Research Article

Bioprotective Properties of Algae *Caulerpa racemosa*: *In-vitro* Antimicrobial and Antioxidant Potential

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ABSTRACT

The antibacterial and antioxidant activity of green algae *Caulerpa racemosa* was collected from Kilakarai region located between 9.23135° N, 78.7844° E, Ramanathapuram District, Tamil Nadu, India. The algal extract was prepared from different solvents namely aqueous, ethanol, methanol, acetone and was tested for their antibacterial activity against three human pathogens and two fish pathogens using disc diffusion methods and antioxidant properties such as DPPH radical scavenging activity and ABTS radical scavenging activity and hydroxyl radical scavenging activity. The antibacterial activity showed effective inhibition against human pathogenic bacteria. The highest zone size (22.00±1.0mm) was observed in human pathogen *Staphylococcus aureus* and the fish pathogen was in *Aeromonas hydrophila* (20.0±0.5mm). The antioxidant potential of the ethanol extract was significantly higher in DPPH radical scavenging activity (75.35µg/ml), ABTS radical scavenging activity (80.33µg/ml) and hydroxyl radical scavenging activity (77.13 µg/ml). This study indicated the potential use of green algae, in particular, *Caulerpa racemosa* indicating that these extracts may apply potential antibacterial compounds or as a health-promoting feed for aquaculture and natural antioxidant.

Keywords: *Caulerpa racemosa*, Antioxidant, DPPH, ABTS, BHT, ROS.

INTRODUCTION

The diversity of marine organisms has become an inspiration for researchers to identify novel marine natural products that could eventually be developed into therapeutics or pharmaceutical products. In fact, many structurally diverse natural products isolated from marine organisms are reported to exhibit an amazing array of biological activities, particularly antibacterial, antiviral, antifungal, anticancer, antioxidant and anti-inflammatory activities. Seaweeds act as probable bioactive compounds that can be used for pharmaceutical applications.[1, 2]

Marine seaweed have been also known as a potential source of antioxidants[3, 4] and usually consumed as a readily available whole food, especially surrounded by coastal communities.[5, 6] Fatty acids, enzymatic and non-enzymatic antioxidant properties of having been investigated in *Caulerpa* species.[7-9] Macroalgae also contain bioactive substances like polysaccharides, proteins, lipids, minerals, certain vitamins and polyphenols, with antibacterial, antiviral, and antifungal properties.[10, 11]

Marine organisms inhabit intertidal areas which are considered harsh environments due to tidal fluctuations.[12, 13] These environmental stresses would ultimately lead to the generation of free radicals and reactive oxygen species (ROS). However, marine organisms are usually protected and unharmed despite the presence of these stresses due to the presence of protective mechanisms mediated by enzymes or antioxidant compounds.[12, 14] Reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and result in extensive oxidative damage which can lead to age-related degenerative diseases.[15, 16]

In our current study, *Caulerpa racemosa* was evaluated for its antibacterial efficacy of human and fish pathogens and *in vitro* antioxidant of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), 2, 2 -azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS) and hydroxyl radical scavenging activity using different solvents of aqueous, ethanol, methanol and acetone extracts.

MATERIALS AND METHODS

Collection of seaweed material

Green algae used in this study *Caulerpa racemosa,* were freshly collected from the Kilakarai region located between 9.23135° N, 78.7844° E, Ramanathapuram District, Tamil Nadu, India. Seaweed taxonomically identified and the voucher specimen was stored in the Central Marine Fisheries Research Institute (CMFRI), Tuticorin, Tamil Nadu, India. The collected samples were washed in running water for 10 min, transported to the laboratory and shade dried (35±3 °C) for 36 h. The shade dried seaweeds were powdered and used for further experiments.

Preparation of seaweed extracts

The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples (5 g) and packed in Soxhlet apparatus and extracted successively with aqueous, ethanol, methanol and acetone for 10 h.[17] The crude extracts were weighed and deep-frozen (-20 °C) until tested.

