ISSN: 2642-9020

Research Article

Journal of Marine Science Research and Oceanography

Screening and Partial Characterization of Natural Antioxidants from Seaweeds Collected From, Rameshwaram Southeast Coast of India

Suparna Roy

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Annamalai Nagar, Tamilnadu, India

***Corresponding author**
Suparna Roy, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Annamalai Nagar, Tamilnadu, India, Email: roysuparnacasau@gmail.com

Submitted: 02 Dec 2019; **Accepted**: 10 Dec 2019; **Published**: 09 Jan 2020

Abstract

The aim of this work is to estimate in-vitro antioxidant activities such as total antioxidant capacity, free radicals (DPPH) scavenging activity, hydrogen peroxides (H_2O_2) scavenging activity, total reducing power scavenging activity and total phenols, of various extracts of seaweeds such as aqueous, acetone, ethanol, methanol, and pigments content by spectrophotometric method, of 33 seaweeds species among which 11 Chlorophyta, 11 Phaeophyceae and 11 Rhodophyta, collected during 2016 from two stations viz. Olaikuda and Vadakkadu, at Rameshwaram, southeast coast of India. Among four different extracts aqueous extracts from all seaweeds had minimum activity than acetone, methanol and ethanol. The Rhodophyta and Phaeophyceae had high antioxidant activity in comparing to Chlorophyta. The highest total antioxidant activity was found in acetone extract from Turbinaria decurrens (98.97±0.00%), followed by its methanol extract (98.81±0.60%) and ethanol extract (98.58±0.53%). The highest reducing power and H_2O_2 scavenging activity were found in acetone extract of Caulerpa racemosa (383.25±1.04%), and methanol extract from Caulerpa racemosa var. macrophysa (24.91±0.49%). The methanol extract from Caulerpa scalpelliformis contained the highest total phenol (85.23±0.12%). The Chloro-a and Chloro-b contents were the highest in Gracilaria foliifera (13.69±0.38% mg/gm dry wt.) and Caulerpa racemosa var. macrophysa (9.12 ±0.12% mg/gm dry wt.) likewise carotenoid was also the highest in Gracilaria foliifera (0.054±0.0003% mg/gm dry wt.) and Caulerpa racemosa var. macrophysa (0.04 ±0.002% mg/gm dry wt.). The partial characterization of the extract which contents the highest activity was done by UV-Visible Spectrophotometer, FTIR, and NMR. The functional groups and all the possible compounds present in the extract were partially characterized. It can be concluded from this study, that some seaweed extract can be used for natural antioxidant production, after further characterization to negotiate the side effect of synthetic, market available antioxidants.

Keywords: Seaweeds, Antioxidant, Total Phenol, Pigment, Olaikuda, Vadakkadu, Rameshwaram.

Introduction

Lipid peroxidation and oxidative stress is the major cause in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis, cancer, etc. Oxidative stresses are initiated by free radicals, especially reactive oxygen species (ROS) species such as superoxide anions, hydroxyl radicals and hydrogen peroxide. The most living species have efficient defence systems to prevent themselves against from the oxidative stress induced by the ROS [1]. In the past decade, antioxidants have shown their role in the prevention of various diseases, in which free radicals are, implicated [2]. Antioxidant is an inhibitor of lipid peroxidation. So, the intake of various antioxidants reduced the risk of such kind of chronic diseases. There are some toxicity and safety concern of the commercially available synthetic antioxidants (Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), and Butylated hydroxyquinone (TBHQ), so natural antioxidants have drawn more attention [3, 4]. The phenolic compounds of plants are reported to have high antioxidant activity and ability to scavenge reactive oxygen species and free radicals. The phenolic compounds of berries have a potential effect in an exhibition of antioxidant activity on human low-density lipoprotein (LDL) and liposome oxidation [5]. Some seaweed such as *Codium fragile*, *Ulva lactuca* and *Eisenia arborea* have been reported to have high antioxidant activities with DPPH free radical and ABTS radical scavenging activity, ferrous reducing power and total antioxidant activity [6]. Some brown seaweed from Visakhapatnam coast such as *Padina tetrastromatica*, *Sargassum ilicifolium* and *Sargassum vulgare* have been evaluated for their antioxidant activities which showed that *Padina tetrastromatica* exhibited higher levels of radical scavenging activity [7]. In our study, we estimated the total phenol and antioxidant activity of seaweeds and evaluated their interrelations, to find out the most

potent seaweed species for future natural antioxidants.

Materials and Methods

Seaweed Collection and Processing

Total 33 species of seaweed were collected during 2016 from two stations viz. Olaikuda and Vadakkadu, at Rameshwaram, southeast coast of India. All the collected species are identified with the help of CMFRI taxonomy key and taxonomy manuals. The algal samples were washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. The water was drained off and the algal material was spread on news paper to remove excess water. They were a shade dried. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored and used for further analysis. The sampling location map [Fig1].

Figure 1: Map showing study area of Olaikuda and Vadakkadu coast, Rameshwaram, southeast coast of India

Antioxidant Capacity Assays

Seaweeds Sample Preparation

This procedure was little modified on the protocol mentioned in Mensor et al. 2001 [8]. The seaweed powder of 500 mg was mixed with 10 ml of methanol, ethanol, acetone and water and kept in 24 hours in the dark at room temperature and centrifuged at 5000 rpm for 15 minutes and supernatants were collected in separate vial and kept in - 4℃ for further use.

Phosphomolybdate Assay (Total Antioxidant Capacity)

Total antioxidant capacities of crude methanol, ethanol acetone and water extracts were assayed by Phosphomolybdate method using ascorbic acid as a standard [9]. Briefly, the 7.45 ml of H2So4 (0.6mM solution), sodium sulphate of 0.9942 g (28mM) in addition to 1.2359 gm ammonium molybdate (4mM) mixed in 250 ml distilled water to prepare the TAC reagent. The 300 µl of seaweed extracts were mixed with 3 ml of TAC reagent. The reaction mixtures were incubated at 95°C for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695nm against a blank on an UV-visible spectrophotometer. Total antioxidant capacity was expressed as the number of equivalents of ascorbic acid in milligrams per gram of extract. A typical blank contained 1 ml of the reagent solution along with an appropriate volume of the solvent and incubated under similar conditions. The antioxidant capacity of the plant extract solution was estimated using following formula:

Total antioxidant capacity, TAC (%) =
[(control absorbance – sample absorbance)/ (control absorbance)] x 100

DPPH radical scavenging activity assay

The scavenging activity of methanol, ethanol, acetone and water extracts of seaweeds was determined according to standard protocol (Surana et al. 2016). Briefly, 2.0 ml of 0.16 mM DPPH solution in methanol was added to the test tube containing 2.0 ml aliquots of sample. The mixture was vortex for 1 minute and kept at room temperature for 30 minutes in the dark. The absorbance of all the sample solutions was measured at 517 nm. Sample blank and control samples were performed according to the method. Scavenging activity of DPPH free radical was calculated using the following equation

$$\text{Percentage inhibition} = \frac{(\text{Acontrol- Atest})}{\text{Acontrol}} \times 100$$

Where Asample is the Absorbance of DPPH solution & tested sample, Asample blank is the absorbance of the sample only without DPPH solution. Synthetic antioxidant Ascorbic acid was used as positive controls.

Hydrogen Peroxide (H_2O_2) Scavenging activity assay

Briefly 40mM H_2O_2 concentration was prepared in phosphate buffer (PH 7.4) and the H_2O_2 concentration was determined spectrophotometrically by measuring the absorbance with extinction co-efficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Extracts (100 µg/ml) in distilled water and ascorbic acid (20-100 µg/ml) as positive control were added to 0.6 ml of 40 mM H_2O_2 solution and the absorbance of H_2O_2 was determined at 230nm at after 10 minute incubation against blank solution containing phosphate buffer without H_2O_2. The % of scavenging activity of H_2O_2 was calculated above.

$$\%\text{ of scavenging activity of } H_2O_2 = \frac{\text{ACont.} - \text{Atest}}{\text{ACont.}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Total reducing capacity of seaweed was determined according to the (Ruch et al. 1989) & Oyaizu (1986): Seaweeds extracts (100µg/ml) in distilled water and 1% Potassium ferrocyanide were mixed with phosphate buffer (0.2 M, PH 6.6) and the mixture was incubated at 50˚C for 20 minutes, 2.5 ml of 10 % of Trichloracetic acid (TCA) was added to the reaction mixture which was centrifuged at 1000×g for 10 minute. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml), Fecl3 (0.5 ml 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid (20-100 µg/ml) was a positive control. The higher the absorbance of the reaction mixture, the greater is the reducing power

% reducing power = (Sample absorbance at 700 nm/1.2) × 100.

Determination of total phenol content

Preparation of seaweeds samples

The 500 mg seaweed powder was dissolved in ethanol, methanol, acetone and water, and kept for 24 hours and after 15 minutes centrifugation at 5000 rmp, the supernatant was collected for the estimation of total phenol.

Total phenol content was estimated by Folin Ciocalteu's method

The 1 ml of aliquots ethanol, methanol and acetone extracts and standard Gallic acid (10, 20, 40, 60, 80, 100 µg/ml) were positioned into the test tubes and 5ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to10 ml with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue colour was developed.

