Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

# Antioxidant activity of the seaweed, *Caulerpa racemosa* - *In vitro* assessment

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## ABSTRACT

Natural sources of antioxidants is a fascinating context of research in recent years. Accordingly more seaweed species are proved to be a promising source of bioactive compounds that possess various biological activities . The present study was designed to evaluate the *in vitro* antioxidant activity of *Caulerpa racemosa*, a green seaweed. The chloroform extract of *Caulerpa racemosa* was studied *in vitro* for scavenging of DPPH, ABTS, hydroxyl radicals, superoxide anions and the ferric reducing antioxidant power. The extract showed an impressive scavenging of DPPH, ABTS, superoxide anion, hydroxyl free radicals and noticeable ferric reducing power. The antioxidant activity increases with increasing amounts of extract. The present study provides evidence that the chloroform extract of *Caulerpa racemosa* is a potential source of natural antioxidant.

**KEYWORDS** antioxidant, *Caulerpa racemosa*, DPPH, ABTS, FRAP, hydroxyl, superoxide anion.

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## INTRODUCTION

Free radicals and reactive oxygen species (ROS) are produced normally for homeostasis and cellular signalling. They are gaining importance since they are involved in many disease conditions including atherosclerosis, cardiac hypertrophy, cancer, rheumatoid arthritis, hypertension, malaria and neurodegenerative disorders[1]. Free radicals, are unstable elements that can take part in any chemical reactions[2]. Reactive oxygen, sulfur and nitrogen species are free to produce superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide. These compounds may cause diseases as diverse as cancer, diabetes, Alzheimer, and Parkinson through inducing oxidative stresses, cell damage and death[2,3].

In all living organisms, reactive oxygen species (ROS) and nitrogen reactive species (NRS) are normally produced with important roles in cellular signalling and homeostasis. Under stressing conditions (e.g., UV radiation, heat exposure, grazing pressure, wounding), the levels of reactive species can increase dramatically, acting as cascade triggers for defence systems which under extreme situations can cause irreversible oxidative damage to all cellular components, including proteins, amino acids, lipids, and nucleic acids[4,5,6,7]. Thus, oxidative stress has been associated with several diseases, such as cancer, hypertension, diabetes, atherosclerosis, neurological and inflammatory disorders[8,9,10,11,12,13,14]. Several synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are commercially available but they are restrained due to their side-effects. Furthermore, they were found to be carcinogenic and toxic in animal models[18]. These explorations augmented the necessity to identify natural antioxidants that are safe and inexpensive. The evolution of alternative natural antioxidants that are from plant source, is of great importance for human health and holds reasonable commercial potential[19].

Recent studies reveal that seaweeds are rich in bioactive compounds in the form of carotenoids, phycocyanins, polyphenols, phycobilins, polysaccharides and vitamins. They are also rich in minerals, polysaccharides, proteins and vitamins along with antioxidant activity which would elevate their value in the human diet as food and pharmaceutical supplements[20]. Strong antioxidant properties have been demonstrated in marine algal extracts[21,22], protective effects against carbon tetrachloride induced liver injury[23], antimicrobial activity[24], anti-proliferative activity towards He La cells[25], and antiviral properties[26]. Seaweeds are traditionally consumed due to its high nutritional value, apart from that they are now considered as a rich source of antioxidants[27].

*Caulerpa racemosa* (Forsskål) J. Agardh 1873 is a green alga belonging to the family Caulerpaceae and order Bryopsidales, which mainly grows in tropical regions, although some varieties may be found in subtropical regions[28]. It is usually served raw as salad or eaten cooked in

South East Asian countries and also used as animal feed. In folk medicine, this macro algae is used to treat hypertension and rheumatism[29]. The bio-efficacy of this alga is antibacterial and larvicidal[30] and antioxidant & ant proliferative values[31]. In view of the above, the present study was to evaluate the in vitro antioxidant activity of the seaweed.

## MATERIALS AND METHODS

### *Collection and processing of seaweed material*

Fresh samples of the macro algae, *Caulerpa racemosa* were collected from the Southeast coast of India mainly in the intertidal region of Mandapam, Ramanathapuram District, Tamil Nadu (9° 22' N, 78° 52 ' E) (Figure.1). The collected seaweed samples were washed thoroughly with tap water followed by distilled water until the debris and associated epiphytes were removed. After subsequent washing, the seaweed was shade dried for 3-weeks. Then the dried seaweed was powdered in a blender and stored for further use.

