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In Vitro Antioxidant Activity Of Red Seaweeds Of Methanolic Extracts From Rameshwaram Coast

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Abstract

Natural extracts of marine macroalgae are widely recognized for their antioxidant.The aim of this study was to evaluate the antioxidant activity of methanolic extracts of two marine algae, *Haloplegma duperreyi* and *Halymenia floresii.* The extracts were assayed, DPPH free radical scavenging capacity, Hydroxyl and ABT+ radical scavenging assay in both algae. Methanolic extracts of both algae were found to exhibit significant antioxidant activity. Among two seaweeds, *Haloplegma duperreyi* exhibited a good capacity for DPPH free radical scavenging capacity, Hydroxyl and ABT+ radical scavenging assay (54.20± 0.50, 56.20 ±0.70, and 39.59.27 ±0.30% inhibition at 500 µg mL-1 respectively) .

Keywords: *Antioxidant activity, Haloplegma duperreyi , Halymenia floresii , Marine algae and Natural extracts.*

INTRODUCTION

Many researchers have attracted on marine organisms, such as algae and lichen, as potential sources of biologically active compounds due to their unique chemical structures, complicated and difficult synthesis, and diverse range of molecules.(1)

Bioactive compounds in seaweed include pigments, lipids, proteins, polysaccharides, and phenolics, having biological activities including antioxidant, antibacterial, anticancer, anti-inflammatory, anti-diabetic, and anti-obesity effects.(2)

Free radicals have been claimed to play an important role in affecting human health by causing many diseases (e.g., heart diseases, cancer, hypertension, diabetes and atherosclerosis (3)An antioxidant's main property is the capacity to capture free radicals and reduce the risk of chronic illnesses like cancer, heart disease, arthritis, and central nervous system damage. (4)

In the past decade, anti-oxidants have shown their relevance in the prevention of various diseases, in which free radicals are implicated. Additionally, red macroalgae are known to contain several potential antioxidants, such as phenol, carotenoids, and tocopherols.(5,6)

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The present study evaluated the antioxidative potential of selected red seaweeds *Haloplegma duperreyi, Halymenia floresii* by measuring the antioxidant activity and correlate with total content of phenol and flavonoid compounds in methanolic extracts. The antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrasyl (DPPH),Hydroxyl scavenging and ABT+ radical scavenging activity.

MATERIALS & METHODS

MEDIA AND CHEMICALS

General laboratory chemicals, culture media, reagents and solvents for bioassay were purchased from Hi-Media, Merck and Sigma Aldrich Chemicals.

Seaweed Collection

The two fresh red seaweeds: *Haloplegma duperreyi (*Mont.) *and, Halymenia floresii* (Clemente) C.Agardh were collected from the from the intertidal region of Mandapam, Ramanathapuram District, Tamil Nadu, Southeast coast of India (9° 22′N, 78° 52′E). The collected materials were transferred to dark polythene bags to avoid photo degradation and transported to the laboratory. Also, the samples were further sent for authentication to the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India.

Fig. 1. ***Haloplegma duperreyi***

Fig .2. ***Halymenia floresii***

SEAWEED EXTRACTION

The seaweeds were primarily rinsed with seawater to get rid of dirt, sand particles, shells, and epiphytes. Then washed with running tap water followed by distilled water and allowed to dry under shade. The shade dried samples were powdered using a milling machine and subjected for extraction using different solvents like methanol, chloroform, ethyl acetate, hexane and aqueous in Soxhlet apparatus. First, the powdered samples were put into a 20g thimble using handmade filter paper. Then, the thimble was carefully placed inside the extractor chamber and poured with selected solvent at 1:10 ratio. The reservoir round bottom flask was heated to 60°C in a heating mantle. At least 15 refluxes were run on each sample and the resultant solvent extract was condensed using a rotary evaporator (Buchi, Bangalore, India) under vacuum at reduced temperature. The resultant precipitant was collected in a glass container for further analysis and stored at -20°C.