After incubation, absorbance was measured at 750 nm spectrophotometer using Perkin Flmer precisely Lambda 25 UV/Vis Spectrophotometer and UV-Spectrophotometer (UV-2600 SHIMADZU). The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data onto total phenolic content of seaweeds are expressed as mg of Gallic acid equivalent weight (GAE) / 100 g of dry mass (Bhalodia et al., 2011; Patel et al., 2010).

Estimation of pigments

The pigments such as chlorophyll a, chlorophyll b and carotenoid were estimated according to standard protocol (Arnon, 1949). The seaweed powder of 500 mg was homogenised in a mortar and pestle with 10 ml of 80% acetone and centrifuged at 5000 rpm for 15 minutes and supernatants were stored in a refrigerator for pigment analysis. The absorbance was measured spectrophotometrically at 645 and 663 nm for chlorophyll a, chlorophyll b and total chlorophyll; the extracts were used for carotenoid estimation at 480 nm (Kirk & Allen, 1965). The content of pigments was estimated from the following formulas:

$$\text{Chloro 'a' (mg/g for wt.)} = \frac{12.7\times A663 - 2.69\times A645}{a \times 1000 \times W} \times V$$

$$\text{Chloro 'b' (mg/g for wt)} = \frac{22.9 \times A645 - 4.68 \times A\,663}{a \times 1000 \times W} \times V$$

$$\text{Carotenoid (mg/g for wt)} = 4 \times (A480) \times 10 / 500$$

Preliminary Characterization

The every species of seaweed which content the highest, total antioxidant capacity, free radical DPPH scavenging activity, H2O2 scavenging activity and Fe reducing power scavenging activity among the Chlorophyta, Rhodophyta and Phaeophyceae was scan from 300nm to 700nm with UV-Spectrophotometer (UV-2600 SHIMADZU) for its preliminary characterization.

Partial Characterization of Natural Antioxidants from Seaweeds

The preliminary identification of probable peaks were analysed by scanning between 300nm to 700nm by using UV-Visible Spectrophotometer. Further characterization of the composition of seaweed extract was elaborated with FT-IR spectroscopy and NMR (nuclear magnetic resonance) spectroscopy.

UV-Visible Spectrophotometer

The ethanol extract of *Caulerpa racemosa var*. *macrophysa*, acetone extract of *Turbinaria decurrens* and methanol extract of *Gracilaria opuntia* was analysed to spectrometric scan of 300 nm to700 nm for preliminary identification. The highest DPPH scavenging activity was found in the methanol extract of *Valoniopsis pachynema*, *Polycladia indica* and *Gracilaria opuntia*, methanol extract of *Turbinaria ornata* and acetone extract of *Caulerpa racemosa* contained the highest ferrous reducing activity. The graphs indicated the presence of specific compounds which was further characterized to identify their functional groups with the help of Fourier Transform Infrared (FT-IR) Spectroscopy and Nuclear magnetic resonance (NMR) Spectroscopy.

Fourier Transform Infrared (FT-IR) Spectroscopy

The extracts were air dried and the powder was taken with potassium bromide (KBr) and mixed well to make pellet and used for analysis of Fourier Transform Infrared (FT-IR) Spectroscopy to identify the functional groups present in various extracts.

Nuclear Magnetic Resonance (Nmr) Spectroscopy

The extracts of three seaweeds which contain highest antioxidant capacity as the ethanol extract of *Caulerpa racemosa* var. *macrophysa*, acetone extract of *Turbinaria decurrens* and methanol extract of *Gracilaria opuntia* were analysed for NMR Spectroscopy. The dissolution solution was chloroform. The structural identification was done by Nuclear magnetic resonance (NMR) spectroscopy.

Statistical Analysis

Each seaweed sample was analysed in triplicates and the data were expressed as mean ± standard deviation. The Pearson correlation was estimated to know the correlation between total phenol content, total antioxidant activity, the DPPH scavenging activity, hydrogen peroxide (H_2O_2) scavenging activity, ferric reducing antioxidant power (FRAP) and pigments of the different extracts of 33 species of seaweeds.

Results and Discussions

Total Antioxidant Capacity of Seaweeds

The aqueous, ethanol, methanol and acetone extracts of total 33 seaweeds species including 11 Chlorophyta, 11 Phaeophyceae and 11 Rhodophyta were used to analyse the total antioxidant capacity. The aqueous extract showed the lower TAC than extracts of methanol, ethanol and acetone. Among 33 seaweeds, Rhodophyta and Phaeophyceae had high activity in compared to Chlorophyta. The total antioxidant activity of Chlorophyta varied from 39.34 ± 1.09 % to 98.23 ± 0.95 %. The ethanol extract of *Caulerpa racemosa var. macrophysa* had the highest TAC of 98.23 ± 0.95 %. But the water extract (39.34 ± 1.09 %) and methanol extract (42.49 ± 0.62 %) of *Caulerpa racemosa var. macrophysa* showed minimum activity. Similarly, ethanol extract of *Valoniopsis pachynema*, *Ulva fasciata*, and *Chlorodesmis hildebrandtii* had 98.05 ± 0.99% TAC; 98.05 ± 1.00% TAC and 97.74 ± 0.99 % TAC. The total antioxidant capacity varied in Phaeophyceae from 80.20 ± 0.99% to 98.97 ± 0.00 %. The acetone and ethanol extracts of *Turbinaria decurrens* had 98.97 ± 0.00 % TAC and 98.81 ± 0.60 % TAC, followed by 98.63 ± 0.13 % in ethanol extract of *Turbinaria conoides*, 98.34 ± 0.75 % in *Sargassum cristaefolium*, 98.55 ± 0.54 % in *Hydroclatharus clathratus* and 97.87 ± 0.79 % in *Cystoseira indica*. In Phaeophyceae, ethanol extracts of all seaweeds showed comparatively high total antioxidant capacity, followed by methanol, acetone and aqueous extracts. Among Rhodophyta, some extracts exhibited very high total antioxidant capacity such as methanol extract of *Gracilaria foliifera* (98.52 ± 0.49 %), followed by *Kappaphycus alvarezii* (98.41 ± 0.70 %), *Digenea simplex* (98.32± 0.67 %) and *Amphiroa anceps* (98.16± 0.30 %).

The ethanol extract of *Aghardhiella subulata* had high activity (97.65 ± 1.00%) but its aqueous extract had comparatively low total antioxidant capacity (81.15 ± 1.10 %). The methanol extract of some red seaweed had high total antioxidant capacity such as *Gracilaria foliifera* (98.52 ± 0.49 %), *Kappaphycus alvarezii* (98.41 ± 0.70 %), *Digenea simplex* (98.32 ± 0.67 %) and *Amphiroa anceps* (98.16 ± 0.30 %) [Fig. 2].

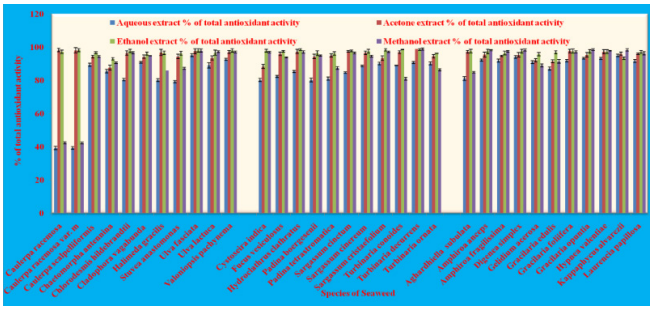


Figure 2: Showing the total antioxidant capacity of seaweeds

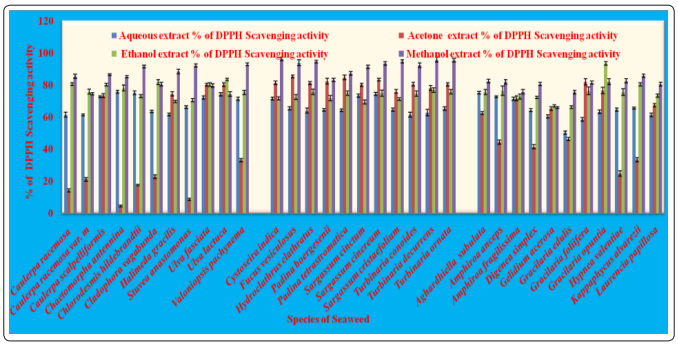


Figure 3: Showing the DPPH scavenging activity of seaweeds

Dpph Scavenging Activity of Seaweeds

The DPPH scavenging activity of four different extracts such as aqueous, ethanol, methanol and acetone of 11 Chlorophyta varied widely. The DPPH radical scavenging activity was 4.54 ± 0.59 % in acetone extract of *Chaetomorpha antennina*; similarly 8.71 ± 0.74 % and 14.30 ± 1.07 % was acetone in extract of *Phyllodictyon amastomonas* and *Caulerpa racemosa*, which was comparatively lower DPPH scavenging activity. The highest free radical DPPH scavenging activity among Chlorophyta was found in methanol extract of *Valoniopsis pachynema* (92.78 ± 0.83%), followed by methanol extract of *Phyllodictyon amastomonas* (92.21 ± 1.07 %), methanol extract of *Chlorodesmis hildebrandtii* (91.43 ± 0.77 %), and methanol extract of *Halimeda gracilis* (88.36 ± 1.37 %).

