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Evaluation of biochemical composition and *in vitro* antioxidant properties of selected seaweeds from Kanyakumari coast, Tamil Nadu, India

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ABSTRACT

To analyze the antioxidant profile and biochemical components of six Indian seaweeds (Ulva fasciata, Chaetomorpha antennina, Spiridia hypnoides, Amphyroa anceps, Sargassum wightii, Chnoospora maxima) from Kanyakumari coast. Among these six seaweeds the carbohydrate content was high in Chnoospora maxima (55.86±0.75%) and least in Amphyroa anceps (25.76±0.88%) and protein was high in Sargassum wightii (16.34±0.04%) and low in Amphyroa anceps (7.86±0.01%). The lipid content was recorded high in Chnoospora maxima (0.54±.005%) and less in Ulva fasciata (0.21±.003%). In the methanol extract of seaweeds, the phenolic content was recorded high in Chnoospora maxima (19.351±0.323 mg GAE/g) and less in Amphyroa anceps (4.456±0.212 mg GAE /g) and the maximum antioxidant activity was observed in Chnoospora maxima (12.756±0.187mg AAE/g) and least in Amphyroa anceps (3.985±0.121 mg AAE /g). In FRAP, maximum inhibition was observed in Chnoospora maxima (20.045±0.321%) and less in Amphyroa anceps (6.097±0.312%). The highest inhibition of hydrogen peroxide radical activity was shown in Sargassum wightii (21.652±2.172%) and low in Amphyroa anceps (6.231±0.356%).The DPPH scavenging capacity was highly recorded in Sargassum wightii (11.512±0.076%) least in Chaetomorpha antennina (1.562±0.054%). The Deoxyribose scavenging activity was maximum present in Sargassum wightii (9.541±0.032%) and less in Chaetomorpha antennina (1.332±0.07%).

Key words: Antioxidant activity, Biochemical compounds, DPPH, Deoxyribose, and Gallic acid, Phenolic compounds.

INTRODUCTION

In botanical terminology, macrophytic marine algae or seaweeds have holdfasts, stipes and blades (or fronds) instead of roots, stems and leaves. Their holdfasts function simply as anchors, and do not extract nutrients as do the roots of higher plants hence, seaweeds absorb and concentrate nutrients directly from seawater (Hillison, 1977). Seaweeds are examined as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a wide range of biological activities. The red and the green species are rich in carbohydrates where as the brown seaweeds are rich in soluble fiber and iodine. They are an excellent source of vitamins such as A, B_1, B_{12}, C, D and E, riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na, K (Dhargalkar and Pereira, 2005). Serious human health disorders such as atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders, and some types of cancer, as well as aging are caused by the uncontrolled production of free radicals such as superoxide anion (O_2), hydroxyl radical (HO^+) and hydrogen peroxide (H_2O_2) (Ruberto *et al.,* 2001). These free radicals are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. They may attack cellular macromolecules such as membrane lipids, proteins, and DNA (Valko, *et al.,* 2005). To defend cellular bio molecules in biological systems, balance

between oxidant formation and endogenous antioxidant defense mechanisms exist. If this balance is disturbed, it can produce oxidative stress. Antioxidants have also been of interest to health professionals because they help the body to protect itself against damage caused by reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) and reactive chlorine species (RCS) associated with degenerative diseases. Antioxidant activities have been attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity, radical scavenging, etc. (Frankel and Meyer 2000; Huang *et al.,* 2005).

Although seaweeds are exposed to the adverse environmental conditions such as light and high oxygen concentrations that lead to the formation of free radicals, and other strong oxidizing agents, they do not have any serious photodynamic damage in vivo. Thus, it can be said that seaweeds are able to generate the necessary compounds to protect themselves from external factors such as pollution, stress and UV radiation. This fact suggests that marine algae, like photosynthesizing plants, have antioxidative mechanisms and compounds which act as antioxidant agents. Hence, the present study was intended to analyze the antioxidant activity and biochemical components of six seaweeds (*Ulva fasciata, Chaetomorpha antennina, Spiridia hypnoides, Amphyroa anceps, Sargassum wightii, Chnoospora maxima*) from Kanyakumari coast.