**Figure1 Dried seaweed, *Caulerpa racemosa***

### *Preparation of seaweed solvent extracts*

About 10g of powdered seaweed material was soaked in dichloromethane and chloroform, respectively for 3days at room temperature with mild shaking. Then the solvent was filtered with Whatman filter paper (125mm). This was repeated 3-4 times until the extract turned colorless. The extracts were collected and stored in refrigerator at 4$^{o}$C for further analysis.

### *Determination of Antioxidant activity*

The antioxidant activity of the seaweed extract was characterized by five complementary biochemical methods to accurately determine the antioxidant potential. The mechanism responsible

for antioxidant is different for each method used. Therefore, different antioxidant assays and extract concentrations (0.2, 0.4, 0.6, 0.8 and 1mg/ml) were analyzed. All antioxidant assays were performed in triplicates, and the absorbance was read with a UV-vis microplate spectrophotometer (Epoch Biotek, USA). Results are expressed as antioxidant percentage and calculated from the standard curve. Ascorbic acid is an antioxidant, which is used as a standard in all the antioxidant assays.

### *DPPH· radical scavenging assay*

The antioxidant activity of the seaweed extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH· (1, 1-Diphenyl-2-picryl-hydrazyl) according to the method of Braca et al, (2001). Samples and standard (ascorbic acid) were taken in various concentrations and the volume was adjusted to 100µL with methanol. About 3mL of 0.1mM methanolic solution of DPPH· was added to the aliquots of samples and mixed well. Negative control was prepared by adding 100µL of methanol in 3mL of 0.1mM metabolic solution of DPPH·. The tubes were allowed to stand in dark for 30mins at room temperature. The absorbance of the sample was measured at 517nm against the blank.

### *Ferric Ion Reducing Power (FRAP) assay*

FRAP assay is based on the ability of the antioxidants to reduce Fe3+ to Fe2+ in the presence of 2,4,6- tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe2+ -TPTZ complex with an absorption maximum at 593nm (Dudonné et al., 2009). Different concentration of sample extract and standard (Ascorbic acid) is added to 2.7mL of FRAP reagent (10 parts of 300 mM acetate buffer (pH-3.6), 1 part of 10mM TPTZ solution and 1 part of 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution) and the reaction mixture is incubated at $37^0$C in dark for 30 min. The absorbance of the samples and control was measured at 593 nm. The antioxidant capacity of based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mM.

### *$ABTS^{\cdot+}$ radical scavenging activity*

The $ABTS^{\cdot+}$ [2,2′-azino-bis(3ethylbenzthiazoline-6-sulfonic acid)] radical cation scavenging activity of the seaweed extract was determined according to Rufino et al (2007). Briefly, 7.0mM ABTS was reacted with 2.45mM potassium persulfate for 12-16h in the dark to yield the $ABTS^{\cdot+}$ radical cation. The $ABTS^{\cdot+}$ radical cation was diluted with 50% ethanol for an initial absorbance of ≈0.700 ± 0.05 at 734nm. The photometric assay was conducted by adding 0.9mL of ABTS solution to different concentrations of samples and incubated for 15min. Absorbance was read at 734 nm after

15-min incubation in the dark. The antioxidant activity of the test samples was calculated by the following equation:

$$\text{Antioxidant activity} = [(A_c - A_t)/A_c] \times 100,$$

where $A_c$ is the control absorbance of ABTS, and $A_t$ is absorbance of the sample. It is expressed as µmol.

## *Superoxide anion radical scavenging activity*

The superoxide anion radical scavenging activity of the sample was detected according to the method[33]. Briefly, 0.1 ml of extract was mixed with 1 ml nitrobluetetrazolium (NBT) solution (156 µM in 0.1 M phosphate buffer, pH 7.4) and 1 ml NADH solution (468 µM in 0.1 M phosphate buffer, pH 7.4). The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 µM in 0.1 M phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 5 min, and the absorbance was measured at 560 nm in spectrophotometer against blank sample (phosphate buffer).

## *Hydroxyl radical scavenging activity*

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compound by Fe3+–Ascorbate–EDTA–$H_2O_2$ system (Fenton reaction) according to this method. The generation of OH is detected by its ability to degrade deoxyribose to form products, which on heating with TBA forms a pink colored chromogen. Reaction mixture contained 60 µl of 1.0 mM FeCl2, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer, 150 µl of 0.17 M H2O2, and 1.5 ml of sample at various concentrations. The reaction was initiated by the addition of $H_2O_2$. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity was calculated.

$$\% \text{ Hydroxyl scavenging activity} = (A_0 - A_{1)} / A_0 \times 100$$

where $A_0$ was the absorbance of the control (without sample) and $A_1$ was the absorbance of the sample or standard.