The methanol extract of seaweeds showed comparatively high free radical DPPH scavenging activity, followed by ethanol, aqueous and acetone extracts. Among 11 Phaeophyceae, the highest DPPH free radical scavenging activity was found in methanol extract of *Cystoseira indica* (96.35 ± 1.12 %), followed by *Turbinaria decurrens* (95.83 ± 1.60 %), *Turbinaria ornata* (95.40 ± 1.03 %), *Sargassum cristaefolium* (94.61 ± 0.99 %) and *Hydroclatharus clathratus* (94.65 ± 0.89 %). The DPPH free radical scavenging activity varied from 61.54 ± 1.54 % to 96.35 ± 1.12 %. All the four extracts of brown seaweeds showed comparatively higher free radical DPPH scavenging activity than red and green seaweeds. Likewise, the methanol extracts of brown seaweeds had comparatively higher activity than other three extracts for all 11 species of Phaeophyceae. For Rhodophyta, the DPPH free radical scavenging activity varied from 24.78 ± 1.66 % to 93.47 ± 1.09 %. The aqueous extract and acetone extract had low TAC in compared to ethanol and methanol extracts. The results showed that all the acetone extracts of 11 red seaweeds had less TAC. The maximum activity was found in ethanol extract of *Gracilaria opuntia* (93.47 ± 1.09 %), followed by methanol extract of *Kappaphycus alvarezii* (85.69 ± 1.05 %), methanol extracts of *Hypnea valentiae* (82.57 ± 1.24 %) and *Aghardhiella subulata* (82.36 ± 1.11%). The minimum activity was found in some of the seaweeds species such as acetone extracts of *Kappaphycus alvarezii* (33.50 ± 1.25 %), *Hypnea valentiae* (24.78 ± 1.66 %), and acetone extract of *Digenea simplex* (41.48 ± 1.22 %), *Gracilaria edulis* (46.25 ± 1.01 %) and *Amphiroa anceps* (44.46 ± 1.23 %) [Fig. 3].

Hydrogen Peroxide (H_2O_2) Scavenging Activity of Seaweeds

The four extracts such as aqueous, acetone, ethanol and methanol were used for the assay of percentage of H_2O_2 scavenging activity. Among 33 species of seaweeds the percentage of H_2O_2 scavenging activity varied from 0.20 ± 0.10 % (*Sargassum cinereum*) to 24.91 ± 0.49 % (*Caulerpa racemosa var. macrophysa*). Among all four extracts for all species methanol extracts had maximum percentage of H_2O_2 scavenging activity. Some of the green seaweeds contained the high percentage of H_2O_2 scavenging activity such as methanol extracts of *Caulerpa racemosa var. macrophysa* (24.91 ± 0.49 %), followed by *Caulerpa racemosa* (20.78 ± 0.47 %), *Caulerpa scalpelliformis* (17.64 ± 0.39 %), *Cladophora vagabunda* (16.71 ± 0.53%) and *Ulva lactuca* (16.32 ± 0.00 %). Some of the Ulvophyceae contained minimum percentage of H_2O_2 scavenging activity such as ethanol extracts of *Caulerpa racemosa* (1.95 ± 0.57 %), *Caulerpa racemosa var. macrophysa* (1.16 ± 0.35 %) and *Ulva fasciata* (2.81 ± 0.61 %). Among 11 species of Phaeophyceae, H_2O_2 scavenging activity varied from 0.20 ± 0.46 % to 19.22 ± 0.27 %.

The methanol extracts of *Turbinaria ornata* contained maximum activity (19.22 ± 0.93 %), followed by *Padina tetrastromatica* (16.80 ± 0.31 %) and *Turbinaria decurrens* (14.86 ± 0.14 %). Some of the seaweeds contained less amount of H_2O_2 scavenging activity such as ethanol extract of *Padina tetrastromatica* (0.37 ± 0.16 %), followed by *Cystoseira indica* (0.57 ± 0.08 %), *Sargassum cinctum* (1.79 ± 0.45 %) and Padina boergesenii (1.95 ± 0.56 %). For all Florideophyceae, methanol extracts contained maximum H_2O_2 scavenging activity among which methanol extract of *Gracilaria opuntia* (18.81 ± 0.98 %), had the highest activity, followed by *Gracilaria foliifera* (16.82 ± 0.50 %) and *Amphiroa anceps* (16.08 ± 0.44 %). Some of the studied red seaweeds contained very less activity, among which the minimum activity was expressed in ethanol extract of *Gracilaria foliifera* (0.64 ± 0.05 %) and *Kappaphycus alvarezii* (0.64 ± 0.29 %) [Fig 4].

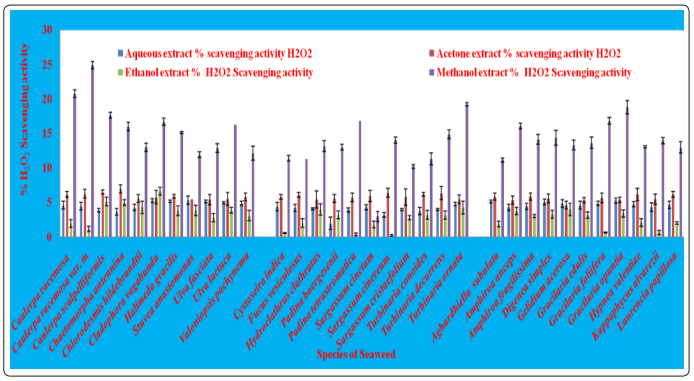


Figure 4: Showing the hydrogen peroxide scavenging activity of seaweeds.

Ferric Reducing Antioxidant Power (Frap) Activity of Seaweeds

The percentage of reducing power among 11species of Ulvophyceae varied from 42.29 ± 0.89 % to 383.25 ± 1.14 %. The acetone extract of all 11 species of Ulvophyceae had the highest activity, followed by aqueous extracts. The FRAP was less in methanol extracts in compared to ethanol extracts. Some of the green seaweed had comparatively higher percentage of reducing power such as acetone extract of *Caulerpa racemosa* (383.25 ± 1.06 %), followed by *Chlorodesmis hildebrandtii* (222.74 ± 1.73 %), *Caulerpa racemosa var. macrophysa* (191.34 ± 0.81 %), *Cladophora vagabunda* (181.90 ± 1.39 %) and aqueous extract of *Chlorodesmis hildebrandtii* (198.40 ± 1.18 %) and *Caulerpa scalpelliformis* (172.33 ± 1.12 %). For Phaeophyceae the reducing power scavenging activity varied from 26.14 ± 0.92 % to 314 ± 2.3 %. Some of the brown seaweeds contained high percentage of reducing power scavenging activity among which the aqueous extract of *Turbinaria ornata* contained the highest (314 ± 2.35 %), followed by *Sargassum cristaefolium* (312.22 ± 1.02 %), *Hydroclatharus clathratus* (303.3 ± 1.15 %). The acetone extract of Sargassum cristaefolium contained also quite high reducing power scavenging activity (191.54 ± 1.39 %), followed by *Padina tetrastromatica* (191.53 ± 1.10 %). In the case of all 11 brown seaweeds among four different extracts, the aqueous extract showed the highest reducing power scavenging activity, followed by acetone extract and the methanol extract recorded for lower reducing power activity and ethanol extracts were also recorded low reducing power scavenging activity. The minimum reducing power activity was found in methanol extract of *Sargassum cinctum* (26.14 ± 0.92 %), followed by *Padina boergesenii* (30.31 ± 0.95 %) and *Sargassum cristaefolium* (33.50 ± 0.75 %).

For red seaweeds, the highest reducing power scavenging activity was found in aqueous extract of *Palisada perforata* (formerly *Laurencia papillosa*) (358.09 ± 1.33 %), followed by Digenea simplex (316.42 ± 1.11 %), methanol extract of *Kappaphycus alvarezii* (178.62 ± 2.89 %), and aqueous extract of *Kappaphycus alvarezii* (175.32 ± 0.05 %), acetone extract of *Aghardhiella subulata* (173.08 ± 0.96 %) and *Laurencia papillosa* (168.19 ± 0.88 %). The minimum reducing power scavenging activity was found in methanol extracts of all seaweeds except *Kappaphycus alvarezii*, followed by ethanol and acetone extract but the aqueous and acetone extracts of all eleven species of seaweeds had high reducing power scavenging activity [Fig. 5].