MATERIALS AND METHODS

2.1 Preparation of sample for use

The seaweeds were collected in June 2013 from the intertidal region of the Kanyakumari coast, Tamilnadu, India and it kept in the polythene bags to prevent evaporation and transported to the lab and washed thoroughly with seawater and then tap water to remove extraneous materials. The samples were shade dried for 2 to 3 days then it was oven dried, powdered and stored in air tight container for future use.

2.2 Extraction

The powdered samples were taken for extraction. 10 g of sample was soaked with 200 ml of methanol for 24 h at room temperature under dark condition. The extraction was repeated thrice, pooled and filtered through Whatman No. 1 filter paper. Each filtrate was concentrated to dryness using rotary evaporator. The dry filtrate was lyophilized and stored in cool dark place for further analysis.

1.3 Biochemical analysis

2.3.1 Estimation of protein

The protein was estimated using Biurette method (Raymont *et al.,* 1964). To 5 mg of sample, 1ml of distilled water, 4 ml of biurette reagent were added and incubated for 30 min. in the room temperature. After that mixture was centrifuged for 10 min at 4000 rpm. The optical density of the supernatant solution was measured in a spectrophotometer at 540 nm. The protein was calculated by using BSA Serum Albumin) as standard and expressed as mg/g protein.

2.3.1 Estimation of Carbohydrate

The Carbohydrate content was estimated by Anthrone method (Roe, 1955). Seaweed sample was soaked in 80% ethanol and was centrifuged at 4000 rpm. 5ml of anthrone reagent was added to the 0.5 ml of supernatant. The tubes were kept in a boiling water bath for 15 minutes and kept in a dark room for 10 minutes. The developed colour intensity was read in a spectrophotometer at 650 nm. Carbohydrate content was calculated by referring to a standard D-Glucose and the results are expressed as mg/g sugar.

2.3.3 Estimation of Lipid

The lipid was estimated by using chloroform-methanol mixture as described by (Folch *et al.,* 1957). To 400 mg of seaweed sample, 5 ml of chloroform-methanol (2:1) mixture was added. The mixture was incubated at room temperature for 24 hrs. After incubation, the mixture was filtered using a filter paper. 10 ml of filtrate was collected, which was kept on a hot plate. The chloroform methanol mixture was evaporated. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample

2.4 Determination of antioxidant activity

2.4.1 Evaluation of Total phenolic contents

To estimate the total Phenolic contents of methanol extracts were followed by the method of (Taga *et al.,* 1984). To 100 µl of sample was mixed with 2.0 ml of 2% Na_2CO_3, after 2 min. incubation, 100 µl of 50% Folin Ciocalteau's

phenol reagent was added. The reaction mixture was mixed thoroughly and allowed to stand for 30 minutes at room temperature in the dark. The absorbance of all the sample solution was measured at 720 nm using spectrophotometer (Shimadzu, UV-160, Japan). Phenolic content are expressed as Gallic acid equivalent per gram.

2.4.2 Determination of Total Antioxidant activity
Total antioxidant activity was measured following standard method (Prieto *et al.,* 1996) 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM) and 1.2359 g of ammonium molybdate (4mM solution) were mixed together in 250 ml with distilled water and labeled as a total antioxidant capacity (TAC) reagent. 300 µl of extract was dissolved in 3ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

2.4.3 Ferric reducing antioxidant power (FRAP) assay
To determine the reducing power of seaweed extracts was by the standard method (Oyaizu, 1986). 1.0 ml of different extract containing different concentration of samples was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). This mixture was kept in a water bath at 50ºC for 20 minutes. After incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 minutes. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance indicates increased reducing power.