IN-VITRO ANTIOXIDANT ACTIVITY

DPPH RADICALS SCAVENGING ACTIVITY

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm. To determine the antioxidant potential of the selected seaweeds, various concentrations of the sample (4.0 ml) were mixed with 1.0 ml of solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture were shaken vigorously and left

to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control.(7) The percentage of inhibition in DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is absorbance of control reaction, A_1 is absorbance of test compound.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was estimated to determine the antioxidant potential of the seaweeds. A reaction mixture of 3.0 ml volume contained, 1.0 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and 1.0 ml of different concentrations (100 -500 µg/ml) of sample. After incubation for an hour at 37℃, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. Vitamin E was used as positive control.(9) The percentage scavenging effect was calculated as,

$$\text{Hydroxyl radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

$ABTS^+$ radical scavenging activity

$ABTS^+$ decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium per sulphate. The reaction was initiated by the addition of 1.0 ml of diluted $ABTS^+$ reagent to 10 µl of different concentrations of sample (100 -500 µg/ml) and also to 10 µl of methanol as a control. (8) Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated as

$$\text{ABTS radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

RESULTS

In-vitro antioxidant studies

Accordingly, in the present study, based on the phytochemical studies, two algae with high phytoconstituents namely, ***Haloplegma duperreyi*** (Sample A) and ***Halymenia floresii*** (Sample B) were subjected to three different antioxidant assays using the seaweeds' **methanol extract** and the results revealed the antioxidant potential of the seaweeds as follows.

DPPH RADICALS SCAVENGING ACTIVITY

The antioxidant capacity of sulfated polysaccharides from seaweeds is well-studied 9(15) . They are known to posses various antioxidant activities such as scavenging of free radicals ABT+ radical ,hydroxyl and DPPH, reducing antioxidant power 10(16). The antioxidative capacity of all the above two seaweed thallus extracts were determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The extracts from the five different solvents were evaluated at varying concentrations ranging from 100 to 500 µg/ml (Table 1, Fig.3). *H. duperreyi* maximal capacity to scavenge free radicals was calculated as 54.20±0.50% at 500 µg/mL concentration. It was noted that the scavenging activity increased as the concentration increased from 100 to 500 µg/mL and the IC_{50} value was found to be 465.49%. Similarly, the DPPH activity *of H. floresii* was found to be maximum at 500 µg/ml concentration with 52.63±0.30% and the IC_{50} value was calculated to be 479.41 µg/ml. Both the samples were compared with the IC_{50} (340.60µg/ml) value of standard ascorbic acid. The results highlights the antioxidant potential of the selected seaweeds.

Table 1. DPPH scavenging assay

	100(µg/ml)	200(µg/ml)	300 (µg/ml)	400(µg/ml)	500 (µg/ml)

Sample A	11.50± 0.10	23.45 ± 0.55	35.40 ± 0.20	43.10 ± 0.45	54.20 ± 0.50
Sample B	9.45± 0.50	21.63 ± 0.50	32.72 ± 0.40	40.93 ± 0.20	52.63 ± 0.30
Standard (Vitamin C)	15.75 ± 0.10	32.50 ± 0.50	45.40± 0.50	57.20 ± 0.30	72.20 ± 0.50

The experiment was conducted in triplicates (n=3)