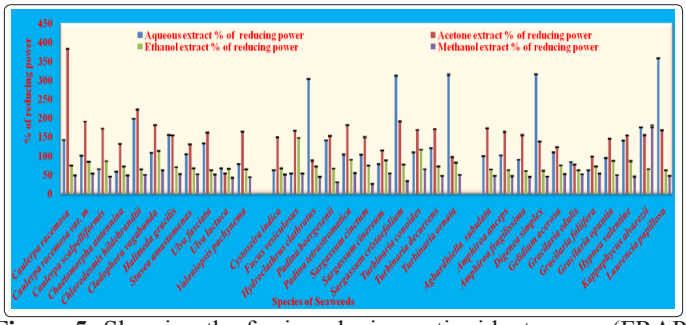
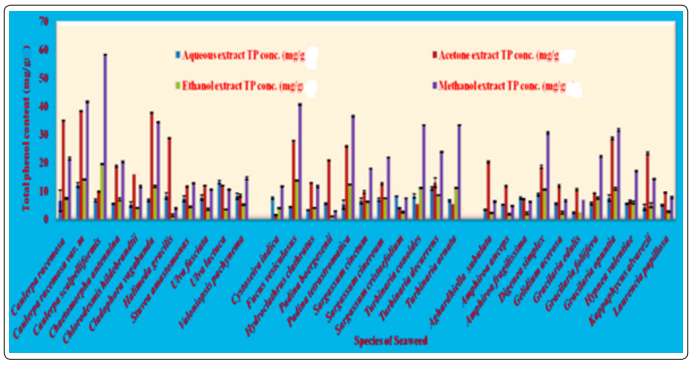


Figure 5: Showing the ferric reducing antioxidant power (FRAP) activity

Total Phenolic Content

Among 11 species of green seaweeds, methanol extract of *Caulerpa scalpelliformis* (58.23 ± 0.12 mg/g) had the highest total phenol content, followed by *Caulerpa racemosa var. macrophysa* (41.62 ± 0.32 mg/g), acetone extract of *Cladophora vagabunda* (37.76 ± 0.19 mg/g) and *Caulerpa racemosa var. macrophysa* (38.32 ± 0.17 mg/g) and *Caulerpa racemosa* (34.93 ± 0.22 mg/g).

The minimum total phenol content was found in ethanol extract of *Halimeda gracilis* (1.44 ± 0.36 mg/g), *Ulva fasciata* (3.59 ± 0.20 mg/g) and *Ulva lactuca* (3.52 ± 0.32 mg/g). Among 11 Phaeophyceae, the maximum total phenol content was found in *Fucus vesiculosus* (40.66 ± 0.26 mg/g), and *Padina tetrastromatica* (36.46 ± 0.03 mg/g). The less total phenol was found in acetone extract of *Cystoseira indica* (1.55 ± 0.23 mg/g), ethanol extract of *Padina boergesenii* (1.06 ± 0.09 mg/g), and ethanol extract of *Sargassum cristaefolium* (2.54 ± 0.12 mg/g). Specifically, the methanol extract of all red seaweeds had comparatively high total phenol content, some of them as 31.56 ± 0.47 mg/g total phenol in Gracilaria opuntia, methanol extract of *Digenea simplex* (30.5 ±0.43 mg/g), followed by acetone extract of *Gracilaria opuntia* (28.65 ± 0.41mg/g) and *Kappaphycus alvarezii* (23.36 ± 0.52 mg/g) and *Aghardhiella subulata* (20.36 ± 0.36 mg/g) [Fig. 52].



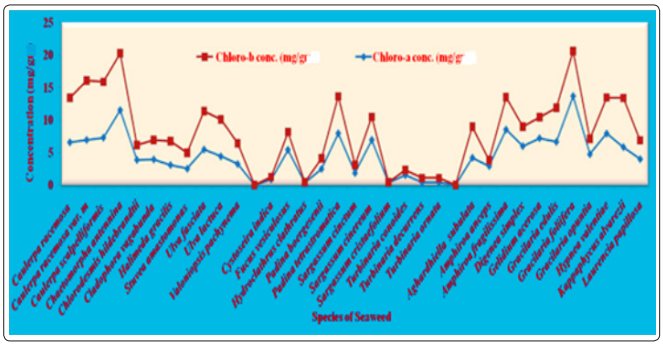
Showing the total phenol content (mg/g dry wt.)

Pigments Content of Seaweeds

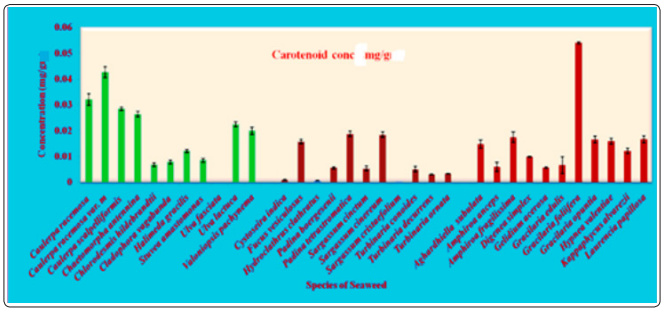
Among 11 Chlorophyta , Chlorophyll-a, content varied from 2.52 ± 0.07 mg/g dry weight *Phyllodictyon anastomosans* (formerly *Stuvea amastomonas*) to 11.52 ± 0.26 mg/g dry wt. (*Chaetomorpha antennina*), consequently Chlorolophyll-b, ranged from 2.28 ± 0.14 mg/g dry wt. (*Chlorodesmis hildebrandtii*) to 9.12 ± 0.12 mg/g dry wt. (*Caulerpa racemosa var. macrophysa*). Some species contained comparatively high Chlorophyll-a, and Chlorophyll-b, such as *Caulerpa racemosa* (Chlorophyll-a - 6.58 ± 0.17 mg/g dry wt.;

and Chlorophyll-b- 6.86 ± 0.11 mg/g dry wt.), *Caulerpa racemosa var. macrophysa* (Chlorophyll-a, - 6.94 ± 0.15 mg/g dry wt. and Chlorophyll-b, - 9.12 ± 0.12 mg/g dry wt.), *Caulerpa scalpelliformis* (Chlorophyll-a, - 7.30 ± 0.15 mg/g dry wt. and Chlorophyll-b, - 8.58 ± 0.37 mg/g dry wt.) and *Chaetomorpha antennina* (Chlorophyll-a, - 11.52 ± 0.26 mg/g dry wt. and Chlorophyll-b, -8.77 ± 0.19 mg/g dry wt.). Some Phaeophyceae, contained adequate amount of Chlorophyll-a, and Chlorophyll-b, such as *Padina tetrastromatica* (Chlorophyll-a, 7.97 ± 0.05 mg/g dry wt. and Chlorophyll-b, -5.96 ± 0.35 mg/g dry wt.), *Sargassum cinereum* (Chlorophyll-a, - 6.95 ± 0.06 mg/g dry wt., Chlorophyll-b, - 3.54 ± 0.08 mg/g dry wt.) and Fucus vesiculosus (Chlorophyll-a, -5.43 ± 0.25 mg/g dry wt. and Chlorophyll-b, - 2.73 ± 0.21 mg/g dry wt.). The Chlorophyll content was comparatively high in red seaweed *Gracilaria foliifera* (13.69 ± 0.38 mg/g dry wt.) and green seaweed *Caulerpa racemosa var. macrophysa* (9.12 ± 0.12 mg/g dry wt.). Among 33 species of seaweeds, the lowest Chlorophyll-a, (0.30 ± 0.09 mg/g dry wt.) and Chlorophyll-b (0.12 ± 0.01 mg/g dry wt.) was found in *Hydroclatharus clathratus*. In the case of 11 Florideophyceae, the Chlorophyll-a, content varied from 2.93 ± 0.09 mg/g dry wt. to 13.69 ± 0.38 mg/g dry wt.; likewise, Chlorophyll-b content ranged from 0.92 ± 0.08 mg/g dry wt. to 6.94 ± 0.04 mg/g dry wt.

Some species of Rhodophyta also had comparatively high Chlorophyll-a, and b such as *Amphiroa fragilissima* (Chlorophyll-a, -8.54 ± 0.43 mg/g dry wt. and Chlorophyll-b, - 4.99 ± 0.01 mg/g dry wt., *Gelidium acerosa* (Chlorophyll-a, - 7.21 ± 0.05 mg/g dry wt. and Chlorophyll-b, - 3.21 ± 0.02 mg/g dry wt. and *Hypnea valentiae* (Chlorophyll-a, -7.93 ± 0.09 mg/g dry wt. and Chlorophyll-b, - 5.54 ± 0.48 mg/g dry wt.). The carotenoid content was varied from a wide range. The present study showed that green and red seaweed had high carotenoid content than brown seaweed. Remarkably, red seaweed Gracilaria foliifera had highest carotenoid content of .054 ± 0.03 mg/g dry wt., followed by 0.04 ± 0.002 mg/g dry wt. carotenoid content of green seaweed *Caulerpa racemosa var. macrophysa*



Showing the Chlorophylls-a and b content of seaweeds (mg/g dry wt.)



Showing the carotenoid content of seaweeds (mg/g dry wt.)

Statistical Analysis

The standard deviation and co-relation of co-efficient of analysed various antioxidant activities and total phenol content is tabulated in table 8.