## *Statistical analysis*

All the experiments in this study were done in triplicates and the results are expressed as means ± SD.

## RESULTS

### *DPPH· radical scavenging activity*

The DPPH· radical scavenging activity is a sensitive antioxidant assay and is independent of substrate polarity[34], this model is widely used to evaluate antioxidant activities in a relatively short time compared with other methods. Fig 1 shows the DPPH scavenging activity of chloroform extract of *Caulerpa racemosa*. At a concentration of 0.8 and 1mg/ml, the chloroform extract have a 69% and 89% of DPPH scavenging activity as compared to a 80% and 99% decrease with Ascorbic acid, which is used as positive control.

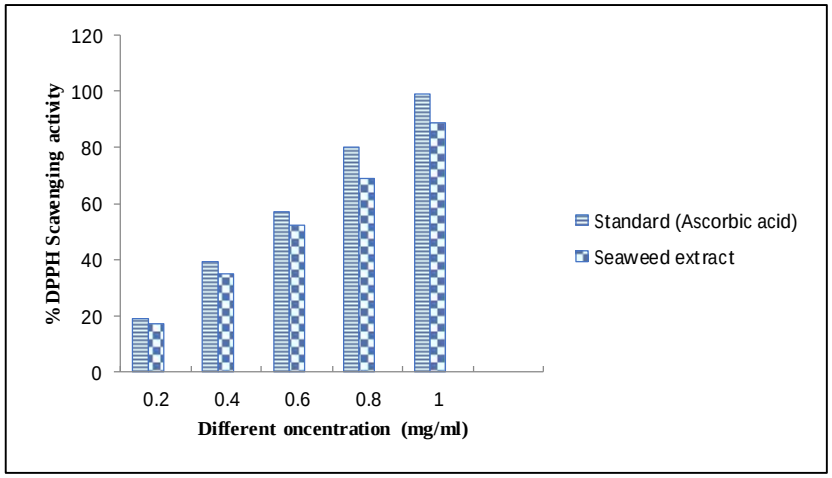


**Fig 2 DPPH radical scavenging activity**

### *ABTS·+ radical scavenging activity*

The free radical scavenging activity involves the reduction of pre-formed radical cation ($ABTS^{\cdot+}$) to ABTS which is proportional to the antioxidant activity. Generation of radical cation ($ABTS^{\cdot+}$) involves the reaction between potassium persulfate and ABTS. Fig 3 shows the ($ABTS^{\cdot+}$) radical scavenging activity of chloroform extract of *Caulerpa racemosa*. At a concentration of 0.8 and 1mg/ml of the seaweed extract, the reduced ABTS was around 72µmol and 78µmol as compared to a 85µmol and 98µmol ABTS by Ascorbic acid.

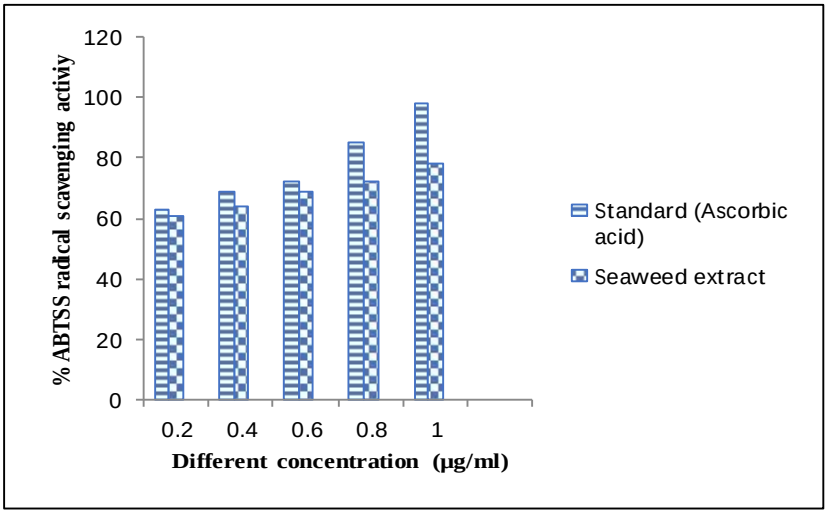


**Fig 3 ABTS$^{+}$ radical scavenging activity**

## *Ferric Ion Reducing Power (FRAP) assay*

The FRAP is another antioxidant assay that is measured using a reaction in which Fe $^{3+}$ is reduced to $Fe^{2+}$. Fig 4 shows that the ferric reducing antioxidant power of the extract increased in a concentration independent manner. The ferric ion reducing antioxidant potential (FRAP) of the seaweed extract was estimated from their ability to reduce TPTZ-Fe (III) to TPTZ-Fe (II). At a concentration of 0.8 and 1mg/ml the FRAP value was 633mM and 837mM Fe (II)/g as compared to 793mM and 992 mM Fe (II)/g in case of the standard, Ascorbic acid.

