Evaluation of Antioxidant Activities of a Marine Brown Alga *Padina gymnospora* (Kutz.) Sond from the Pamban Coast of Rameswaram, Tamil Nadu, India

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ABSTRACT

The present study focused on determining the antioxidant properties of the *Padina gymnospora* ethanol crude extracts, from the brown algae. The evaluation of antioxidant properties was estimated and determined by using five standard methods, namely 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis 3ethylbenzthiazoline-6-sulphonic acid (ABTS), superoxide radical scavenging, ferric reducing antioxidant power (FRAP) assay and hydroxyl radical assay. The tested brown alga had the maximum antioxidant activity was recorded in the ethanol extract of *P. gymnospora*, whereas ethanol crude extract of other algae and diatom showed good antioxidant potential. This alga had a lesser radical scavenging ability than standard ascorbic acid. This study suggests that ethanol crude extracts contain different potential antioxidant compounds capable to scavenge different types of free radicals.

Keywords: Antioxidant activity, DPPH, ABTS, FRAP, *Padina gymnospora*.

I. Introduction

Since ancient times, algae have been used as nutritional and functional food sources in Asian countries like Japan and China have known about the algae are part of their diet [1]. There is evidence in these countries that the incidence of certain kinds of cancer, such as prostate and breast, which is lowered due to regular consumption of seaweeds, the leading cause being their bioactive compounds that may prevent against those diseases. Natural antioxidants are potential with an essential for multifunctional to prevent malfunctioning of oxidation in complex food systems than the synthetic antioxidants as interest. Aquatic plants are naturally gaining importance as a potential source of antioxidants. The ascorbic acid acts as an antioxidant with direct effects to prevent, control, and regulate cellular malfunctions. It is a substrate for the redox enzyme ascorbate peroxidase, which is particularly important in stress resistance in plants. Gershoff [2] reported that ascorbic acid is present at high levels in all parts of plants, particularly in chloroplasts that reach concentrations of 20 mM there. Many synthetic antioxidants are namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutyl hydroxy quinones (TBHQ) available which are the maximum used in different food that they are highly potential health hazards, which were strictly prevented from using in various food products in many countries. The marine algal extracts have also been demonstrated to have strong antioxidant properties, especially, brown seaweeds which show comparatively higher antioxidant activity than green and red [3].

The marine brown algal seaweed *Padina* is a genus of Phaeophyceae under the order of Dictyotales in Algae, which is occurring worldwide in specifically tropical and temperate seas. Global species diversity and distribution ranges, however, remain mostly unknown [4]. The *Padina* has the second most speciose with the 43 recognized species of dictyotalean genus after Dictyota (76 identified species)[5].

II. Materials and Methods

2.1. Collection of Marine Algae

Brown algae were collected from the Pamban coast of Rameswaram, Tamilnadu, India in December 2014. The freshly harvested seaweeds were washed with clean seawater to remove salt, epiphytes, and sand attached to the surfaces of the samples and transported to the laboratory. The samples were carefully washed with tap water. The samples were pulverized into powder and stored at - 80 °C before extraction.

2.2. Chemicals

The required chemicals for DPPH, ABTs, and FRAP were obtained from Sigma Aldrich (Steinheim, Germany). As per the protocol depends reagents were of analytical grade and obtained from Merck (Darmstadt, Germany) included organic solvent for extraction.

2.3. Preparation for Analysis

About 5 g of *Padina gymnospora* powdered seaweed was extracted overnight with 200 ml ethanol at room temperature and centrifuged at 3000 rpm for 10 min. The supernatant was collected in a separate labeled bottle after passing through a Whatman No.1 filter paper and the residue was re-extracted two times under the same conditions. The pooled extracts were dried, frozen, and kept on - 80 °C until analysis. The freeze-dried extracts were re-dissolved in ethanol and used for the analysis.

2.3.1. DPPH radical scavenging activity

Different concentrations of 4ml samples were mixed with 1ml of ethanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM [6]. The mixture had been shaken vigorously and kept to stand for 30 minutes and the absorbance (OD) was taken at 517 nm. Ascorbic acid was used as a control. The percentage of DPPH decolorization of the sample was calculated as per the following equation;

$$\text{DPPH radical scavenging activity (\%)} = [(\text{A control} - \text{A test}) / \text{A control}] \times 100 \quad (1)$$

IC_{50} values (µg/ml) where the inhibitory concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for comparing.

