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A COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITY OF EXTRACTS OF *Lobophora variegate* Less AND *Codium tomentosum* STACKHOUSE

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ABSTRACT

Objective: *In-vitro* antioxidant activities of two selected Indian algal species viz., *Lobophora variegata* (brown algae) and *Codium tomentosum* (green algae) were evaluated.

Methods: DPPH, Hydroxyl, ABTS, Superoxide and Reducing power of crude extracts (petroleum ether, Benzene, Ethyl acetate, Ethanol and Methanol) were determined.

Results

The antioxidant activities of the extracts revealed that they inhibited more than 60% of DPPH, Hydroxyl, ABTS, and Superoxide radicals. The radical scavenging activity, the reducing power increased with the concentration of the extracts. Depending on the type of extract and the antioxidant tests, the 50% inhibitory concentration (IC_{50}) ranged from 20.73 to 32.47 ug/ml. The results showed that all of the extracts had antioxidant properties, with *C. tomentosum* methanol having the highest antioxidant activity.

Conclusion: The present study suggests that *C. tomentosum* could be considered during diseases from oxidative deteriorations.

Keywords: Red algae, ABTS, DPPH, Reducing power, Free radical activity

INTRODUCTION

The generation of reactive oxygen and nitrogen species (ROS and RNS) has been associated to the oxidative deterioration of food as well as the aetiology of a number of human diseases, including atherosclerosis, diabetes, chronic inflammation, neurological disorders, and certain types of cancer **[1]**. Dietary antioxidants are commonly utilized in the prevention of chronic diseases and are added to food to slow down oxidative degradation. Many studies have shown that a high dietary intake of natural phenols with the presence of several types of antioxidants found in plants and seaweeds, such as flavonoids, is strongly linked to a longer life expectancy, a lower risk of developing chronic diseases, and various types of cancer **[2, 3, 4]**. Natural antioxidants present in higher plants, such as alpha-tocopherol, phenols, and beta-carotene, are employed in the food industry to suppress lipid peroxidation and protect the human body from free radicals, slowing the progression of many chronic diseases **[5]**.

There is now a greater interest in marine organisms, particularly algae, as a result of an increasing demand for biodiversity in the search for therapeutic drugs from natural resources. Marine algae algae has been closely associated with human life from ancient times, and has been extensively used as a source of food, feed, fertilizer and medicine, primarily for economically valuable phycocolloids. More than 60 trace elements are found in higher concentrations in marine algae than in terrestrial plants. Protein, iodine, bromine, vitamins, and stimulatory and antibacterial chemicals are also present **[6]**. Marine macroalgae have variable nutritional contents based on species, habitats, age, and environmental circumstances. Marine seaweeds, in particular, are an essential source of a diverse range of bioactive chemicals and a key component in the screening of many pharmaceutical drugs.

Lobophora variegate is a widespread brown algae found in tropical and subtropical shallow water environments, including coral reefs in the Caribbean, the Indian Ocean, and the Red Sea **[7]**. *Codium tomentosum* is a green algae with thick, dark-green tubular strands arranged into branches. It has a sponge-like, somewhat velvety touch. The species is very common around the world **[8]**. Although it is considered edible **[9, 10]** the fact that they grow in wastewater limits their use as a food source or in industrial applications.

MATERIALS AND METHODS

Collection of sample

Fresh algae samples were collected from the coastal region of Gulf of Manner, Rameswaram region, Tamilnadu. The collected samples were washed with sea

water and brought to the laboratory in a plastic bag containing sea water to prevent evaporation.

Preparation of extracts

5 g of the fine powder of each algae was extracted successively with 100 ml of alcoholic and organic solvents (Petroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol) in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for in vitro antioxidant activity.

