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Research Article

INVITRO STUDIES ON ANTIOXIDANT AND HAEMAGGLUTINATION ACTIVITY OF SOME SELECTED SEAWEEDS

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ABSTRACT

Background: Seaweeds are found abundant all along the seacoast and so far 2400 secondary metabolites are derived from it. They are used for various medicinal purposes.

Objective: Free radicals are responsible for aging and causing various human diseases. Antioxidant substances which scavenge free radicals play an important role in the prevention of free radical induced diseases. So, the present study was carried out to estimate the antioxidant activity of the seaweeds.

Materials and method: Effect of seaweed extracts on DPPH radical was measured based no method modified by Lu and Foo and Lai.

Result: Highest activity was found in *Hypnea* extracts where 86.13% of DPPH was scavenged. *Amphiora* showed no activity. When six species of the seaweeds were analysed for their haemagglutinic activity the members of Phaeophyceae such as *Padina tetrastomatica, Acanthophora indica* and *Hypnea* had positive effects against almost all eight blood groups. O+ had positive activity against all six species.

Conclusion: Seaweeds show promising antioxidant and haemagglutinic properties and can be explored further for pharmacological use.

Keyword: Antioxidant, DPPH scavenging, Haemagglutinic activity, Seaweeds.

INTRODUCTION

The presence of antioxidant substances in seaweeds is suggested to be an endogenous defense mechanism as a protection against oxidative stress due to extreme environmental conditions [1]. The anti oxidant compounds include chlorophyll and carotenoid pigments, vitamins like α-tocopherol and phenolic substances [2]. Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical – induced diseases. Recently, the seaweeds extracts and fractions have been considered to be a rich source of antioxidants and different types of antioxidants have been isolated from various species of seaweeds [3-6]. The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolicacid, flavonoid, tannins e.g.), which are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation [7-10].

Antioxidant reduces the risk for chronic disease including cancer and heart disease. Antioxidant like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogen compound such as gallates have strong antioxidant activity.Various antioxidants are present in sea weeds, for example polysaccharides, dietary fibres, minerals proteins, amino acids, Vitamins, polyphenols and carotenoides [11]. Therefore, sea weeds are potential source of novel antioxidants. In addition, natural antioxidants are considered more safe for use as ingredients in medicine, dietary supplements, nutraceuticals and cosmetics with the objective of improving consumer health, reducing the effects of harmful diseases and other broader aspects of immune system function. Direct consumption of seaweed products for their antioxidant composition alone provides a useful alternative to non-natural substances, while simultaneously providing worthwhile nutritional benefits [12]. The principal agents responsible for the protective effects could be the presence of antioxidant substances that inhibit their effects as free radical scavengers, hydrogen donating compounds, singlet oxygen quenchers and metal ion chelators [13].

The proteinaceous secondary metabolites, haemagglutinins cause the clumping or agglutination of red blood cells in vitro [14]. Haemagglutinins cause adverse effects like reduced growth, diarrhoea, decreased nutrient absorption and increased incidence of bacterial infections in humans.

The present study was designed to study the antioxidant activity of four species of seaweeds namely *Amphiora corallina, Ulva fasciata, Acanthophora spicifera* and *Hypnea valentiae*. An attempt was also made to analyse the haemagglutination activity in a few species of seaweeds.

MATERIALS AND METHODS

Sample collection

Few species of sea weeds were collected from south eastern parts of the Tamil Nadu coast especially from Kanyakumari district during November 2010. *Amphiora corallina*, was collected from Kanyakumari. *Ulva fasciata, Acanthophora specifera, Hypnea valentiae* were collected from Muttam.

Preparation of Sample

Sea weeds were carefully cleaned from epiphytes and washed several times with tap and distilled water then shade dried. The dried samples were cut into small pieces and ground into fine power using a dry grinder. The ground samples were sieved to get uniform particle size, then kept in an air-tight container and stored in a freezer (-20^{o}C) for further analysis. Ethanol extracts were obtained using soxhlet apparatus, they were labelled and stored in a deep freezer at a temperature of 20^{o}C until further use.

Free Radical Scavenging Assay

Effect of sea weed extracts on DPPH radical was measured based on the method modified by Lu and Foo (2000) and Lai *et al*., (2001) [15,16].

Preparation of Serial Dilution

For the haemagglutinating activity serial dilutions for each blood group were prepared. Serial dilutions in 5 ml capacity test tubes of 1:2, 1:4, 1:8, 1:16 and 1:32 were prepared by adding 1 ml previously prepared phosphate buffer saline (PBS) pH 7.2 and then adding 0.5 ml of algal extracts to each tube.

Preparation of 2% suspension of erythrocytes:

All groups of human blood A, B, O and AB both Rh + and Rh- were obtained from the Blood donation Camp conducted at our college.