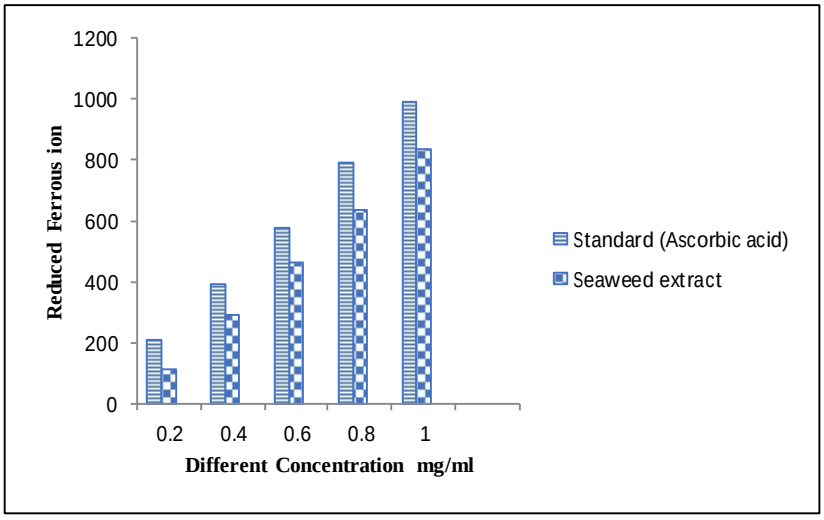


**Fig 4 Ferric Reducing Antioxidant Power**

## *Superoxide anion radical scavenging activity*

The super oxide anion radical scavenging activity of the seaweed extract was shown in the fig 5. The decrease in absorbance at 560 nm with the seaweed extract and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture.

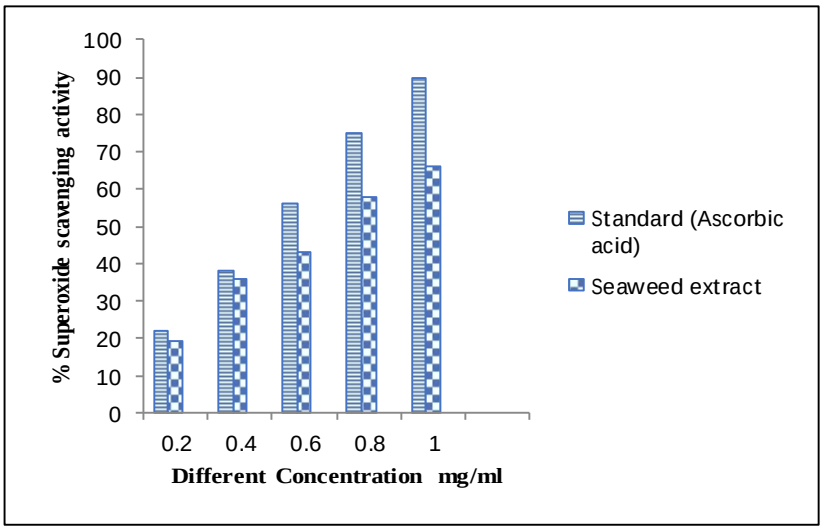


**Fig 5. Superoxide anion radical scavenging activity**

## *Hydroxyl radical scavenging activity*

This assay shows the abilityy of the extract to inhibit hydroxyl radical-mediated deoxyribose degradation in an $Fe^{3+}$-EDTA -ascorbic acid and $H_2O_2$ reaction mixture. The results shown in figure 6 indicates that the percentage of hydroxyl scavenging activity of the seaweed.