2.4.4 Hydrogen Peroxide Radical Scavenging Assay
To determine the scavenging ability of seaweed extracts on hydrogen peroxide by the standard method (Glucin *et al.,* 2004) Hydrogen peroxide (10mM) solution was prepared in the phosphate buffer saline (0.1 M, pH 7.4). 1ml (0.25 mg) of the extract was mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer (Shimazdu, UV - 160) against a blank (without hydrogen peroxide) after 10 minutes of incubation at 37ºC. The percentage scavenging of hydrogen peroxide was calculated using the following formula

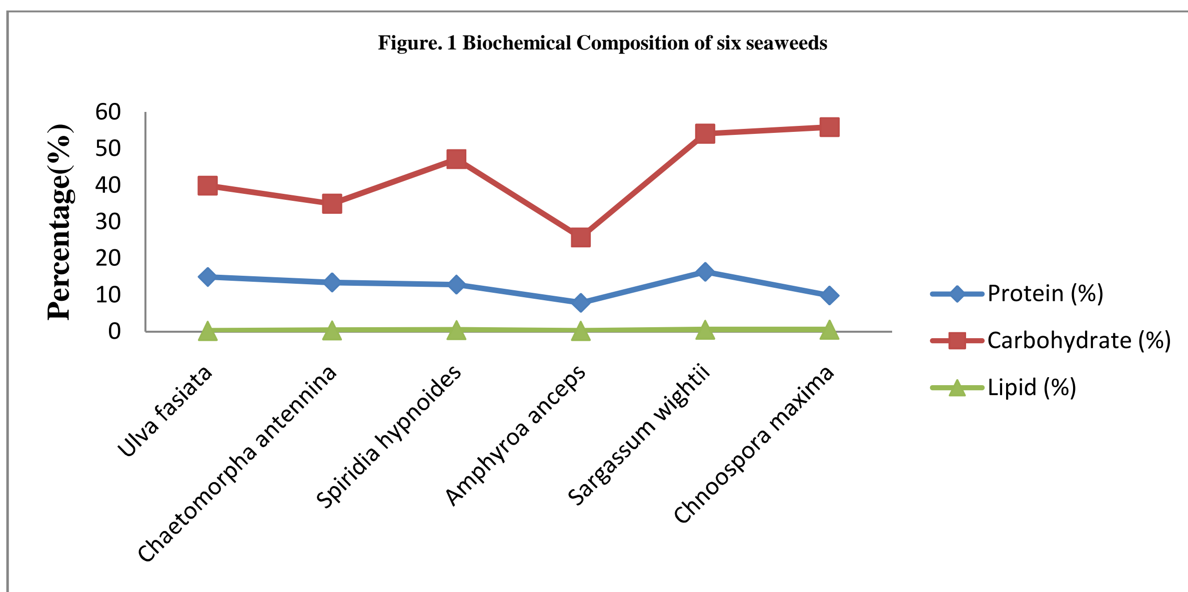
$$\% \text{ Scavenging } (H_2O_2) = \left(\frac{A0\text{-}A1}{A0} \right) X\ 100$$