Native erythrocytes were prepared as follows: Approximately 1 ml of human blood was centrifuged at 3,000 rpm for 5 min at room temperature. The supernatant of serum was discarded and the pellet of erythrocytes was washed three-times with cold PBS at pH 7.2 It was then centrifuged again for 5 min. Finally, a 2% V/V of erythrocyte suspension was prepared to be tested for agglutination.

Assay of Haemagglutinating activity:

Haemagglutinating activity was investigated serially in different dilutions of each algal extract against all the four blood groups. The 0.5 ml 2% erythrocytes suspensions of "A" group was added in all the five dilutions shaken, for few seconds and then allowed to stand for incubation in a water bath at 25° C for 30 minutes, control was also run simultaneously. The test tubes were left to stand at room temperature for 2 hours. Agglutination was observed.

RESULTS

Antioxidant activities of ethanolic seaweed extracts

Amphiora corallina showed negative activity in all the five concentration. The sample was highly turbid. *Ulva fasciata* showed significant activity with the increase of sample concentration. The activity increased from 2.40% at 100µl to 39.79% at 500µl. The antioxidant screening assays were found to be statistically significant at 5% (P>0.05) (Table 1). There was similar increase in the percentage of DPPH scavenged with increase in concentration of *Acanthophora spicifera*. At 100µl the activity was 2.53%, at 200µl it increased to 15.67%.

Further at 300µl the activity was 19.98% and the peak activity (24.37%) was observed at the highest concentration of 500µl. The antioxidant screening assays were found to be statistically significant at 5% (P>0.05) level . *Hypnea valentiae* showed good activity even at very low concentrations. When compared to other samples very minute quantity (from 2.0µl - 40µl) of *Hypnea valentiae* was enough. This also showed relatively good activity with increase in sample concentration.

One way analysis of variance was carried out between sample concentration and DPPH scavenging in the different seaweed species analysed. The result showed a significant (P<0.05, P<0.01) relationship between these two parameters. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples

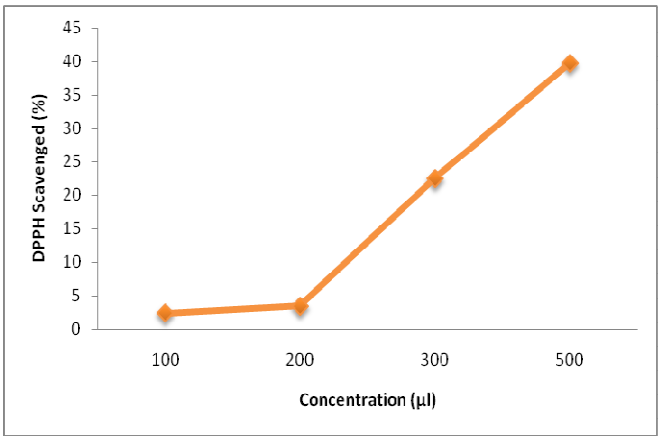

Fig 1: Showing the percentage of DPPH Scavenged by *Ulva fasciata*

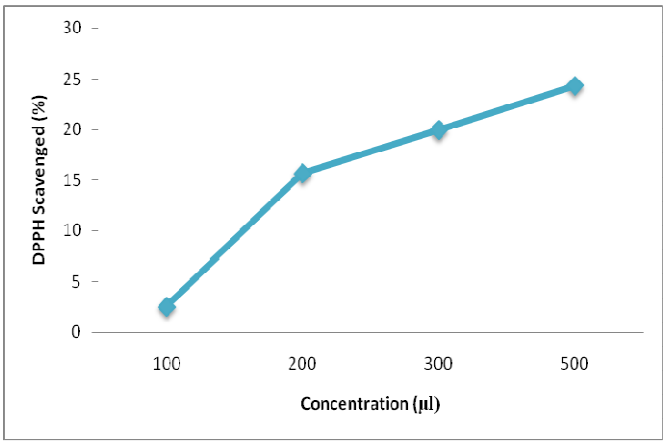

Fig 2: Showing the percentage of DPPH Scavenged by *Acanthophora spicifera*

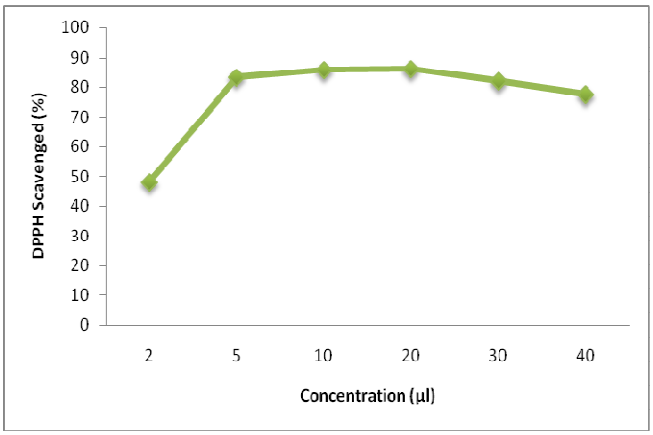

Fig. 3: Showing the percentage of DPPH Scavenged by *Hypnea valentiae*

Haemagglutinic Activity

All 6 species showed a positive activity for O^+ blood group. *Sargassum species* showed no activity against A^+, A^-, B^+, B^-, AB^+, AB^-, O^-blood groups but possible activity in O^+ blood group. *Sargassum polycystum* showed a positive response only in O^+ and no activity against other blood groups. *Padina tetrastromatica* showed a positive activity for all eight different blood samples. (A^+, A^-, B^+, B^-, AB^+, AB^-, O^-, O^+)

Acanthophera spicifera showed a positive activity for all eight different blood samples. A^+, A^-, B^+, B^-, AB^+, AB^-, O^-, O^+. *Gracilaria corticata* showed a positive activity for two different blood samples. O^+ and AB^+. *Hypnea valentiae* on the other hand showed positive activity for all blood groups. (Table 2 to 5)

Table 2: Showing haemagglutinic activity against blood group A^+ & A^- in different species of seaweeds.

Seaweeds	Blood Group A^+						Blood Group A^-					
	1:2	1:4	1:6	1:8	1:16	1:32	1:2	1:4	1:6	1:8	1:16	1:32
Saragassum species	-	-	-	-	-	-	-	-	-	-	-	-
Saragassum polycystum	-	-	-	-	-	-	-	-	-	-	-	-
Padina tetrastromatica	+	+	+	+	+	+	+	+	+	+	+	+
Gracilara corticata	-	-	-	-	-	-	-	-	-	-	-	-
Hypnea valentiae	+	+	+	+	+	+	+	+	+	+	+	+
Acanthophora spicifera	+	+	+	+	+	+	+	+	+	+	+	+

Table 3: Showing haemagglutinic activity against blood group B^+ & B^- in different species of seaweeds

Seaweeds	Blood Group B^+						Blood Group B^-					
	1:2	1:4	1:6	1:8	1:16	1:32	1:2	1:4	1:6	1:8	1:16	1:32
Saragassum species	-	-	-	-	-	-	-	-	-	-	-	-
Saragassum polycystum	-	-	-	-	-	-	-	-	-	-	-	-
Padina tetrastromatica	+	+	+	+	+	+	+	+	+	+	+	+
Gracilara corticata	-	-	-	-	-	-	-	-	-	-	-	-
Hypnea valentiae	+	+	+	+	+	+	+	+	+	+	+	+
Acanthophora spicifera	+	+	+	+	+	+	+	+	+	+	+	+

Table 4: Showing haemagglutinic activity against blood group AB^+ & AB^- in different species of seaweeds

Seaweeds	Blood Group AB^+						Blood Group AB^-					
	1:2	1:4	1:6	1:8	1:16	1:32	1:2	1:4	1:6	1:8	1:16	1:32
Saragassum species	-	-	-	-	-	-	-	-	-	-	-	-
Saragassum polycystum	+	+	+	+	+	+	-	-	-	-	-	-
Padina tetrastromatica	+	+	+	+	+	+	+	+	+	+	+	+
Gracilara corticata	+	+	+	+	+	+	-	-	-	-	-	-
Hypnea valentiae	+	+	+	+	+	+	+	+	+	+	+	+
Acanthophora spicifera	+	+	+	+	+	+	+	+	+	+	+	+

Table 5: Showing haemagglutinic activity against blood group O^+ & O^- in different species of seaweeds

	Blood Group O^+						Blood Group O^-					
	1:2	1:4	1:6	1:8	1:16	1:32	1:2	1:4	1:6	1:8	1:16	1:32
Saragassum species	+	+	+	+	+	+	-	-	-	-	-	-
Saragassum polycystum	+	+	+	+	+	+	-	-	-	-	-	-
Padina tetrastromatica	+	+	+	+	+	+	+	+	+	+	+	+
Gracilara corticata	+	+	+	+	+	+	-	-	-	-	-	-
Hypnea valentiae	+	+	+	+	+	+	+	+	+	+	+	+
Acanthophora specifera	+	+	+	+	+	+	+	+	+	+	+	+

DISCUSSION

The mean total antioxidant and free radical scavenging activities of *Hypnea valentiae* show varying degrees of free radical scavenging activity. DPPH radical scavenging activity was seen to increase as the concentration of the sample increased. At 500µl the % of DPPH scavenged was 39.79 and at sample concentration of 20µl the % of DPPH scavenged was 86.13 for standards. These findings are in agreement with the results obtained by Lu and Foo [15].