Antioxidant activity

DPPH radical scavenging activity

The DPPH is a constant free radical and is extensively used to measure the radical scavenging activity of antioxidant component. This process is based on the reduction of DPPH in methanol solution in the company of a hydrogen donating antioxidant due to the arrangement of the non-radical form DPPH. Using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) the free radical scavenging action of all the extracts was assessed as per the previously reported process. DPPH of 0.1 mM solution in methanol was prepared. 1 ml of this solution was poured into 3 ml of the solution at different concentrations (50, 100, 200, 400 and 800 µg/ml). The mixtures were shaken dynamically and allowed to stand at room temperature for 30 minutes **[11]**. After that the absorbance was measured at 517nm using a UV-VIS spectrophotometer (Genesys 10 UV: Thermoelectron corporation). Ascorbic acid was employed as the standard. The lesser absorbance values of reaction mixture identify higher free radical scavenging action. Using the subsequent formula the ability to scavenge the DPPH radical was computed.

DPPH scavenging activity (% inhibition) = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the outcomes were averaged.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging properties was analysed according to the method described by Halliwell *et al*., **[12]**. Stock answers of $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), EDTA (1 mM), H2O2 (10 mM) and Deoxyribose (10 mM) were put in distilled deionized water. The assay was executed by adding 0.1 ml EDTA, 0.1 ml H_2O_2, 0.01 ml of $FeCl_3$, 0.36 ml of deoxyribose, 1.0 ml of the extract of diverse concentration (50, 100, 200, 400 & 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for

1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging achievement of the extract is accounted as % inhibition of deoxyribose. The degradation is figured by using the succeeding equation

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging action was calculated as elucidated by Srinivasan *et al.*, **[13]**. The superoxide anion radicals were made in 3.0 ml of Tris - HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris - HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was estimated at 560nm against a blank sample, ascorbic acid. The percentage inhibition was determined by using the following equation. **Superoxide radical scavenging activity = (A0 - A1) / A0 X 100**

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were achieved in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation ($ABTS^{+}$)

ABTS assay was supported on the slightly modified technique of Huang *et al.* **[14]**. By reacting 7 mM ABTS solution with 2.45 mM potassium persulphate, ABTS radical cation ($ABTS^{+}$) was prepared. This mixture is permitted to be in the dark at room temperature for 12-16 hrs previous to use. With ethanol to an absorbance of 0.70 + 0.02 at 734 nm the $ABTS^{+}$ solution was added. Following this trolox standard to 3.9 ml of diluted $ABTS^{+}$ solution or addition of 100 µL of sample, absorbance was calculated at 734 nm by Genesys 10S UV-VIS (Thermo scientific) accurately after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

$ABTS^{+}$ radical cation activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were repeated thrice and the end results were averaged.

Reducing Power

The reducing power of the extract was established by the method of Kumar and Hemalatha **[15]**. 1.0 ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed up with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50^{o} C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerated centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

RESULTS

The DPPH radical scavenging capacity by crude (P.ether, Benzene, Ethyl acetate, ethanol and methanol) extracts of the algae *L.variegata* and *C.tomentsum* was evaluated at different concentrations of the extracts and the results are illustrated in **Figure 1a & b**. The methanol extract of *C.tomentsum* (129.49% ± 0.92%) exhibited a strong DPPH activity, followed by the methanol extract of *L.variegata* (112.41% ± 0.81%), ethanol of *C.tomentsum* (121.48% ± 0.40%), Benzene of *L.variegata* (102.27% ± 0.39%) at a concentration of 800 ug/ml. The concentration of *C.tomentsum* methanol extract needed for 50 % inhibition (IC_{50}) was 37.56 µg/ml, while ascorbic acid needed 30.45 mg/ml **(Table 1 & 2)**.