Sample A: ***Haloplegma duperreyi***

Sample B*:* ***Halymenia floresii***

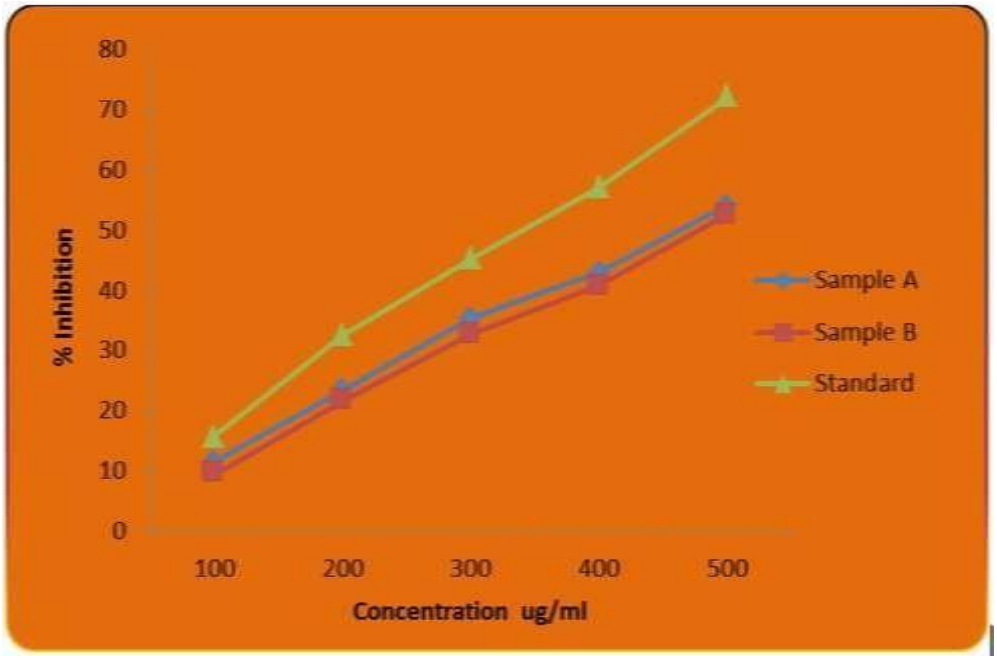

Fig. 3 . DPPH scavenging assay of *: H. duperreyi* and *H. floresii*

\IC$_{50}$ value of Sample A : 465.49 µg/ml
IC$_{50}$ value of Sample B : 479.41 µg/ml
IC$_{50}$ value of Vitamin C (standard) : 340.60µg/ml

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of H. duperreyi and *H. floresii* were evaluated. Among the two seaweeds, ***H. floresii*** showed the highest radical scavenging activity of **59.50 ± 0.10%** at 500 µg/mL concentration, while the ***H. duperreyi*** extract showed maximum hydroxyl radical scavenging activity of **56.20 ± 0.70%** at the highest concentration of **500 µg/mL** (Table 2 , Fig 4). The results revealed a concentration dependent significant scavenging activity. The IC$_{50}$ values of the both the extracts were calculated and compared with the standard Vitamin E.

The hydroxyl radical scavenging effect of the samples is determined by using Fenton reaction. it was reported that sulfated polysaccharides exhibit moderate or no defense against hydroxyl radical assay. (11)

Table. 2 Hydroxyl Radical Scavenging Assay

Test Sample	% inhibition				
	100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
Sample A	13.50± 0.70	27.69 ± 0.50	35.40 ± 0.50	46.39 ± 0.30	56.20 ± 0.70
Sample B	10.45± 0.30	24.70 ± 0.50	37.60 ± 0.80	48.52 ± 0.50	59.50 ± 0.10
Standard (Vitamin E)	17.58 ± 0.50	39.28 ± 0.30	48.38± 0.70	61.45 ± 0.60	77.30± 0.40

The experiment was conducted in triplicates (n=3)
Sample A: ***Haloplegma duperreyi***
Sample B: ***Halymenia floresii***