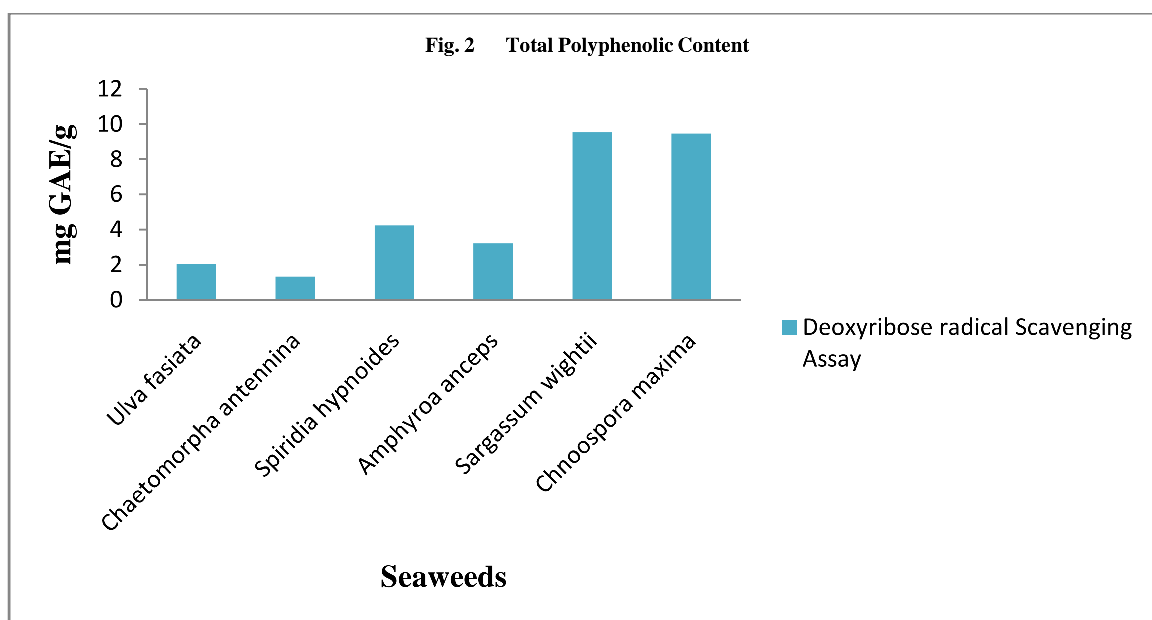
2.4.5 Deoxyribose radical scavenging activity
To determine the scavenging ability of solvent extracts against deoxyribose radical was determined by the method (Chung *et al.*, 1997). 2.0 ml of sample were added to the mixture of 2.0 ml of $FeSO_4$ $7H_2O$ (10mm), 0.2 ml EDTA (10mM) and 2.0 ml deoxyribose (10mM). The volume was made upto 1.8 ml with phosphate buffer (0.1 M, pH 7.4) and to that 0.2 ml H_2O_2 (10mM) was added. The mixture was incubated at 37ºC under dark for 4 h. After incubation, 1ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then left to stand under boiling water bath for 10 minutes. The colour developed was measured at 532 nm. Scavenging activity (%) was calculated using the equation given by Heo *et al.,* (2005).

2.4.5 DPPH radical scavenging activity
The scavenging effects of samples for DPPH radical were monitored according to the method of (Yen and Chen, 1995). Briefly, 2.0 ml of aliquot of test sample was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 minute and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the formula given by Duan *et al.,* (2006). Synthetic antioxidants, Gallic acid and Ascorbic acid were used as positive controls.

RESULTS

3.1 Biochemical Composition
3. 1. 1 Protein content
In the present work, the protein content ranged between 7.86±.01 and 16.34±.04%. The maximum protein value was observed in seaweed *Sargassum wightii* (16.34±.04%) followed by *Ulva fasciata* (14.98±.09%), *Chaetomorpha antennina* (13.45±.04%), *Spiridia hypnoides* (12.87±.08%), *Chnoospora maxima* (9.87±.08%) and *Amphyroa anceps* (7.86±.01%).