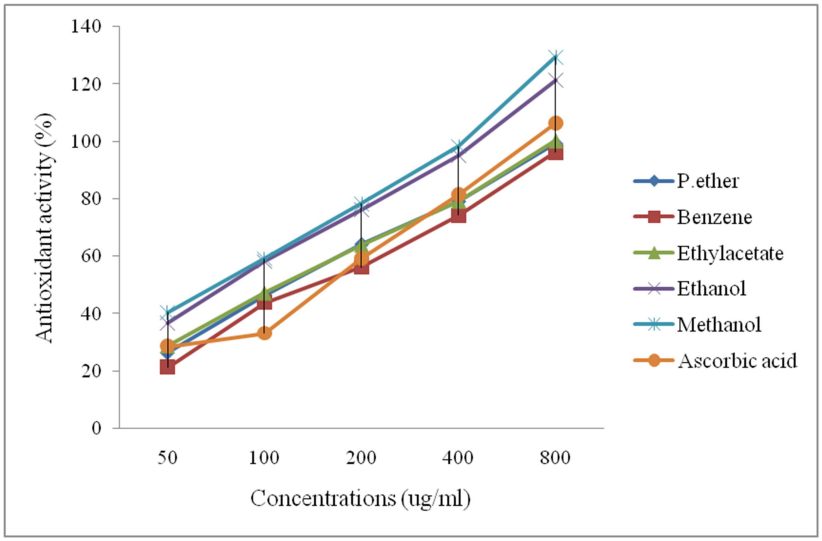
The hydroxyl radical inhibition activities of *C.tomentosum* and *L.variegata* are presented in **Figure 2a & b**. There was a concentration-dependent increase in inhibition in the extracts assayed. The methanol extracts of both algae showed a significant difference at all concentrations. The methanol extract of the *L.variegata* showed significantly higher radical inhibition at all concentrations. Methanol extract of the *L.variegata* had significantly higher scavenging activity of 32.36%, 44.16%, 70.40%, 98.20% and 133.26% at 50, 100, 200, 400 and 800 µg/ml. There was also a significant difference in radical inhibition by the ethanol extracts of the *C.tomentosum* and *L.variegata* at all concentrations. The ethyl acetate extract of the *L.variegata* had significantly higher radical inhibition compared to other extracts. The concentration of *C.tomentosum* and *L.variegata* methanol extracts needed for 50 % inhibition (IC_{50}) was 33.08 and 35.05ug/ml respectively, while ascorbic acid needed 32.47 µg/ml **(Table 1 & 2)**.

The results of ABTS radical scavenging activity of *C.tomentosum* and *L.variegata* at various concentrations are shown in Figure 3a&b. There was a concentration-dependent response in this assay. All the solvent extracts showed great

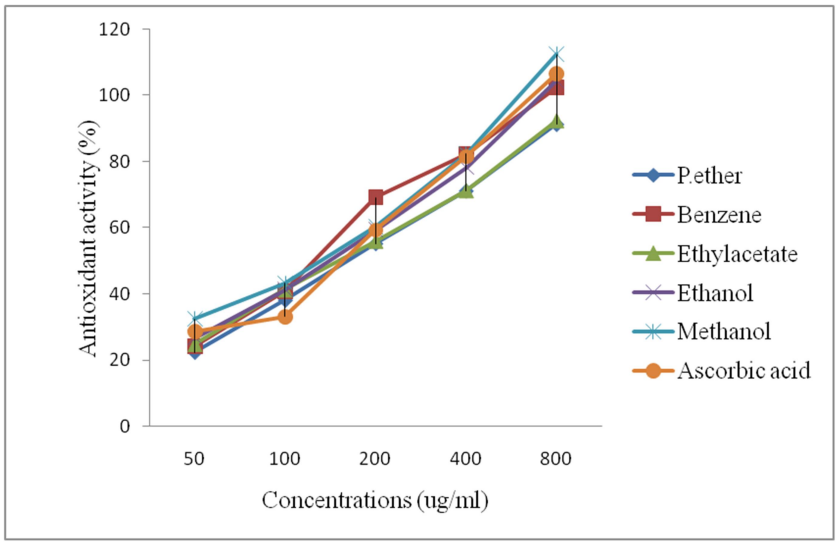
ABTS radical scavenging activity at low concentrations. Methanol extract of the *C.tomentosum* had a significantly higher radical scavenging activity of 130.29% at 800µg/ml. The ethanol extracts of both *C.tomentosum* and *L.variegata* showed no significant difference at all concentrations. A significant difference was observed in the ethyl acetate extract of *C.tomentosum*s at all concentrations. The quantity of *C.tomentosum* and *L.variegata* methanol extract required to produce 50% inhibition of ABTS radical 29.82 and 28.81 µg/ml whereas 26.14 µg/ml **(Table 1 & 2)** needed for trolox.