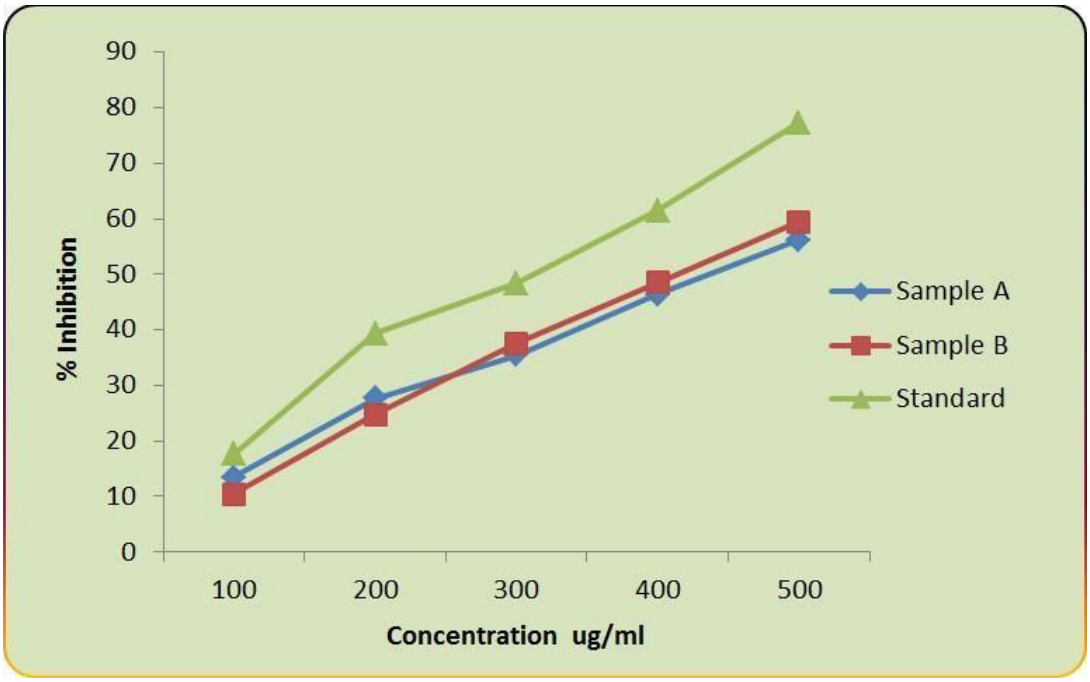

Fig. 4 Hydroxyl Radical Scavenging Assay of : ***H. duperreyi*** **and** ***H. floresii***

IC_{50} value of Sample A : 434.26 µg/ml
IC_{50} value of Sample B : 413.75 µg/ml
IC_{50} value of Vitamin E (standard) : 316.18 µg/ml

$ABTS^{+}$ radical scavenging assay

In this assay, the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), which has a dark blue colour, was reduced by the antioxidant components present in the algal extracts into colourless ABTS, and was measured spectrophotometrically. In ***H. duperreyi***, the scavenging activity was found to maximum at the highest concentration (59.27 ± 0.30%) seconded by ***H. floresii*** (55.29 ± 0.50). The inhibition rate showed a proportional increase with increase in concentration from 100 -500 µg/ml (Table 4.20,Fig. 4. 19). The IC_{50} value of *H. duperreyi* was calculated as **416.47 µg/ml** and for *H. floresii* the IC_{50} value was **428.75 µg/ml**. These values were compared with the standard Vitamin C.

Table 3 ABTS^{+} radical scavenging assay

Test Sample	% inhibition				
	100 (µg/ml)	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**500 (µg/ml)**
Sample A	12.62± 0.55	27.60 ± 0.50	38.38 ± 0.50	48.26 ± 0.50	59.27 ± 0.30
Sample B	11.48± 0.50	25.27 ± 0.30	35.49 ± 0.30	47.28 ± 0.30	55.29 ± 0.50
Standard (Vitamin C)	15.38 ± 0.45	38.62 ± 0.30	45.35± 0.50	57.27 ± 0.50	69.30± 0.50

The experiment was conducted in triplicates (n=3)
Sample A: ***Haloplegma duperreyi***
Sample B*:* ***Halymenia floresii***