Table 8: Showing Correlation co-efficient of different extracts of seaweeds

Correlation co-efficient aqueous extracts					
Pearson Correlation	% of TAC	% of DPPH	% of RP	% H_2O_2 SA	TP
% of TAC	1				
% of DPPH SA	0.150506542	1			
% of RP	0.11437974	-0.218310414	1		
% H_2O_2 SA	0.066671074	-0.134770442	0.050339563	1	
TP	-0.196364544	0.130789383	-0.101636142	0.13473727	1
Correlation co-efficient acetone extracts					
Pearson Correlation	% of TAC	% of RP	% of RP	% H_2O_2 SA	TP
% of TAC	1				
% of DPPH SA	0.094149305	1			
% of RP	0.317469889	-0.363951354	1		
% H2O2 SA	0.054192047	-0.145710317	0.308491052	1	
TP	0.220577813	-0.329076604	0.439569942	0.111440952	1
Correlation co-efficient of ethanol extracts					
Pearson Correlation	% of TAC	% of DPPH	% of RP	% H_2O_2 SA	TP
% of TAC	1				
% of DPPH SA	0.094149305	1			
% of RP	0.317469889	-0.363951354	1		
% H2O2 SA	0.054192047	-0.145710317	0.308491052	1	

TP	0.220577813	-0.329076604	0.439569942	0.111440952	1
Correlation co-efficient of methanol extracts					
Pearson Correlation	% of TAC	% of DPPH	% of RP	% H_2O_2 SA	TP
% of TAC	1				
% of DPPH SA	0.152663419	1			
% of RP	0.020792649	-0.019039521	1		
% H2O2 SA	-0.553583986	-0.325381458	0.11059627	1	
TP	-0.260515834	0.161532641	0.050225313	0.425080828	1

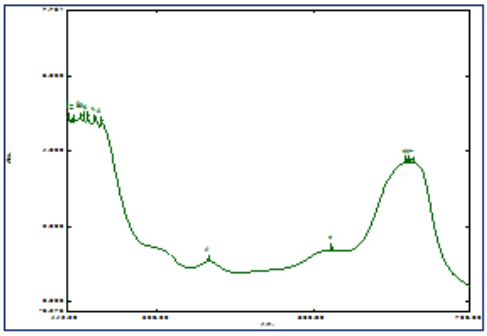
Partial Characterization of Natural Antioxidants from Seaweeds

Seaweed extracts were characterized with the help of UV-Visible Spectrophotometer, Fourier Transform Infrared (FT-IR) Spectroscopy and NMR (Nuclear Magnetic Resonance) Spectroscopy.

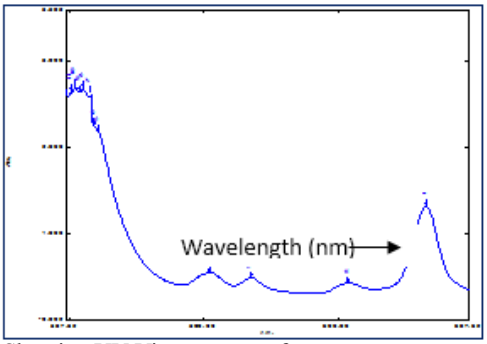
Uv-Visible Spectrophotometer

The scan (300 nm to700 nm) was done for the seaweed extracts such as the ethanol extract of *Caulerpa racemosa var. macrophysa* [Fig. 55], acetone extract of *Turbinaria decurrens* [Fig. 56], and methanol extract of *Gracilaria opuntia* [Fig. 57] which contained the highest total antioxidant capacity. The highest DPPH scavenging activity was found in the methanol extract of *Valoniopsis pachynema*, *Cystoseira indica* and *Gracilaria opuntia* [Fig. 58], methanol extract of *Turbinaria ornata* contained the highest ferrous reducing activity [Fig. 59] and acetone extract of *Caulerpa racemosa* [Fig. 60].

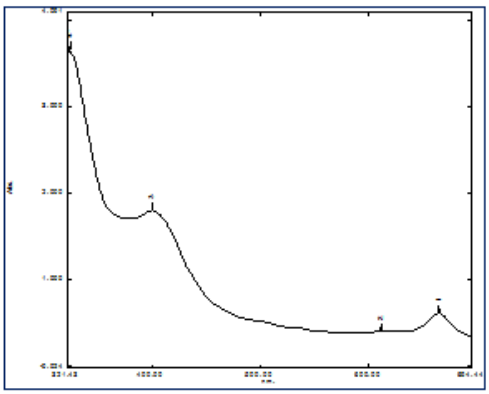
The peaks of UV-Vis spectrum was indicated the presence of some compounds in the extracts. The possible functional groups were identified with the help of Fourier Transform Infrared (FT-IR) Spectroscopy and the present possible compounds were identified by Nuclear magnetic resonance (1HNMR) Spectroscopy.



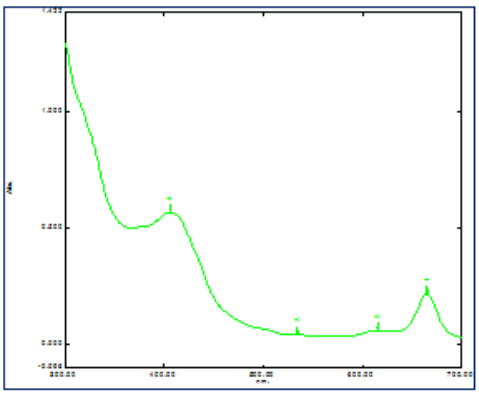
Showing UV-Vis spectrum of ethanol extract of *Caulerpa racemosa var. macrophysa*.



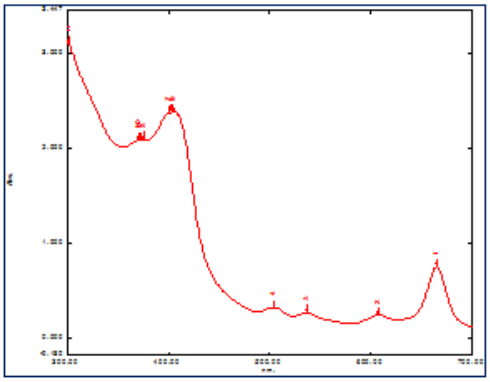
Showing UV-Vis spectrum of acetone extract of *Turbinaria decurrens*.



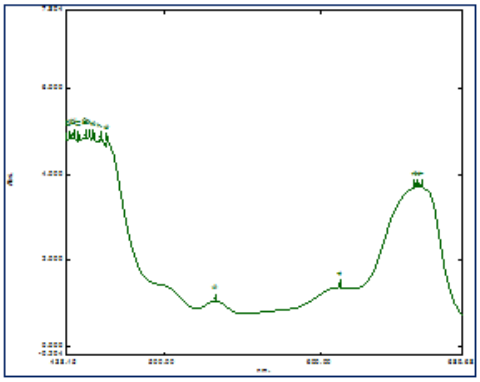
Showing UV-Vis spectrum of methanol extract of *Gracilaria opuntia*.



Showing UV-Vis spectrum of ethanol extract of *Gracilaria opuntia*.



Showing UV-Vis spectrum of methanol extract of *Turbinaria ornata*.



Showing UV-Vis spectrum of acetone extract of *Caulerpa racemose*

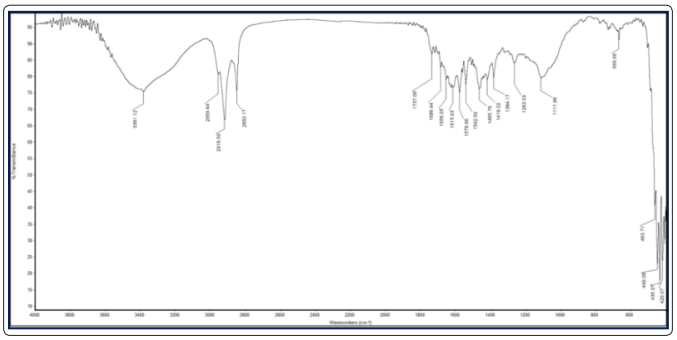
X-axis = Wavelength (nm), Y- axis = Absorbance

Identification of Functional Groups Present In the Various Extracts with Highest

Antioxidant Capacity

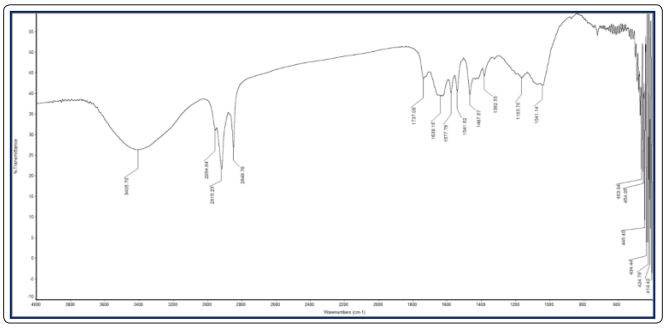
The total antioxidant capacity was highest in ethanol extract of *Caulerpa racemosa var. macrophysa*, acetone extract of *Turbinaria deccurens* and methanol extract of *Gracilaria opuntia*. The highest DPPH scavenging activity was found in methanol extract of *Valoniopsis pachynema*, the methanol extract of *Cystoseira indica*, and ethanol extract Gracilaria opuntia. The H_2O_2 scavenging activity was the highest in aqueous extract of *Caulerpa racemosa var. macrophysa*, methanol extract of *Turbinaria decurrens* and methanol extract of *Gracilaria opuntia*.