The superoxide radical inhibitory effect of various crude extracts of *C.tomentosum*and *L.variegata* comparable to the standards (Ascorbic acid) is presented in **Figure 4a & b**. Extracts from three solvents demonstrated a strong scavenging activity against superoxide radical radical compared to standards. There was no significant difference in the radical scavenging activity of the methanol extracts of *C.tomentosum* and *L.variegata* at all concentrations. Methanol extract of the *L.variegata* had a significantly higher scavenging activity of 132.20% compared to methanol extract of the *C.tomentosum* (130.24%). The ethanol extracts of *C.tomentosum* and *L.variegata* showed a significant difference in radical scavenging activity at all concentrations assayed. The IC_{50} value of methanol extract of *C.tomentosum* and *L.variegata* on superoxide radical was found to be 32.82 and 30.81 mg/ml and 29.45 mg/ml for ascorbic acid, respectively **(Table 1 & 2)**.

The reducing power of the *C.tomentosum*and *L.variegata* from the five solvent extracts and standard drugs is shown in **Figure 5a & b**. The reducing power of the solvent extracts on ferric to ferrous gradually increased with increase in concentration. The reducing capacity of methanol extracts of *C.tomentosum* and *L.variegata* within a concentration showed no significant difference in all concentrations tested. Methanol extract of the *L.variegata* had a significantly higher mean reducing power than methanol extract of the *C.tomentosum*.