These results are also supported by the study of Dutty and Power [17]. who described that different samples in different solvents give different antioxidant potentials. Previous studies reported that ethanolic extracts of samples displayed high antioxidant potential. However, other ethanolic Chinese plant extracts showed little antioxidant potential [17]. Antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential.

In a study carried out by Mollar [18,19]. ethanolic and water extracts are the most widely employed solvents due to their more hygienic characteristics. Thus it can be assumed that each seaweed possess different antioxidant potential in different extraction mediums. The results obtained on total antioxidant activity of ethanolic extract was supported by the findings from DPPH radical scavenging activity.

This finding is in agreement with the previous results reported by Lu and Foo [15]. Where, flavonoids from apple showed the highest antioxidant activity using β-carotene bleaching method and DPPH scavenged assay. This phenomenon could be explained from the observation that the ethanol extract of *Hypnea valentiae* which contained high phenolic content but showed very low antioxidant activity.

Khalil [20]reported that all samples of papaya demonstrated purple bleaching reaction at increased concentration showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration. Some other researchers reported that the extracts exhibited a concentration dependant antiradical activity by quenching DPPH radical [21,22] .

Around 8 species of seaweeds collected from Kanyakumari District were tested for haemagglutinic activity, out of which only 3 exhibited positive activity (Table 3 to 6). However, according to Blunden et al [23]. only 19% species of the British seaweeds showed positive results for haemagglutination.

The brown and red seaweeds of 4 species respectively furnished a positive test for haemagglutinins. However, Fabregas *et al*., [24] observed a positive activity in 28 species of Spanish brown seaweeds. Phaeophyceae (brown seaweeds) *Acanthophora spicifera, Hypnea valentiae, Padina tetrastromatica, Gracilaria corticata, Sargassum sp., Sargassum polycystum* responded positively with the Haemoagglutination test.

Baqir *et al*., [25] studied the haemagglutination against erythrocytes of blood groups A, B, AB and O and reported a positive activity in 12 and 5 species of green brown and red seaweeds.In other cases our results agreed with those of Blunden *et al* [23] who reported that the tropical species such as *Acanthophora spicifera, Botrocladia leptopoda,*

Cendoceras clavutum 2 species of *Hypnea, Sarcinemo filiformis, Scinaia cornplanta* and *Solieria chordalis* also agglutinated the RBCs.

In the present study, a positive activity was found in *Hypnea valentiae, Padina tetrastromatica* and *Acanthophora spicifera*.This result is also supported by Alam and Usmanghani [26]. If a comparative account of haemagglutinin test for different species of Phaeophyta and Rhodophyta is chalked out then half of the species belonging to Rhodophyta exhibited the activity, while the seaweeds of Phaeophyta showed the activity in only one third of the investigated species.

Natural products have proved to be a great source of new biologically active compounds. Marine algae that are found in abundance along the shore of Kanyakumari were analysed for their potential to agglutinate human erythrocytes. Of the eight different species that were screened, only one extract did not agglutinate human blood cells.

Direct competition of seaweed products for their antioxidant composition alone provides a useful alternative for non-natural substances, while simultaneously providing worth while nutritional benefits. (Cornish and Garbary) [12] According to Marinova and Yanishlieva, [27] antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential. The difference in the DPPH radical scavenging activity of each sample in different extracts implies that the extracting solvent used would affect the radical scavenging potency. The water soluble natural antioxidants from another seaweed *S. thunbergii* exhibited the DPPH free radical scavenging activities, and the scavenging activity of the radicals increased with increasing concentrations of the extract.(Park *et al*., [28])The antioxidant activities of commercial enzyme extracts from *Sargassum* sp. exhibited more prominent effects in hydrogen peroxide scavenging, which was approximately 90% at 2 mg/ml. The *Ulva fasciata* samples have more effective antioxidant activity when compared to the *Chaetomorpha antennina*. And the percentage of scavenging was found to be about 83.95% *for U. fasciata* and 63.77% for *C. antennina* sample [29]. Thoudam *et al*. [30]studied the antioxidant activity of *Sargassum muticum* using various solvents such as benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether. Among the various extracts, methanolic extracts was found to have highest DPPH scavenging and total antioxidant activity.Studies on seaweeds such as *Fucus vesicuosus* and *Ascophyllum nodosum* found out that methanolic extract of *Fucus vesicuosus* and *Ascophyllum nodosum* scavenged DPPH radicals by 31.2% and 25.6% respectively. *Sargassum* sp.was found to have the highest free radical scavenging property [31].

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