2.3.2. $ABTS^+$ radical scavenging activity

ABTS [2, 2'-azino-bis (3-thylbenzothiazoline-6-sulphonic acid)] was obtained by reacting to 7 mM [7]. ABTS stock solution with 2.45 mM potassium persulfate and the mixture were left to stand in the dark at room temperature for 12 - 16 h before use. The samples were reduced to the concentration ranges from 10 to 50µg/ml. The reaction was subjected by the addition of 1ml of diluted $ABTS^+$ to the different concentrations of *P. gymnospora* as a sample and 10 ml ethanol as control. The absorbance was measured after 10 min incubation at 730 nm. The ABTS radical scavenging activity was expressed as

$$\text{\% of ABTS radical cation inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \quad (2)$$

2.3.3. Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition[8]. The 3 ml reaction mixture contained 50 ml of 1M NBT, 150 ml of 1M NADH with or without sample, and Tris buffer (0.02M, pH 8.0). The reaction was started by adding 15 ml of 1M PMS to the mixture and the absorbance change was recorded at 560 nm after 2 minutes. The inhibition percentage was calculated against a control without the extract.

2.3.4. Hydroxyl radical scavenging activity

The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO4, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate, and varying concentrations of the sample[9]. After incubation at 37 °C for 60 minutes, the absence of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as the scavenging activity.

$$\text{Hydroxyl radical scavenging activity } \mathbf{(\%)} = [1-(A_1-A_2)/A_0] \times 100 \quad (3)$$

Where A_0 was the observance of the control (without extract), A_1 was the observance in the presence of the extract, and A_2 was the observance without sodium salicylate.

2.3.5. Ferric-reducing antioxidant power (FRAP) assay

The 10 mM of 2, 4, 6-tripyridyl-s-triazine (TPTZ) stock solution in 40 mM HCL, 20 mM $FeCl_3.6H_2O$ and 0.3M acetate buffer (pH 3.6) was prepared [10]. The FRAP reagent was a mixture of 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution, and 25 ml acetate buffer. It was freshly prepared and warmed to 37ºC. FRAP reagent (900 ml) was mixed with 90 ml distilled water and 30 ml of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 minutes and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyltriazine (Fe_3^+-TPTZ) complex was reduced to ferrous (Fe_2^+) form. The absorption at 540 nm was recorded.

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation(SD).

3. Results and Discussion

An organic chemical compound 2,2-diphenyl-1-picrylhydrazyl abbreviated form is DPPH. It is a stable free radical molecule with a dark-colored crystalline powder. Cotelle *et al*., [11] reported that the DPPH had been used enormously as a free radical chemical to evaluate reducing substances. The salient feature of DPPH is the purple color, usually disappeared while an antioxidant compound is present in the medium. Thus, molecules with antioxidant can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases the more potent antioxidant activity of the extract. The seaweed extracts consisted of many primary and secondary metabolites like phenols, flavonoids; steroids, etc., which are responsible for helping to prevent many diseases due to their free radical scavenging activities [12]. The seaweed *P. gymnospora* exhibited significant dose-dependent inhibition of

DPPH activity. This alga had a lesser activity than the standard of ascorbic acid. The results were presented in Table 1, the IC_{50} value of ascorbic acid, and the algal sample was 30.78µg/ml, and 39.98µg/ml., respectively.

Table 1: DPPH Inhibitory Activity of *Padina gymnospora*

Concentrations (µg/ml)	% of Inhibition	
	Ascorbic Acid (Standard Antioxidant)	DPPH
10	38.29 ± 1.12	35.22 ± 0.01
20	42.55 ± 1.32	39.66 ± 0.12
30	46.88 ± 1.45	43.52 ± 0.14
40	53.69 ± 2.22	50.12 ± 1.01
50	58.45 ± 2.34	55.45 ± 1.25

Data represented as mean ± SD (n=3)

ABTS assay is a simple indirect method for determining the activity of natural antioxidants. Roginsky and Lissi, [13] reported that ABTS radical is rather stable, still, it reacts energetically with an H-atom donor, such as phenolics, been converted into a non-colored form of ABTS. The ABTS radical cation-scavenging assay performed showed that the antioxidant activity increases with an increase in the concentration. The results showed (Table 2) that *P. gymnospora* showed a lesser behavior than standard ascorbic acid of 30.38µg/ml and 25.85 µg/ml, of IC_{50}, respectively.