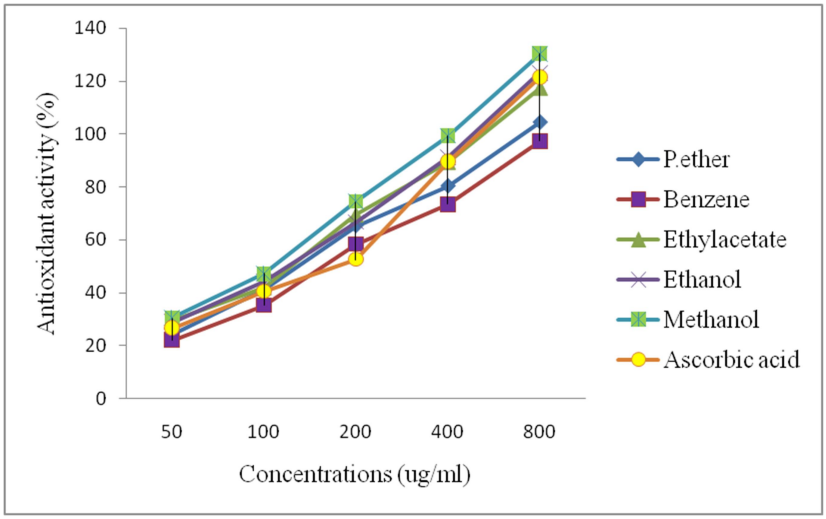


C.tomentosum

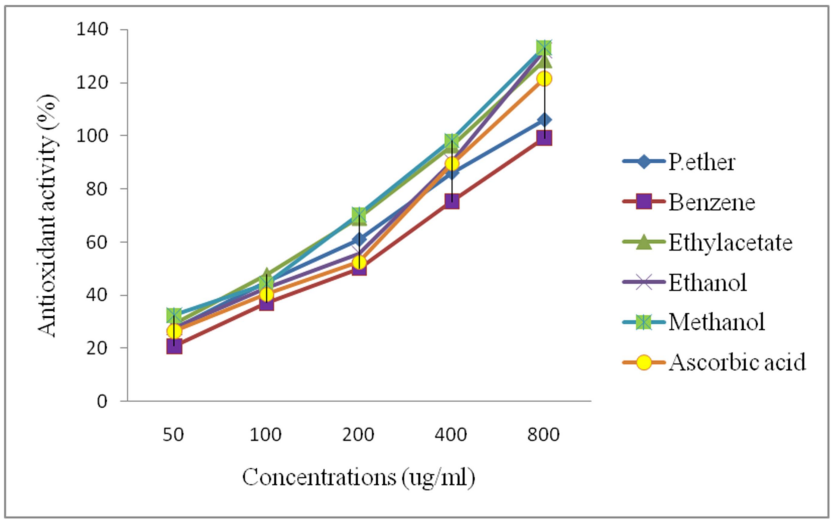


L.variegata

Figure 1: DPPH radical scavenging activity of *L.variegata* and *C.tomentosum*

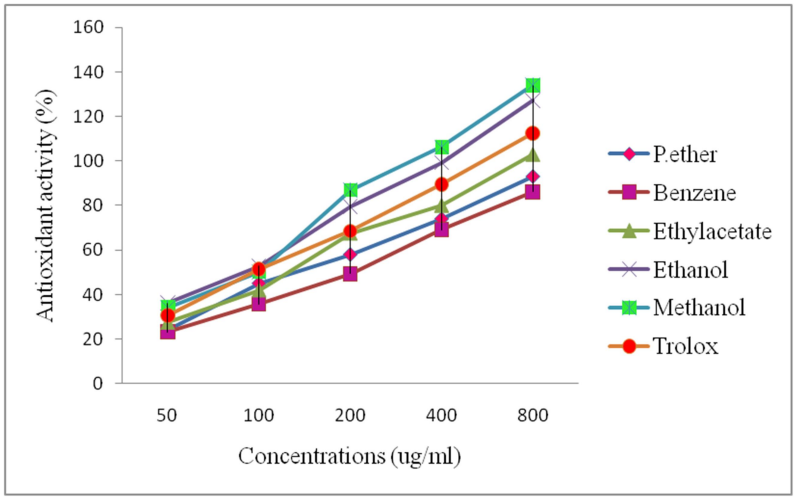


C.tomentosum

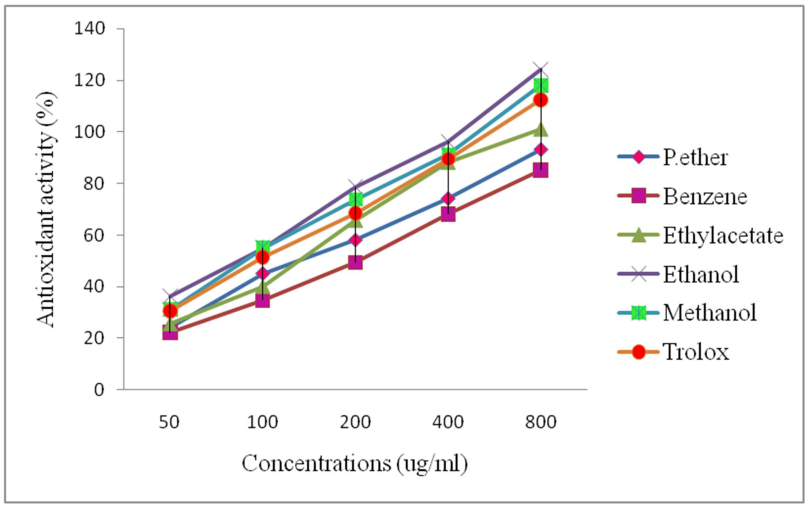


b. *L.variegata*

Figure 2: Hydroxyl radical scavenging activity of *L.variegata* and *C.tomentosum*