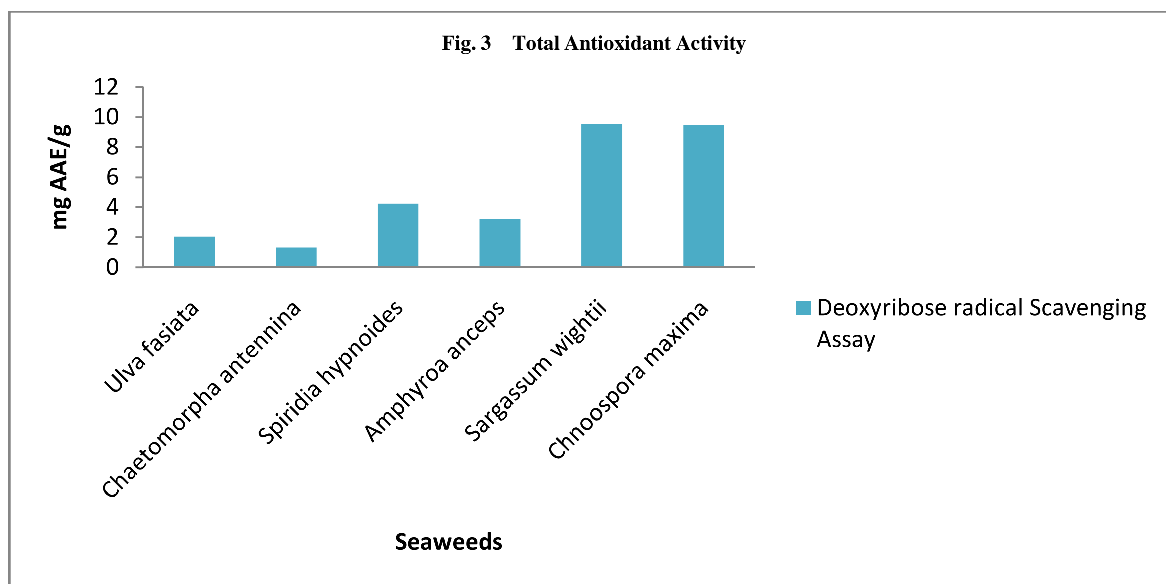


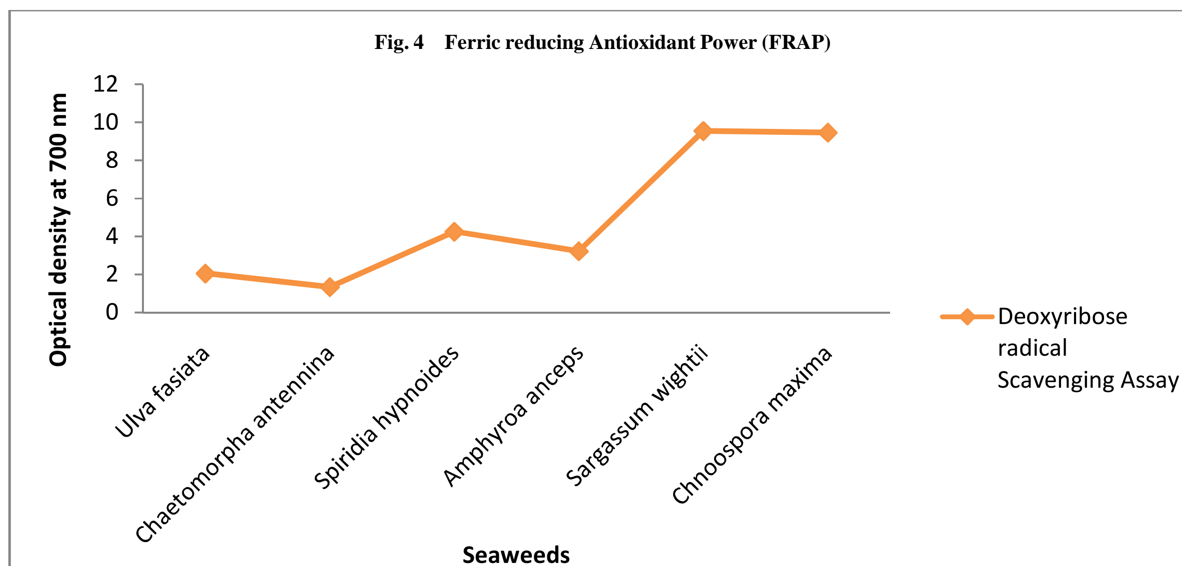
3. 1. 2 Carbohydrate content

The maximum carbohydrate content was recorded in *Chnoospora maxima* (55.86±0.75%), followed by *Sargassum wightii* (54.09±0.66%), *Spiridia hypnoides* (47.09±1.2%), *Ulva fasciata* (39.86±0.22%), *Chaetomorpha antennina* (34.96±0.85%) whereas the minimum value was observed in *Amphyroa anceps* (25.76±0.88%).