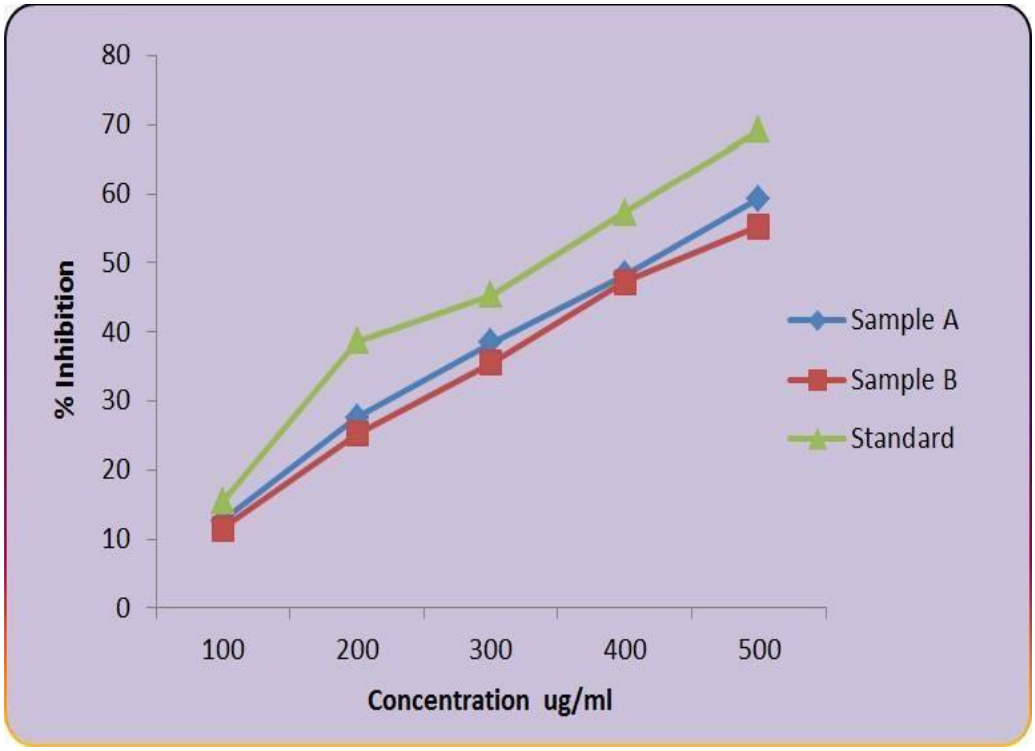

Fig 5 ABTS^{+} radical scavenging assay of *H. duperreyi* and *H. floresii*
IC_{50} value of Sample A : 416.47 µg/ml
IC_{50} value of Sample B : 428.75 µg/ml
IC_{50} value of Vitamin C (standard) : 345.61 µg/ml

DISCUSSION

Many studies have proved that seaweeds are a potential source of natural antioxidants. 12(10) demonstrated that phenolic compounds, which include flavonoids, phenol, and tannin, have been found in significant amounts in the *Portieria* species. It has been suggested that the presence of

phytoconstituents like flavonoids, tannins, and polyphenols prevents a variety of diseases through their free radical scavenging activity. The benefits of ascorbic acid are perhaps well recognised to be increased by flavonoids. The antioxidant properties of steroids, terpenoids and saponins have been the subject of numerous reports. In fact, that seaweeds contain numerous secondary metabolites is very strong evidence of their medicinal potential, among which phenolic compounds have effective therapeutic values (11).13 Furthermore, among the many phytochemicals recovered from red seaweeds, phenolic compounds, particularly flavonoids, phenolic acids, sulphated polysaccharides, and carrageenans, have been identified as the significant contributors to the antioxidant activity of these red algae species *Porphyra tenera* (12,13), *Gelidiella acerosa* showed presence of enhanced antioxidant and phenolic activity. (14)

CONCLUSION

Many biological species found in the maritime environment can produce an enormous variety of compounds with therapeutic properties.In these research, we concluded that the methanol extract of *Haloplegma duperreyi* possesses marine herbal potential because the indication of phenolic component that play pivotal role in marine macro algae *Halymenia floresii* that compared with methanol extracts and it also showed the capacity of antioxidant in methanol extract is suspected the potent antioxidant in macro algae . Thus, it could be benefited as a source of marine natural medicine and cosmeceuticals.

Of course, further research is needed to investigate in depth the most potent macroalgae extracts' molecular mechanisms and bioactive compounds accounting for the antioxidant and anticancer activities in human cells and in vivo experiments.

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