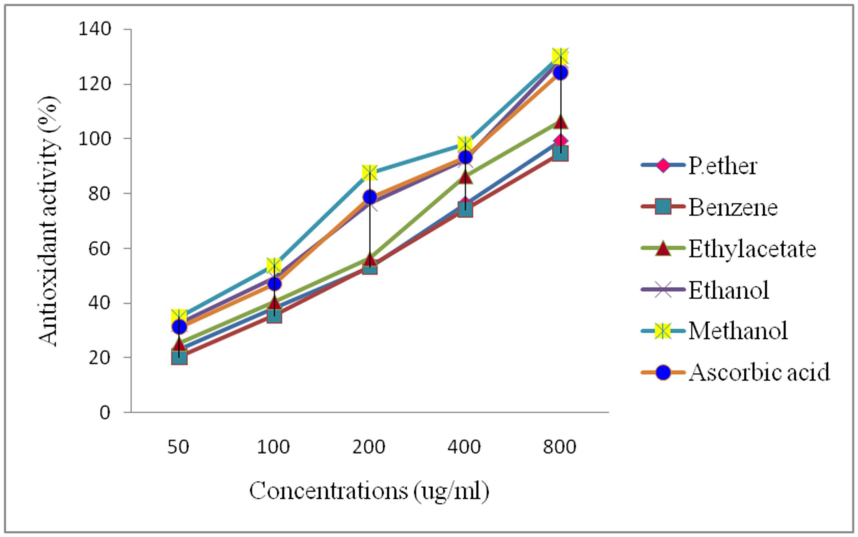


a.*C.tomentosum*

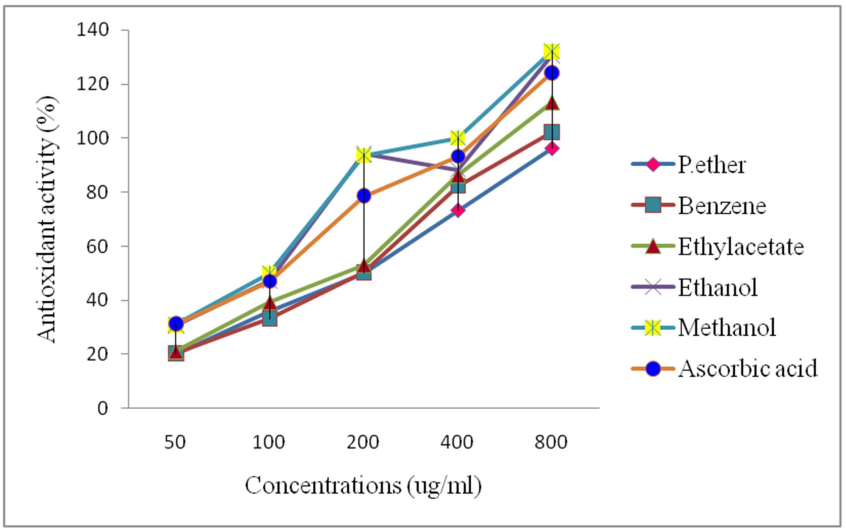


L.variegata

Figure 3: ABTS radical scavenging activity of *L.variegata* and *C.tomentosum*

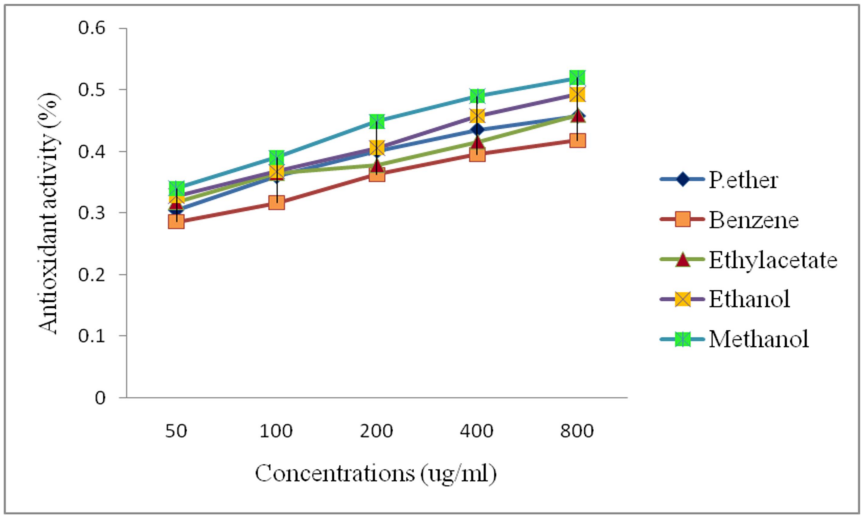


a.*C.tomentosum*

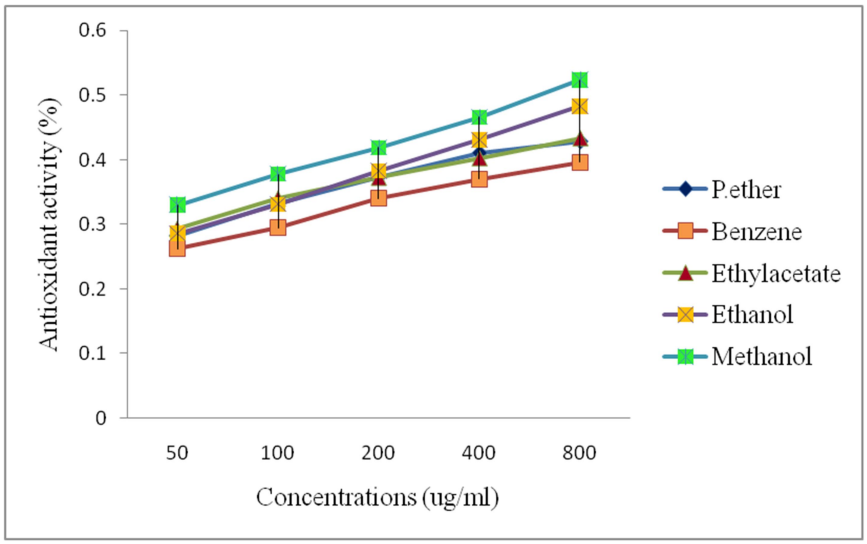


L.variegata

Figure 4: Superoxide radical scavenging activity of *L.variegata* and *C.tomentosum*



a.*C.tomentosum*



b. *L.variegata*

Figure 5: Reducing power assay of *L.variegata* and *C.tomentosum*

Table 1: IC_{50} values of crude extracts of *C.tomentosum*

Tests	IC50 values					
	Petroleum Ether	Benzene	Ethyl Acetate	Ethanol	Methanol	Ascorbic acid/Trolox*
DPPH	28.84	27.99	28.23	36.99	37.56	30.45
Hydroxyl	27.27	25.24	30.24	32.88	33.08	32.47
Superoxide	24.65	23.43	25.10	31.70	32.82	29.45
*ABTS	25.30	22.74	25.02	27.58	29.82	26.14

Table 2: IC_{50} values of crude extracts of *L.variegata*

Tests	IC50 values					
	Petroleum Ether	Benzene	Ethyl Acetate	Ethanol	Methanol	Ascorbic acid/Trolox*
DPPH	28.76	29.98	27.14	30.16	31.48	30.45
Hydroxyl	29.24	28.16	33.21	35.89	35.05	32.47
Superoxide	24.69	23.41	25.09	30.68	30.81	29.45
*ABTS	22.30	20.73	24.73	29.56	28.81	26.14

DISCUSSION

Marine sources are providing to be a viable alternative for bioactive chemicals. Marine algae are now widely utilized as nutritional supplements for both animals and humans, and they are quickly absorbed. Dietary fibres from macroalgae have a wide range of activities, including antioxidant, antibacterial, antimutagenic, anticoagulant, and anticancer properties. The antioxidant activity of *C.tomentosum* and *L.variegata* have been quantified by the DPPH, ABTS, Superoxide radical, Hydroxyl radical and reducing ability.

Antioxidant molecules can quench DPPH free radicals (by providing hydrogen atoms or by providing electrons, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/ bleached product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a

decrease in absorbance at 517 nm. The DPPH method, which dates back nearly 50 years, is commonly used to evaluate antioxidant activity and test the potential of substances to act as free radical scavengers or hydrogen donors. *C.tomentosum* had the highest DPPH activity, which was also significantly different from the *L.variegata*. There was a consistent increase in radical inhibition with a corresponding increase in extract concentration, indicating that the extracts ability to scavenge DPPH radicals is dose dependent. At 800 µg/ml, the inhibitory effects of extract on DPPH radicals increased with increasing concentration (50-800 ug/ml), with the highest inhibition in *C.tomentosum* (129.49%) and the lowest in *L.variegata* (112.41%) **(Figure 1a & b)**. The level of inhibition seen in this study is higher than that reported by Seenivasan *et al*. [16] for *Sargassum wightii* Greville ex J. Agardh, a free radical scavenger with significant scavenging activity. At 2 mg/ml, Foon *et al*. **[17]** found 46.07 and 56.03 % of DPPH activity in *Padina* sp. and *Eucheuma cottonii* Weber-van Bosse, respectively.