3.1.3 Lipid content

The higher amount of lipid was present in *Chnoospora maxima* (0.54±.005%), followed by *Sargassum wightii* (0.51±.002%), *Spiridia hypnoides* (0.42±.004%), *Chaetomorpha antennina* (0.34±.006%), *Ulva fasciata* (0.21±.003%), and the low value was recorded in *Amphyroa anceps* (0.21±.001%).





3.2 Antioxidant Activity

3. 2. 1 Total phenol content

In the methanol extracts of six seaweeds, the total phenolic content was recorded high in *Chnoospora maxima* (19.351±0.323 mg GAE/g), followed by *Sargassum wightii* (16.482±0.412 mg GAE /g), *Spiridia hypnoides* (8.921±0.102 mg GAE /g), *Chaetomorpha antennina* (6.342±0.154 mg GAE /g), *Ulva fasciata* (5.987±0.321 mg GAE /g), and low value was recorded in *Amphyroa anceps* (4.456±0.212 mg GAE /g) shown in Figure 2.

3. 2. 2 Total antioxidant activity

In the six seaweeds were the maximum value of antioxidant activity was shown in *Chnoospora maxima* (12.756±0.187mg AAE/g), followed by *Sargassum wightii* (11.897±0.176 mg AAE /g), *Ulva fasciata* (9.562±0.324 mg AAE /g), *Chaetomorpha antennina* (8.987±0.183 mg AAE /g), *Spiridia hypnoides* (7.347±0.154 mg AAE /g) and less value was observed *Amphyroa anceps* (3.985±0.121 mg AAE /g) denoted in Figure 3.