Table 2: ABTS Inhibitory Activity of *Padina gymnospora*

Concentrations (µg/ml)	% of Inhibition	
	Ascorbic Acid (Standard Antioxidant)	ABTS
10	33.29 ± 1.25	30.22 ± 0.02
20	46.22 ± 1.45	42.56 ± 1.21
30	50.11 ± 1.68	48.33 ± 2.12
40	57.94 ± 2.11	55.98 ± 3.05
50	63.45 ± 2.88	59.36 ± 3.15

Data represented as mean ± SD (n=3)

Superoxide anion radical is one of the most durable reactive oxygen species among the free radicals that are generated after the oxygen is taken into living cells. Superoxide anion changes to other harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radicals, which induce oxidative damage [14]. The decrease in the absorbance at 560 nm with the *P. gymnospora* thus indicates the consumption of superoxide anion in the reaction mixture. The antioxidant activity of the ethanol crude extract determined by superoxide anion radical assay varied, as seen in Table 3. The reducing power was found to be higher in ethanol extract. At a concentration of 43.16µg/ml of *P. gymnospora* of IC_{50} superoxide anion radical generated, which showed lesser scavenging activities than the standard ascorbic acid (38.44µg/ml of IC_{50} value).

Table 3: Superoxide Inhibitory Activity of *Padina gymnospora*

Concentration (µg/ml)	% of Inhibition	
	Ascorbic Acid (Standard Antioxidant)	Superoxide
10	27.54 ± 1.35	24.26 ± 1.02
20	35.69 ± 1.52	32.55 ± 1.22
30	42.12 ± 1.87	40.12 ± 1.34
40	51.44 ± 2.44	49.55 ± 2.55
50	57.56 ± 2.67	54.64 ± 2.97

Data represented as mean ± SD (n=3)

Ferric-reducing antioxidant power (FRAP) is another important indicator of the antioxidant potential of either a compound or an extract with a solvent [15]. The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors, which could be reduced the oxidized intermediate compounds of lipid peroxidation processes, hence acting as primary and secondary antioxidants [16]. The antioxidant activity of the ethanol crude extract determined by FRAP assay varied, as seen in Table 4. The reducing power was found to be higher in ethanol extract. At a concentration of 28.14µg/ml in *P. gymnospora* and 21.32µg/ml with ascorbic acid of IC_{50} values.

Table 4: FRAP Inhibitory Activity of *Padina gymnospora.*

Concentration (µg/ml)	% of Inhibition	
	Ascorbic Acid (Standardized Antioxidant)	FRAP
10	36.78 ± 1.08	33.55 ± 0.12
20	45.22 ± 1.21	41.22 ± 1.09
30	57.89 ± 1.37	48.23 ± 1.46
40	61.48 ± 2.01	58.24 ± 1.63
50	68.55 ± 2.55	62.11 ± 1.85

Data represented as mean ± SD (n=3)

Hydroxyl radical iron is the vital, active oxygen and is causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to macromolecules like DNA, lipid, and protein [17].

In this present study, the algal sample was found to scavenge O_2 significantly and in a dose-dependent manner and may protect the DNA, protein, and lipid from damage. Earlier, a study in this alga from the intertidal regions of Pondicherry coast, India by Saranya *et al*., [18] reported that *P. gymnospora* extracts as a good source for the development of antioxidant agents. The results for the hydroxyl scavenging assay were shown in Table 5. The concentrations for 50% inhibition were found to be 29.74 and 21.89µg/ml for the *P. gymnospora* and ascorbic acid, respectively.

Table 5: Hydroxyl Radical Scavenging Inhibitory Activity of *Padina gymnospora*

Concentration (µg/ml)		% of Inhibition
	Ascorbic Acid (Standardized Antioxidant)	Hydroxyl
10	**33.87 ± 1.06**	**31.14 ± 1.00**
20	**48.22 ± 2.02**	**41.55 ± 1.07**
30	**55.98 ± 2.22**	**49.58 ± 1.21**
40	**62.84 ± 2.76**	**54.78 ± 1.34**
50	**67.33 ± 3.11**	**61.23 ± 1.39**

Data represented as mean ± SD (n=3)

4. Conclusion

In this communication, we are reporting the first time that ethanolic extraction method by using antioxidant activity. In the present study, the *Padina gymnospora* (alga) ethanolic extractions were showing significant results in the various antioxidant activities. The experimental findings proved that this alga extract is an excellent source of bioactive compounds with a wide variety of applications and will be used in natural antioxidants in different food and pharmaceutical industry products.

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