500.19mg/mL) and BHT(IC500.13 mg/mL). It is of note that the ability of the EtOAc fraction of *Portieria hornemannii* to scavenge hydroxyl radical was significantly greater than that of α-tocopherol (IC50 0.25 mg/mL) ($p < 0.05$).

H_2O_2 is a reactive non-radical pro-oxidant and is of potential biological significance because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it may convert into more reactive species, such as singlet oxygen and HO. radicals. The H_2O_2 scavenging activities of EtOAc of *Portieria hornemannii* were found to be significantly higher ($p<0.05$) (IC501.85 and 2.16mg/mL, respectively) than the *Padina gymnospora* (IC50 3.77 mg/mL) and *Codium tomentosum* (IC50 4.6 mg/mL) (Table 3). Prior reports showed the greater H_2O_2 scavenging activity (~96%) in the EtOAc fraction of the brown seaweed [11, 13], thus supporting the fact that the polar fractions of brown seaweeds harbor a rich source of natural antioxidant principles.

Table- 1: DPPH activity of three seaweeds at different concentration

Samples in %	Concentration in µg/ml			
	50	100	150	200
STD	66.41±0.05	69.42±0.01	73.3±0.01	80.54±0.01
PH (E)	65.21±0.11	68.16±0.21	72.19±0.23	79.24±0.21
PG (E)	64.26±0.01	67.89±0.01	71.29±0.01	75.89±0.01
CT (E)	61.12±0.01	63.26±0.01	68.12±0.01	70.24±0.01

Table -2: Reducing power of three seaweeds at different concentration

Samples in %	Concentration in µg/ml			
	50	100	150	200
STD	89.19±1.14	74.49±1.01	66.21±1.01	53.33±1.01
PH (E)	87.31±1.01	72.27±0.29	65.12±1.31	52.76±0.79
PG (E)	72.96±0.99	60.62±0.99	57.08±1.01	41.76±0.99
CT (E)	67.51±1.01	54.17±0.99	51.11±1.01	45.96±0.99

Table -3: Hydrogen peroxide scavenging activity of three seaweeds at different concentration

Samples in %	Concentration in µg/ml			
	50	100	150	200
STD	87.16±1.14	81.39±1.11	76.51±1.21	63.37±1.09
PH (E)	84.31±1.21	76.17±1.29	71.42±1.37	65.74±1.79
PG (E)	79.86±1.99	71.67±0.89	67.08±1.01	51.74±0.89
CT (E)	69.71±1.91	64.27±0.79	59.21±1.11	54.86±0.19

The EtOAc fraction of *Portieria hornemannii*(1 mg/mL) exhibited higher absorbance at 700 nm (A700 nm 1.42), indicating a higher reducing power (Table 4). The other species followed the order, *Codium tomentosum* (0.76) >*Padina gymnospora*(0.73) toward reducing ability (Table 4). Similarly, the reducing capacities of the EtOAc of *Codium tomentosum* (A700 nm 0.96 and 0.81, respectively) were found to be higher than those of MeOH extract (A700 nm 0.53) and n-hexane (A700 nm 0.71) fraction (Table 4).

Prior reports suggested that the EtOAc fractions of seaweed harbors reductones, for example, phenolic compounds, which have been shown to impart antioxidant action by breaking the free radical chain by donating a hydrogen atom [10]. There are other studies reporting the presence of reducing agents in brown seaweeds, including *Sargassumsp* collected from different regions [9, 10]. It can also be concluded from these observations that these seaweed species may contain the presence of polyphenols, which can act as reducing agents.

Table-4: Hydroxyl radical scavenging of three seaweeds at different concentration

Samples in %	CONCENTRATION (µg/ml)			
	50	100	150	200
STD	72.46±1.01	78.01±179.03	84.72±1.01	92.21±0.99
PH (E)	71.85±0.19	77.43±1.01	82.16±0.29	90.12±1.09
PG (E)	65.05±1.01	71.28±1.01	78.31±1.01	87.32±1.01
CT (E)	61.75±0.99	67.53±1	71.56±0.99	73.32±1.01

CONCLUSION

In the current study, the antioxidant activities of three seaweed species such as *Portieria hornemannii, Padina gymnospora, Codium tomentosum* were evaluated. The results clearly indicated that all the tested seaweeds in this investigation possess antioxidant activity. *Portieria hornemannii* exhibited high antioxidant activity with a low IC_{50}. Further work is under way in our laboratories which are aimed to investigate the nutraceutical potential of the studied seaweeds for to utilize in food and beverages.

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