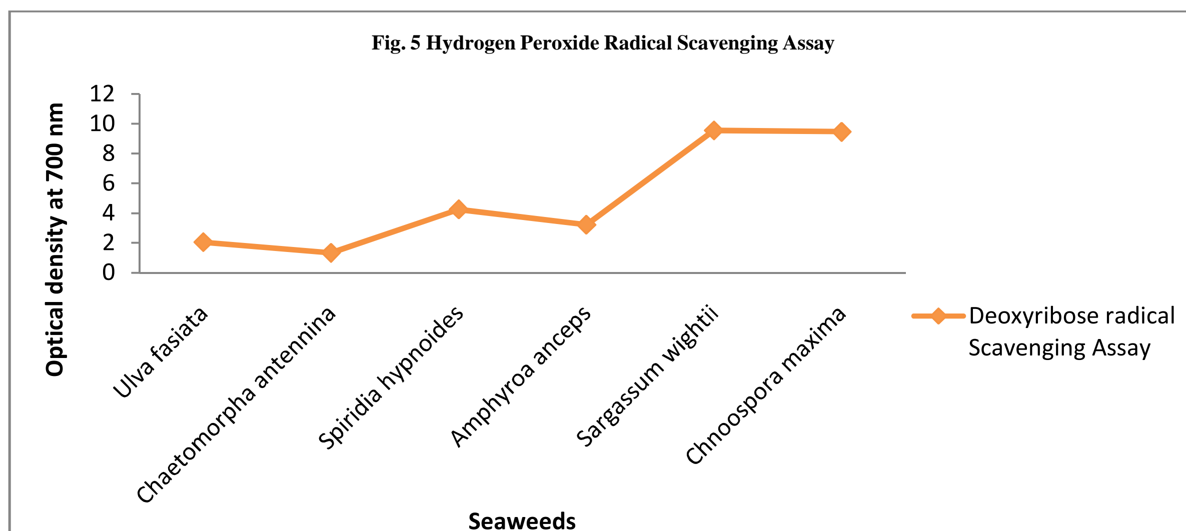


Fig. 5 Hydrogen Peroxide Radical Scavenging Assay

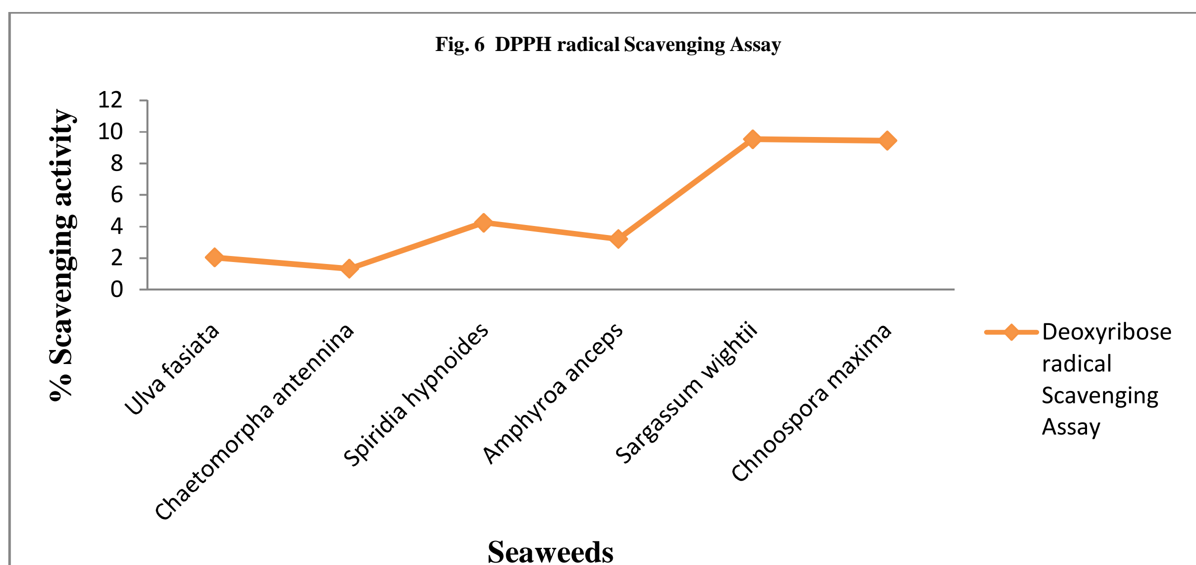


Fig. 6 DPPH radical Scavenging Assay

3.2.3 Ferric reducing antioxidant power assay

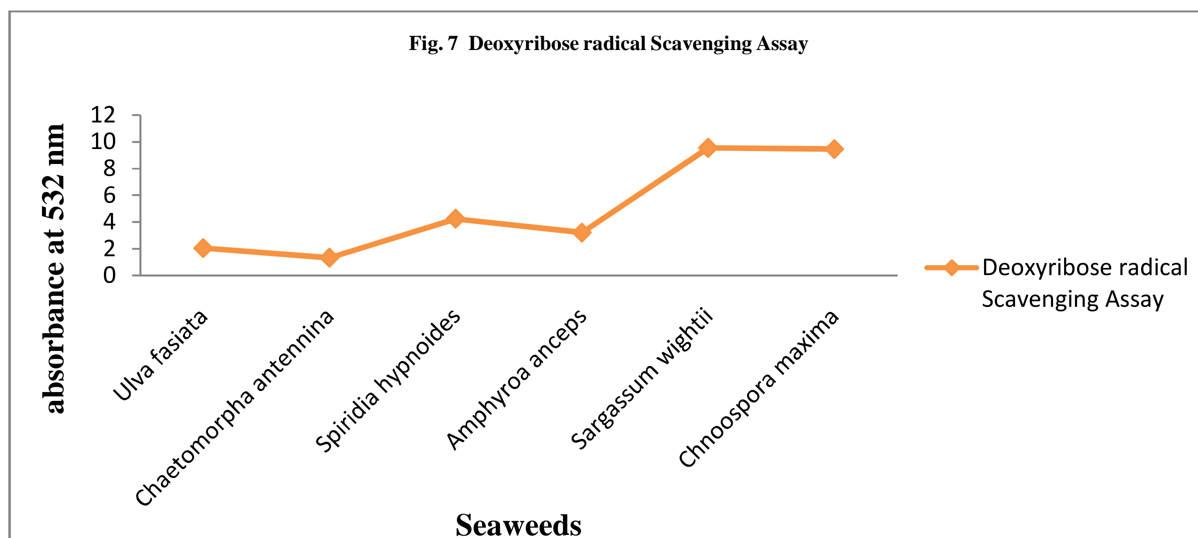
In the methanol extract of these sample were shown their scavenging ability against ferric radical was recorded maximum in *Chnoospora maxima* (20.045±0.321%), *Sargassum wightii* (18.034±0.012%), *Chaetomorpha antennina* (12.034±0.211%), *Spiridia hypnoides* (11.923±0.251%), *Ulva fasciata* (10.342±0.102%), and *Amphyroa anceps* (6.097±0.312%).