The highest ferrous reducing power was found in acetone extract of *Caulerpa racemosa*, methanol extract of *Turbinaria ornata* and aqueous extract of *Laurencia papillosa*. The different type of seaweeds extracts which was extracted by Soxhlet extractor, were collected and kept at room temperature for dry. After complete drying, the dried powder was collected and made it as pellet with mixed with KBr, then analysed for Fourier Transform Infrared (FT-IR) Spectroscopy. The infrared spectrum normally divided depending on stretches, at below 3000 cm^{-1} indicated the presence of carbons which are saturated with functional groups, consequently, stretches and bends produced above 3000 cm^{-1} prevailed for unsaturated carbon. Some broad peak between 3100 and 3600 cm^{-1} produced due to functional group with exchangeable protons such as alcohol, amide, and carboxylic acid. The IR spectrum of the methanol extract of *Caulerpa racemosa var. macrophysa* showed several broad and narrow peaks at different wavelengths. The broad bend at 3381.12 cm^{-1} was due to presence of amine (N-H) and amide (N-H) group. The bends at 2954.64 cm^{-1} and 2918.59 cm^{-1} produced may be due to the presence of alkanes (C-H) and acid (O-H), similarly the peak at 2850.17 cm^{-1} indicated the presence of aldehydes (C-O) group. The bend at 1737.08 cm^{-1} indicated for the presence of aldehydes (C=O) group. The peak at 1686.44 cm^{-1} produced for the presence of carbonyl group (C=O), similarly the peak at 1656.05 cm^{-1} for amide (N-H) and alkenes stretch (C=C). The peak at 1615.63 cm^{-1} and 1578.66 cm^{-1} also produced for amide group. The peak at 1542.59 cm^{-1} and 1384.17 cm^{-1} was present due to nitro (N-O) group, accordingly 1465.76 cm^{-1} for aromatic (C=C) group, 1419.02 cm-1 for alkanes and alkyl halides, similarly 1263.03 cm^{-1} for ester group, 1111.96 cm^{-1} ether group (C-O), the bend at 669.06 cm-1 was produced for the alkyl (C-Br) (Fig. 61).



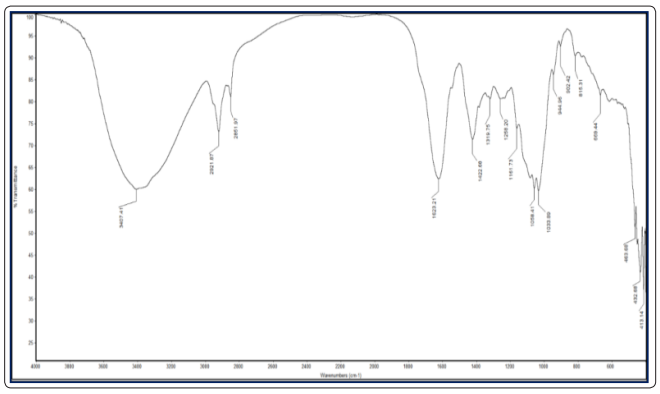
Showing the FT-IR spectrum of the ethanol extract of Caulerpa racemosa var. macrophysa. X-axis represent wave number & Y-Axis represent % of transmittance

The bend at 3405.70 cm^{-1} demonstrated the presence of 'free' hydroxyl bond. The three prominent peaks at 2954.64 cm^{-1}, 2918.23 cm^{-1} and 2849.76 cm^{-1} prevailed in IR spectrum of methanol extract of *Gracilaria opuntia* due to presence of alkanes (C-H) stretching and aldehydes stretch (C-H). The spectrum showed several frequent bends and stretches at 1737.08 cm^{-1} to 1041.14 cm^{-1} which explained the presence of several functional groups. The peaks at 1737.08 cm^{-1} and 1577.78 cm^{-1} produced due to presence of amide (N-H), alkenes (C=C) and secondary amine (N=N). The wide bend at 1541.62 cm-1 demonstrated the presence of nitro group (N-O). The peak at 1467.57 cm^{-1} produced indicating the presence of aromatic group (C=C) and alkanes (C-H) group. The peak at 1382.55 cm^{-1} also demonstrated the presence of nitro (N-O) group and 1163.76 cm^{-1} peak for alkyl halide such as C-F group, 1041.14 cm^{-1} for alkyl halide C-Br group (Fig. 62).



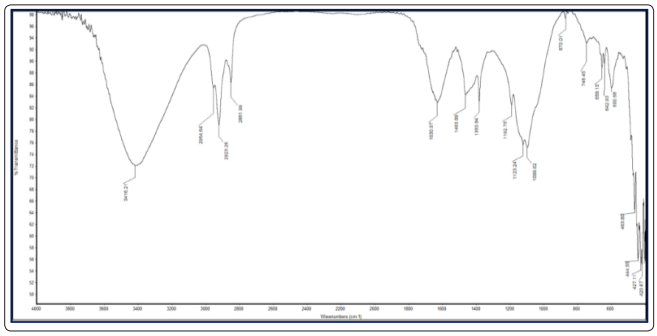
Showing the FT-IR spectrum of the methanol extract of Gracilaria opuntia.
X-axis represent wave number & Y-Axis represent % of transmittance.

The IR spectrum of the acetone extract of *Turbinaria decurrens* showed several broad and narrow bends and peaks at frequent wavelength interval. The wide bend at 3407.41 cm^{-1} demonstrated the existence of amine, amide and free hydroxyl group may be phenol. The peak at 2921.87 cm^{-1} indicated the presence of alkanes (C-H) and aldehydes (C-H). The peak at 1623.21 cm^{-1} demonstrated the presence of amide stretch. The bend at 1422.68 cm^{-1} was produced for alkanes (C-H) group, similarly, 1319.75 cm^{-1} peak for acid (C-O) group, 1258.20 cm^{-1} peak for acid and ester group. The peak at 1161.73 cm^{-1} was for ether (C-O) group, 1058.41 cm^{-1} for alcohol (C-O), and 1033.89 cm^{-1} for ester group. The bends produced at 944.96 cm^{-1} and 902.42 cm-1 for alkyl halide C-F group, 815.31 cm^{-1} and 669.44 cm^{-1} for C-Cl, C-Br alkyl halide group (Fig. 63).



Showing the FT-IR spectrum of the acetone extract of Turbinaria decurrens. X-axis represent wave number & Y-Axis represent % of transmittance

The highest DPPH scavenging activity was found in methanol extract of *Valoniopsis pachynema*, so the extract was dried and analysed for the demonstration of possible functional groups in this extract. The wide bend at 3416.21 cm^{-1} prevailed for free hydroxyl (OH) group, amine and amide (H-N) group. The prominent peaks at 2854.64 cm^{-1} prevailed for alkanes (C-H) stretch and 2923.26 cm^{-1} peak for aldehydes (C-H) group. The peak produced for 2851.99 cm^{-1} aldehydes (C-H) group. The several peaks prevailed in IR spectrum in between 1630.97 cm^{-1} to 1099.02 cm^{-1} which elaborated the presence of functional groups accordingly as 1630.97 cm^{-1} peak for amine (N-H), 1463.89 cm^{-1} peak for aromatic (C=C) and alkanes (C-H) group, 1383.84 cm^{-1} peak for nitro group (N-O), 1192.75 cm^{-1} for halide such as C-F group, 1123.24 cm^{-1} and 1099.02 cm^{-1} peaks for ether and alkyl halides group. The several peaks in between 870.01 cm^{-1} to 600.68 cm^{-1} for various alkyl halides groups such as C-F, C-CL, and C-Br etc [Fig. 64].

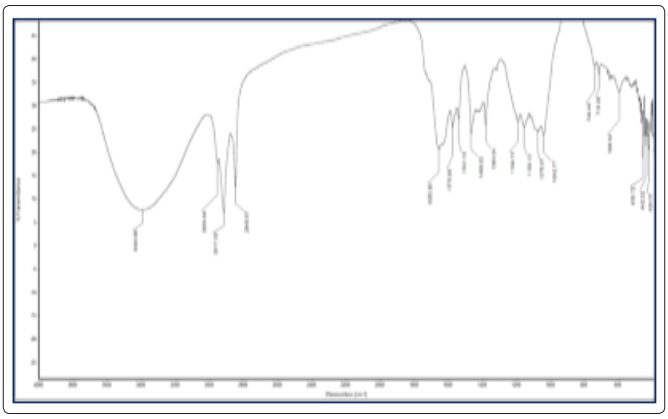


Showing the FT-IR spectrum of the methanol extract of Valoniopsis pachynema. X-axis represent wave number & Y-Axis represent % of transmittance