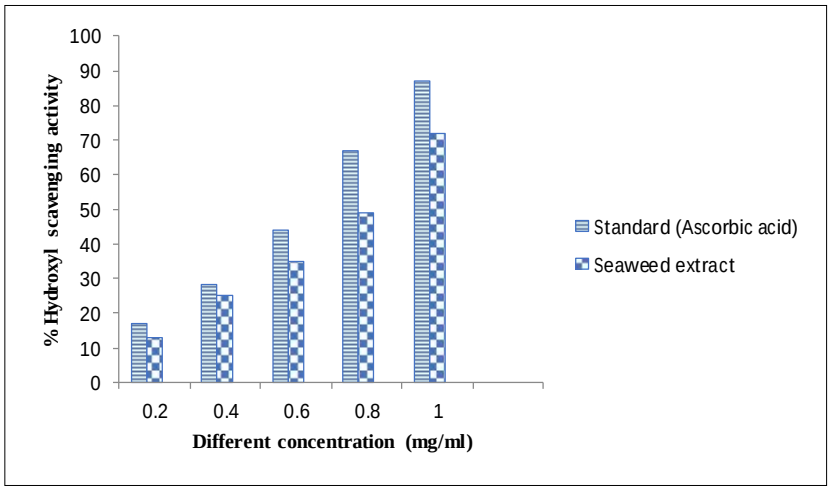


**Fig 6 Hydroxyl scavenging activity**

## DISCUSSION

In living systems, free radicals are constantly generated which interacts with other molecules within the cells. This can cause oxidative stress and damage to tissues and bimolecular leading to various diseases, especially degenerative diseases and extensive lyses. Many commercially available antioxidant drugs possess significant side effects. Traditionally, medicinal plants are used to treat various diseases, but in the middle of the $20^{th}$ century, the use of medicinal plants was reduced to one fourth due to the overuse of synthetic chemicals to treat diseases. Now the situation is reversed, the researchers have considerable interest and are keen in finding natural sources to replace the synthetic ones.

Marine algal extracts have been demonstrated to possess strong antioxidant properties[35]. The chloroform extract of the seaweed posses strong antioxidant activity which is the outcome of the different antioxidant assays performed in this study.

The effect of antioxidants on DPPH radical scavenging is due to the hydrogen donating ability. The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from the antioxidant. In the present study, the chloroform extract of the seaweed showed a concentration dependent antioxidant potential when compared with standard ascorbic acid. The antioxidant potential may be attributed to their free radical scavenging ability.

The free radical scavenging activity was determined by ABTS radical cation decolourisation assay. $ABTS^{\cdot+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate. This pre-formed radical cation was reduced to ABTS by the addition of the seaweed extract, in a concentration-dependent manner. The results were compared with those obtained using the standard ascorbic acid and the amount of ABTS present demonstrates that the extract is a potent antioxidant.

The reducing power of a compound may serve as a significant indicator for a potential antioxidant. In the ferric reducing antioxidant power assay, the antioxidants in the sample reduce $Fe^{3+}$ to blue coloured $Fe^{2+}$ that is monitored by measuring the absorbance. As shown in figure 3, the reducing power of the seaweed extract was as good as the standard ascorbic acid that indicated that the antioxidant compounds are electron donors.

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. It is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping the chain reaction of mitochondria directly react with oxygen and form superoxide anion. Superoxide anion is also very harmful to cellular components. The superoxide radical generated from dissolved

oxygen by PMS-NADH coupling was measured by their ability to reduce NBT to NBT diformazan via superoxide radical. All the concentrations showed scavenging activity on the superoxide radicals.

Hydroxyl radicals are the major active oxygen species causing lipid per oxidation and enormous biological damage. They were produced in this study by incubating ferric-EDTA with ascorbic acid and $H_2O_2$, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. The generation of OH is detected by its ability to degrade deoxyribose to form products which on heating with TBA forms a pink coloured chromogen. When the chloroform extract of the seaweed was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The hydroxyl scavenging activity of the seaweed extract was effective in a concentration dependent manner. Thus the seaweed extract was able to prevent damage to the deoxyribose OH radicals.

## CONCLUSION

In summary, the present study investigates the antioxidant potential of the chloroform extract of the seaweed, *Caulerpa racemosa* by various antioxidant assays, that focus on removing free radicals. The seaweed extract have the ability to quench DPPH and ABTS when compared to the standard, ascorbic acid. The antioxidant activity was further demonstrated by its reducing activity. The seaweed extract also exhibited scavenging activity of both superoxide anion and hydroxyl radical. Further the study reveals that the seaweed has a remarkable antioxidant potential as compared to the standard ascorbic acid.

## ACKNOWLEDGEMENTS

The authors are grateful to the Dean and Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University for providing the necessary facilities to carry out this research work.

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