3.2.4 Hydrogen Peroxide Radical Scavenging Assay

The maximum value of hydrogen peroxide radical scavenging activity was shown in *Sargassum wightii* (21.652±2.172%), followed by *Chnoospora maxima* (19.491±0.987%), *Spiridia hypnoides* (18.419±2.123%), *Ulva fasciata* (11.512±1.231%), *Chaetomorpha antennina* (9.321±0.936%), and less value recorded in *Amphyroa anceps* (6.231±0.356%).

3.2.5 DPPH Radical Scavenging Assay

The DPPH scavenging capacity of these seaweeds was recorded high in *Sargassum wightii* (11.512±0.076%) followed by *Chnoospora maxima* (10.453±0.015%), *Spiridia hypnoides* (6.435±0.092%), *Ulva fasciata* (2.098±0.023%), *Amphyroa anceps* (1.563±0.035%) whereas less value was observed in *Chaetomorpha antennina* (1.562±0.054%).



3. 2. 6 Deoxyribose Radical Scavenging Assay

The maximum value of deoxyribose scavenging activity was recorded in *Sargassum wightii* (9.541±0.032%) followed by *Chnoospora maxima* (9.451±0.986%), *Spiridia hypnoides* (4.245±0.034%), *Amphyroa anceps* (3.213±0.012%), *Ulva fasciata* (2.045±0.098%) whereas the less value was observed in *Chaetomorpha antennina* (1.332±0.07%).

DISCUSSION

Algae are very simple chlorophyll containing organisms, their holdfasts function simply as anchors, and do not extract nutrients as do the roots of higher plants hence, seaweeds absorb concentrate nutrients directly from seawater and red algae produce large amount of polysaccharides around their cells (Bold and Wynne, 1985). Hence, this study was carried to analyze biochemical components and its range present in the selected seaweeds. Due to their low content in lipids (ranged between 0.21±.003 to 0.54±.005 %), high concentration in polysaccharides (55.86±0.75 to 25.76±0.88 %), natural richness in proteins (7.86±.01 to 14.98±.09 %) as well as their content in bioactive molecules, marine algae are known to be a good source of healthy food. Antioxidant activities have been attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity, radical scavenging, etc. (Frankel and Meyer 2000; Huang *et al.,* 2005). In this work, the result shows the brown algae *Sargassum wightii* and *Chnoospora maxima* could be the dominant source of antioxidant activity (11.897±0.176 to 12.756±0.187 mg/g), this suggest the phenolic compound which are enormously high in brown species. The brown algae possess the capability of scavenging free radicals and stabilizing lipid peroxidation due to their hydroxyl groups present in polyphenols like fucoxanthin, carotenoids etc (Yen *et al.,* 1993). However, the absence of oxidative damage in the structural components of macroalgae (i.e., polyunsaturated fatty acids) and their stability to oxidation during storage suggest that their cells have protective antioxidative defense systems (Fujimoto 1990 and Matsukawa *et al.,* 1997).

This fact suggests that marine algae have antioxidative mechanisms and compounds which act as antioxidant agents. As seaweeds and seaweed isolates have the potential to benefit both health and improve food satisfactoriness, reduce the postprandial absorption rates of glucose and lipids in acute human feeding studies, highlighting their potential use in the development of drugs.

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