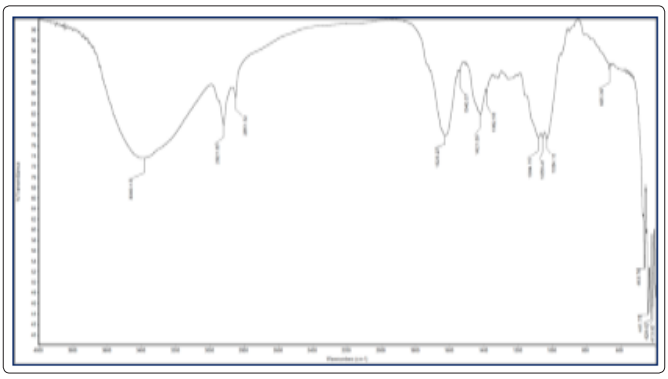
The hydrogen peroxide (H_2O_2) scavenging activity was found maximum in aqueous extract of *Caulerpa racemosa var. macrophysa*, methanol extract of Turbinaria ornata and methanol extract of Gracilaria opuntia. The IR spectrum for *Caulerpa racemosa var. macrophysa* and methanol extract of Gracilaria opuntia was already explained for their maximum total antioxidant capacity and DPPH scavenging activity. The methanol extract of *Gracilaria opuntia* was analysed to IR spectroscopy for explained the functional groups and bonds present in the dried sample of *Gracilaria opuntia*. The IR spectrum revealed some wide and narrow peaks with frequent interval at various wavelengths. The broad and wide peak at 3383.13 cm^{-1} indicated the presence of amine and amide (N-H) group, followed by two narrow peaks at 2921.80 cm^{-1} and 2851.32 cm^{-1} for alkanes (C-H) and aldehydes (C-H) bonds. The peak at 1626.23 cm^{-1} produced for amide (N-H) and alkenes (C=C) bonds. The 1540.57 cm^{-1} and 1382.55 cm^{-1} peaks referred to the presence of nitro (N-O) group. The peak at 1084.75 cm^{-1} produced for ether (C=O), peak at 1058.41 cm^{-1} for the presence of alcohol (C-OH) group.

The prominent peak at 1034.10 cm^{-1} produced for ester group and the bend at 665.39 cm-1 demonstrated the presence of alkyl halide (C-CL) group [Fig. 65]. The H_2O_2 scavenging activity was the highest in the methanol extract of *Turbinaria ornata* and ethanol extract of *Gracilaria opuntia* was analysed for FT-IR spectroscopy to identify the functional groups which were responsible for the highest H_2O_2 scavenging activity. The wide and broad peak at 3393.88 cm^{-1} produced for the presence of 'free' hydroxyl band, amine (N-H) and amide (N-H) group. The peak at 2954.64 cm^{-1} produced for alkanes (C-H) bond, 2917.22 cm^{-1} and 2849.67 cm^{-1} for aldehydes (C-H) bond. The peak at 1655.36 cm^{-1} produced for alkanes group, 1576.99 cm^{-1} and 1468.62 cm^{-1} peak for aromatic (C=C) functional group, 1541.18 cm^{-1} peak for amide (N-H) group. Similarly, the bend 1468.62 cm-1 indicated the presence of alkanes (C-H) group and 1384.04 cm^{-1} peak for nitro (N-O) group, 1194.15 cm^{-1} peak for ester group, 1078.67 cm^{-1} for ether (C-O) group. The peak at 1042.77 cm^{-1} demonstrated the presence of alcohol (C-O) stretch. The several peaks at 744.40 cm^{-1}, 718.06 cm^{-1} and 598.54 cm^{-1} produced for the presence of alkyl halides such as C-Cl, C-Br etc [Fig. 66]. The percentage of ferrous reducing power was found maximum in acetone extract of *Caulerpa racemosa*. The spectrum of dried powder of acetone extract of Caulerpa racemosa showed several wide and narrow peaks at various intervals along the spectrum. The wide and clear bend at 3405.95 cm^{-1} demonstrated

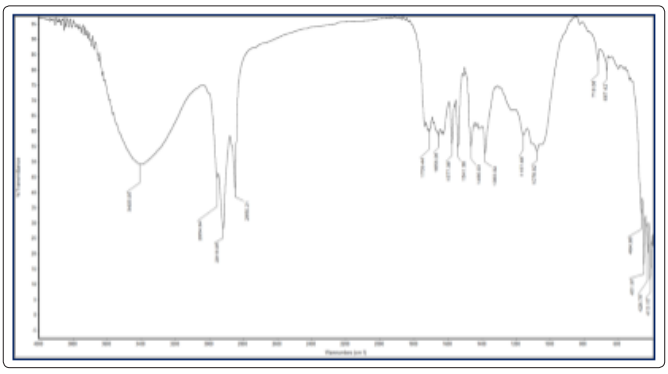
the presence of amine and amide (N-H) group. The stretches at 2954.64 cm^{-1} prevailed for the presence of alkanes (C-H) stretch and 2918.95 cm^{-1} peak for aldehydes (C-H) bond. The bend at 2850.21 cm-1 was indicated the presence of aldehydes (C-H). The bend at 1709.44 cm^{-1} demonstrated the presence of ketone acyclic group, then peak at 1656.06 cm^{-1} indicated the existence of the carbonyl (C=O) bond, accordingly, two narrow bends at 1577.38 cm^{-1} and 1541.38 cm^{-1} for amide (N-H), aromatic (C=C) group and nitro (N-O) group. The two broad peaks at 1157.68 cm^{-1} and 1078.92 cm^{-1} indicated for the presence of ether (C=O) and alcohol (C-O) group. The bends at 718.06 cm^{-1} and 667.42 cm^{-1} indicated for alkyl halide groups [Fig. 67].



Showing the FT-IR spectrum of ethanol extract of *Gracilaria opuntia*



Showing the FT-IR spectrum of the methanol extract of *Turbinaria ornata*

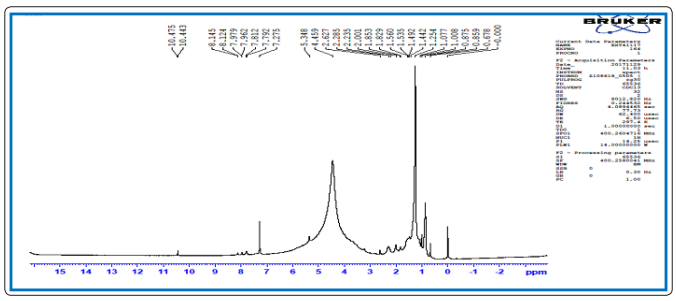


Showing the FT-IR spectrum of the methanolic extract acetone extract of Caulerpa racemosa. *X-axis represent wave number & Y-Axis represent % of transmittance*

The possible structure of compounds for high antioxidant capacity by

Nuclear Magnetic Resonance (1HNMR) Spectroscopy

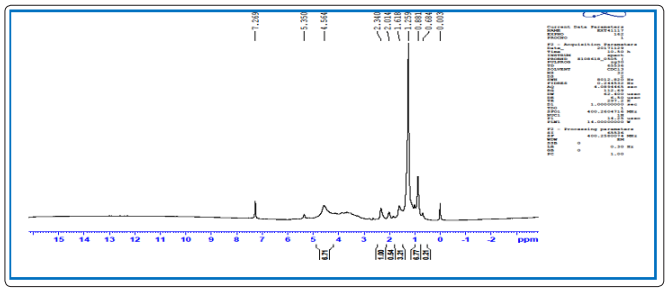
The total antioxidant capacity was highest in ethanol extract of *Caulerpa racemosa var. macrophysa*, acetone extract of *Turbinaria decurrens* and methanol extract of *Gracilaria opuntia*. These particular three extracts were analysed for NMR spectroscopy.



Showing 1HNMR spectrum of ethanol extract of *Caulerpa racemosa var. macrophysa*.

Discussions

The 1HNRM spectrum of the ethanol extract of *Caulerpa racemosa var. macrophysa* showed several signals between 0 ppm to 12 ppm. Generally, the presence of signals between 0 ppm to 1.5 ppm demonstrates the presence of protons with Sp3 hybridization CH_3-C group. Similarly, signals present between 1.5 ppm to 2.5 ppm indicate the possibility to allelic groups like C=C-C-H or O=C-C-H, accordingly, signals present between 2.5 ppm to 4.5 ppm is due to presence of heterocarbon like X-C-H where x may be N, O, F, and Cl. The signals show at 4.5 to 6.5 ppm due to presence of vinylic type of hydrogen aromatic group as –C=C-H. The signals at 6.5 to 8.5 ppm are due to possibly presence of Sp2 hybridized aromatic benzene compounds. Towards the downfield, signals between 9.0 ppm to 12.0 ppm indicates the presence of aldehydes (O=C-H) or carboxylic acids (CO=H) [Fig. 68].