The most reactive free radical is the hydroxyl radical, which is generated when superoxide anions and hydrogen peroxides combine with metal ions like copper and iron. Nearly all types of biomolecules, including proteins, DNA, polyunsaturated fatty acids, and nucleic acids, can be damaged by hydroxyl radicals **[18]**. **Figures 2a & b** demonstrated that the scavenging activity of alga extracts and the standard antioxidant (Ascorbic acid) was concentration-dependent, which was consistent with previous findings **[19, 20]**. The researchers discovered that alga extracts were effective in scavenging hydroxyl radicals in a dose-dependent manner. Hydroxyl radicals have been shown to be capable of removing hydrogen atoms from membranes and causing lipid peroxidation. We expected that the selected alga extracts would have antioxidant effects against lipid peroxidation on biomembranes and would scavenge hydroxyl radicals during the initiation and termination stages of peroxy radicals based on the findings.

The $ABTS^+$ assay is a useful technique for determining hydrogen-donating and chain-breaking antioxidants. The ABTS radical is stable and combines with an H-atom donor to produce a non-colored version of ABTS without the need for an intermediary radical. The ability of an antioxidant species to transfer electrons or hydrogen atoms to inactivate the $ABTS^+$ radical cation is reflected in its decolorization **[21]**. A concentration-dependent scavenging of ABTS by the crude methanol extract of *C. tomentosum* was found to be 129.49 percent in the current study. In contrast to normal trolox, the crude methanol extract of both alga

samples demonstrated dose-dependent inhibitory action in the ABTS scavenging assay. The findings of this investigation are consistent with those of previous investigations published by Pandithurai and Murugesan **[22]**; Hadeel *et al*. [23] and Ana Jesus *et al.* **[24]**.

In organisms, including humans, superoxide anion radicals are produced as a result of cellular oxidation. It decomposes to form stronger oxidative species, such as hydrogen peroxide and hydroxyl radicals, through dismutation and other sorts of reactions, while being a relatively weak oxidant **[25]**. Superoxide radicals and their derivatives are toxic to cells, causing DNA and cell membrane damage. As a result, it's critical to scavenge SOA radicals. It is also the source of the free radicals. Figure 4a & b exhibited the scavenging effect of superoxide anion on various algal extracts. Superoxide scavenging activity of *L.variegata* exhibited a maximum of 132.20% inhibition at the concentration of 800µg/ml, which is higher than ascorbic acid (124.12%). Qi *et al*. **[26]** observed considerable superoxide anion scavenging activity in a polysaccharide recovered from *Ulvapertusa* and Kuda *et al*. **[27]** reported strong superoxide anion scavenging activity in four algal water extracts.

Reducing power, which is an index for antioxidant activity, can be measured using a reaction in which Fe 3+ in potassium ferricyanide is converted into Fe 2+. The ferric reducing assay quantifies the ability of an antioxidant capacity to reduce a reactive oxygen species against that species oxidative power. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants **[28]**. As shown in **Figure 5a & b**, reducing power of all the extracts increased in a dose dependent manner. In both *L.variegata* and *C.tomentosum* species, methanol extracts were found to have higher reduction capacity than their extracts. The findings showed that extracts from *L.variegata* and *C.tomentosum* have stronger antioxidant activity. However, more research is needed to isolate the antioxidant components or to determine the biological activity of these extracts in vitro or in vivo.

Conflict of interest

The authors declare that they have no competing interests.

Sources of funding

Nil

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