Showing 1HNMR spectrum of acetone extract of Turbinaria deccurens

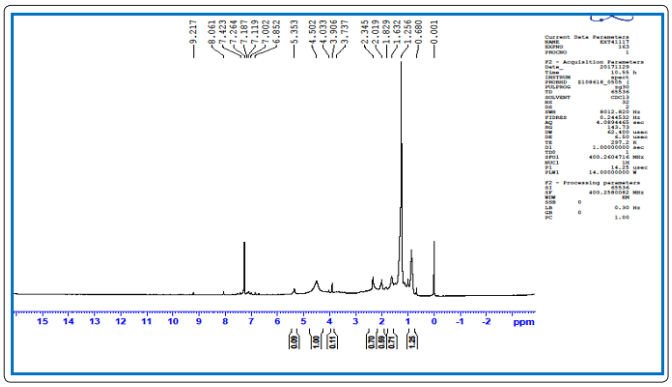
Discussions

The acetone extract of extract of *Turbinaria decurrens* showed some major signals between 0 δppm to 8 δppm. The type of spectrum indicates that the possible compound is aromatic type. Some major signals at 7.25 δppm, 4.5644 δppm, 1.25 δppm and 0.851 δppm developed due to presence of aromatic proton, fluorine, methylene ($-CH_2$) and methyl proton respectively (Fig. 68). In this present spectrum, the signal at 7.275 δppm demonstrated the presence of aromatic protons or Sp2 aromatic benzene group or it may be solvent peak, similarly signal peak at 4.45 δppm produced due to presence of C-F (or), OH proton (or) aniline group. The signals at 1.25 δppm and

1.077 δppm was indicated the presence of CH2 and methyl (CH3) proton. The type of spectrum indicates that the possible compound is aromatic type. The compound structure will be predicted after critical cutinisation of the results of Thin Layer Chromatography of all fractions of the extract and the 2D NMR analysis of each fraction of compound. The present possible functional groups which were present in samples will be predicted by FTIR spectrum and NMR spectrum (Fig. 69).

Discussions

The NMR spectrum of methanol extract of *Gracilaria opuntia* had several signals which indicated the presence of aromatic compounds as spectrum is 07 type. There are several peaks of NMR spectrum of *Gracilaria opuntia* demonstrated, the presence of the aromatic compounds. The signals at 7.26 δppm, 1.25 δppm and 0.68 δppm probably demonstrated the presence of aromatic proton, methylene proton and –OH proton. For future identification of compounds responsible for high antioxidant activity, will be analysed with Gas Chromatography-Mass Spectroscopy, Thin Layer Chromatography and 2D NMR to the complete the structural configuration of compound (Fig. 70).



Showing the [1]HNMR NMR spectrum of methanol extract of *Gracilaria opuntia*

The aim of the NMR study is try to identify the compounds presents in this particular extracts. The structural identification of compounds present in the extracts will be further characterized with the help of further analysis of the extracts by advanced techniques. The possible functional groups of a particular compounds present in the extracts are added according to explanations.

Statistical Analysis

The Pearson correlation co-efficient indicated that some extracts had positive correlation and some extracts had negative correlation between different types of antioxidant activities and total phenol content (table1).

General Discussions

Presently, some marine macro algae due to their excellent nutritive compositions is gradually finding place in the market as food items and food ingredient [17]. Some seaweed has excellent medicinal applications [18]. So, seaweeds are becoming as a demand in the food industry and pharmaceutical industry [19]. Seaweeds have comparatively high antioxidant rather than land plants [20]. It is now a leading requirement of substitution of synthetic antioxidants with naturally occurring safer antioxidants as the synthetic antioxidants have unfavourable side effects and there are stronger restrictions on their [21, 22]. So, to reduce the risk of developing of the deadliest diseases, the intake of natural antioxidant of biological origin will be the best preventive measured. Globally, many types of seaweed have been reported for their antioxidant activities, such as Chernane (2014), evaluated some seaweed for their antioxidant activity (*Ulva rigida, Enteromorpha intestinalis, Fucus spiralis and Bifurcaria bifurcata*) from the Moroccan coast and Ismail (2016), worked on *Ulva fasciata* (Chlorophyta), *Sargassum linifolium* (Phaeophyta) and *Corallina officinalis* (Rhodophyta) and reported that DPPH scavenging activity was the highest in the *U. fasciata* (81.3%), followed by *S. linifolium* (79.8%) then *C. officinalis* (72.6%) [23, 17]. The methanolic extracts of four *Chaetomorpha* species such as *C. aerea, C. crassa, C. linum and C. brachygona* have been evaluated for the antioxidant activity which had been shown that *Chaetomorpha linum* had the highest antioxidant potential [24]. In southeast India, some works have been done on antioxidant activity of seaweed. It has been reported that *Gelidium acerosa* had the highest DPPH scavenging activity (72.5 ±2.78%) among studies seaweeds collected from Tamilnadu coast [25]. From our study, it had been reported that some seaweed species had comparatively very high antioxidant activity in comparing to other reports.

Conclusions

Some seaweed extract had been recorded to have high antioxidant activities and the preliminary characterization indicates the presence of some compounds which may be used as future natural antioxidant after further characterization advanced techniques.

Acknowledgement

Author thanks Dr. P. Anantharaman, Professor for his laboratory support. Author thanks to Dean and Director, C.A.S in Marine Biology, Faculty of Marine Sciences, Annamalai University. Author acknowledges her sincere thanks to all higher authorities of Annamalai University.

Conflict of Interes

There are no conflicts of interest to be declared.

Funding

Author is thankful to the Department of Science and Technology (DST), Govt. of India for their financial support and kind co-operation.

References

1. Hazra B, Sarkar R, Biswas S, Mandal N (2010) Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of Terminalia chebula, Terminalia belerica and Emblica officinalis. BMC Complem Altern M 10: 20-35.
2. Lee H H, Lin C T, Yang L L (2007) Neuroprotection and free radical scavenging effects of Osmanthus fragrans. J Biomed Sci 14: 819-827.
3. Ito N, Hirose M, Fukushima S, Tsuda H, Shira T, et al. (1986) Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem. Toxicol 24: 1071-1082.
4. Wattenberg L W (1986) Protective effects of 2 (3)-tert-butyl-4-hydroxyanisole on chemical carcinogenesis. Food Chem. Toxicol 24: 1099-1102.
5. Heinonen I M, Meyer A S, Frankel E N (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein

and liposome oxidation. J Agric Food Chem 46: 4107-4112.
6. Raja R, Hemaiswarya S, Arunkumar K, Carvalho I S (2016). Antioxidant activity and lipid profile of three seaweeds of Faro, Portugal. Braz J Bot 39: 9-17
7. Rajagopal S V, Raman B V, Reddy B, Rao M R, Sivakumar K (2008) Antioxidants and Free Radical Scavenging Activity of Brown Algae of Visakhapatnam Coast. Asian Journal of Chemistry 20: 5347-5352.
8. Mensor L L, Menezes F S, Leitao G G, Reis A S, Dossantos T, et al. (2001) Antioxidant activity determination by radical scavenging activity. Phytother Res 15: 127.
9. Lallianrawna S, Muthukumaran R, Ralte V, Gurusubramanian G, Kumar S, et al. (2013) Determination of total phenolic content, total flavonoids content and total antioxidant capacity of Ageratina adenophora (Spreng) King & H Rob. Sci Vis 13: 4.
10. Surana A R, Kumbhare M R., Wagh R D (2016) Estimation of total phenolic and total flavonoids content and assessment of in vitro antioxidant activity of extracts of Hamelia patens Jacq Stems. Research Journal of Phytochemistry 10: 67-74.
11. Ruch R J, Cheng S J, Klaung J E (1989) Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10: 1003-1008.
12. Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 44: 307-15.
13. Bhalodia N R, Nariya P B, Acharya R N, Shukla V J (2011) Evaluation of in vitro antioxidant activity of flowers of Cassia fistula Linn. International Journal of Pharm Tech Research. IJPRI 3: 589-599.
14. Patel V R, Patel P R, Kajal S S (2010) Antioxidant activity of some selected medicinal plants in Western Region of India. Advances in Biological Research 4: 23-26.
15. Arnon D (1949) Copper enzymes in isolated chloroplasts polyphenol oxidase in Beta vulgaris. Plant Physiology 24.
16. Kirk J, Allen R (1965) Dependence of chloroplast pigment synthesis on protein synthesis effect of actidione. Biochemical and Biophysical Research Communications 21: 523-530.
17. Holdt Ito N, Hirose M, Fukushima S, Tsuda H, Shira T, et al. (1986) Studies on antioxidants Their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem Toxicol 24: 1071-1082.
18. Tom D Dillehay, C Ramírez, M Pino, M B Collins, J Rossen, et al. (2008) Monte Verde: Seaweed, Food, Medicine, and the Peopling of South America. Science 320: 784-6.
19. Smith A J (2004) Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of Applied Phycology 16: 245-262.
20. Veeraperumah S, Namasivayam S K, Pitchai M, Perumal P, Ramasamy R, et al. (2012) Antioxidant properties of sequential extracts from brown seaweed Sargassum plagiophyllum C Agardh. Asian Pacific Journal of Tropical Disease 2: S937-S939.
21. Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity Songklanakarin. J Sci Technol 26: 211-219.
22. Chu Y (2000) Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agricul 80: 561-566.
23. Chernane H (2014) Evaluation of antioxidant capacity of methanol extract and its solvent fractions obtained from four Moroccan macro algae species. European Scientific Journal 10.
24. Farasat M, Khavari Nejad R A, Nabavi S M B, Namjooyan F (2013) Antioxidant Properties of Some Filamentous Green Algae (Chaetomorpha Genus). An international Journal of Brazilian archives of biology and technology 56: 921-927.
25. Devi K P, Suganthy N, Kesika P, Pandian S K (2008) Bio protective properties of seaweeds In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complementary and Alternative Medicine 8: 38.