Antibacterial activity

Test microbes

The human pathogenic bacterial strains are *Escherichia coli* (MTCC 2939), *Pseudomonas aeruginosa* (MTCC 2453) and *Staphylococcus aureus* (MTCC 9706) and fish pathogens, *Aeromonas hydrophila* (MTCC 1739) and *Vibrio vulnificus* (MTCC 1146) were used for this experiment. The pathogens were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTEC), Chandigarh, India. It was subcultured and used for the antibacterial assay.

Disc diffusion method

The antibacterial activity of seaweed extracts was assessed by the disc diffusion technique.[18] Mueller Hinton agar (MHA) plates were prepared and individually swabbed with pathogenic bacteria. The sterile discs (6 mm) were placed over the surface of the agar plates. Preparation of seaweed extract (1 mg/ml) was added on the discs at various concentrations (50, 100, 250 and 500 µg/ml). A disc containing standard concentrations of the antibiotic Ciprofloxacin (20 µg/disc) was used as positive control. The agar plates were incubated for 24 h at 37 ºC and the inhibition zones were measured in millimeter and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

Statistical analysis

All the values were expressed as Mean ± Standard Deviation (SD). The statistical significance was evaluated by two-ways Analysis of Variance (ANOVA) using SPSS version 20 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Post-hoc analysis, Duncan.[19]

***In vitro* antioxidant activity**

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated using the method of Liyana-Pathirana and Shahidi.[20] Appropriate dilutions of the extracts (1 mg/ml) was mixed with, 1 ml of 0.135 mM methanolic solution of DPPH radical. Absorbance was measured at 517 nm after 30 min of reaction. BHT was used as reference standard and the inhibition percentage was calculated using the following formula:

Percentage of inhibition= [(Abs $_{control}$-Abs $_{sample}$/Abs $_{control}$)] × 100

2, 2 -azino-bis (3-ethylbenzthiazoline)-6- sulfonic ($ABTS^{+}$) radical scavenging activity

Determination of 2, 2 -azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS) radical scavenging ability of algal extracts was carried out by the method of Re *et al*.[21] Previously, 7 mM ABTS solution and 2.4 mM potassium persulphate solution were prepared separately. Equal amount of two stock solutions were mixed and allowed to stand for 12 h in dark at room temperature. About 1 ml of diluted ABTS.+ solution react with seaweed extracts (1mg/ml) after 10 min the absorbance was measured UV-spectrophotomatrically at 734 nm against the blank solution. ABTS free radical inhibition was calculated by following

Percentage of inhibition= [(Abs $_{control}$-Abs $_{sample}$/Abs $_{control}$)] × 100

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined according to a slightly modified method of the 2-deoxy-ribose oxidation methods.[22] Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. A reaction mixture containing 0.2 ml each of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA and 10 mM 2-deoxyribose was mixed with 0.2 ml of the extracts solution, and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture until the total volume reached 1.8 ml. Then 0.2 ml of 10 mM H_2O_2 was finally added to the reaction mixture and incubated at 37° C for 4 h. After the incubation 1 ml of 2.8% TCA (trichloracetic acid) and 1.0% TBA (thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. Absorbance was measured at 532 nm.

% Radical scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$)/(Abs $_{control}$)] × 100

RESULTS AND DISCUSSION

The different solvent extracts of *Caulerpa racemosa* (Table 1) exhibited the inhibition activity of human pathogens, the maximum zone was found in the ethanol extract against *S. aureus* (22.0±1.0) followed by *P. aeruginosa* (20±0.5) and *E. coli* (18.0±0.5) at 500µg concentrations. The ethanol extract was observed to be statistically significant with an efficient yield of higher inhibitory activities against human pathogens compared to methanol, acetone and aqueous. Further from the results obtained it was observed that all the extract prepared with ethanol could record higher inhibitory activities against *S. aureus.* Shimaa *et al.*[23] reported the inhibition activity of *S. vulgare* against *P. aeruginosa* and *K. pneumonia*. The highest antimicrobial activity was in ethanol extract against *K. pneumoniae*. The present study concurs with Karthikaidevi *et al.*[24] stated that among the solvents, ethanol extract of seaweeds exhibited a higher zone of inhibition against both Gram-positive and Gram-negative bacteria. About 100% inhibition activity was obtained by ethanolic extracts of *C. tamaricifolia.*[25] The maximum inhibition was noticed with ethanol extract of S. *lanceolatum*[26] and *S. ilicifolium*[27] against different bacterial strains. Matloub and Awad[28] described the different extracts of *Sargassum* spp. viz., *Sargassum asperifolium*, S. *lanceolatum*, *Sargassum dentifolium* and *Sargassum linifolium* have various antimicrobial activities and can act as promising natural sources of bioactive products. Dhanya *et al*.[29] give evidence that the antimicrobial activity of *Ulva reticulate* extracts against *Staphylococcus aureus,*

Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and *Bacillus subtilis*. Cox *et al.*[30] experienced 40 different seaweeds; of these, seven species revealed high antimicrobial activity against multi-resistant pathogens. Shanmugapriya *et al.*[31] revealed that the tested seaweed *G. corticata* was highly active against Gram-negative bacteria than Gram-positive bacteria. Kolanjinathan *et al.*[32] found that ethanolic extract of *G. edulis* inhibited *E. coli, P. aeruginosa, S. aureus* and *S. faecalis.*

The different solvent extracts of *Caulerpa racemosa* displays the inhibition activity of fish pathogens, the ethanol extract shows the highest zone of inhibitory activity against *A. hydrophila* (20.0±0.5), followed by *V. vulnificus* (16.0±1.0) at 500µg concentration (Table 1). The ethanol extract was observed to be statistically significant with an efficient yield of higher inhibitory activities against fish pathogens compared to methanol, acetone and aqueous. The constant usage of antimicrobial agents in aquaculture has increased more resistant bacterial strains in aquatic environs and may to create risks to consumers.[33] Subsequently early periods, marine plant extracts have been used for treatments of communal infectious diseases, treatments with plants having antibacterial activity are a potential beneficial alternate in aquaculture.[34] Different strains of *V. harveyi* and *V. vulnificus* were weakly inhibited by the ethanol extract of *Asparagopsis taxiformis* from the Sicily Channel.[35] Ethanol was also used for thalli of *Gracilaria fisheri* from India, the extract of which showed a high inhibition against *V. harveyi.*[36] Previous studies of some marine algae belonging to selected species of marine benthic brown; red algae collected from different coastal areas of Alexandria (Egypt) were investigated for their antibacterial and antifungal activities against fish pathogens. Screening of organic solvent extracts from several marine macroalgae, including *Pterocladia capillacea* showed specific activity in inhibiting the growth of five virulent bacterial strains affected by fish species such as *Vibrio anguillarum, Pseudomonas fluorescens* and *Aeromonas hydrophila.*[37, 38]

Free radicals are formed in normal cell metabolism.[39] Quenching these free radicals entails donating electrons and protons, and converting them into more stable and less damaging species by antioxidants. There is no single, widely acceptable assay method for evaluating antioxidant potential, and therefore it is essential that more than one antioxidant assay is performed for evaluating the antioxidant properties of any plant material.[40] 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *Caulerpa racemosa* different solvent extracts such as aqueous, ethanol, methanol and acetone and the synthetic antioxidant (BHT) compounds are shown in Figure 1. The ability of algal extracts to scavenge the reactive metabolites would inhibit the formation of primary and secondary amines oxidation products. In this investigation, *C. racemosa* extracts shows the maximum DPPH radical scavenging activity was increased as the concentration increased (P<0.05). However, significantly higher inhibition activity was in ethanol extract (75.35±2%) followed by methanol (71.76±1.3 %), acetone (66.51±0.9%) aqueous (62.89±1.8%) and compared to BHT (87.21±1.8). The previous reports in the literature of the antioxidant ability of algae, alcoholic and aqueous extracts of seaweeds have been evaluated for DPPH activity.[41, 42] Devi *et al.*[43] mentioned that DPPH radical scavenging ability differed very much and their variety between 5% and 72.5% for *C. hornemanni* and *G. acerosa*, respectively. Souza *et al.*[44] reported that ethanolic extracts of *G. birdiae* and *G. cornea* exhibit the best performance showing a high scavenging activity of 60%. Ethanol extracts indicate their enhanced level of antioxidant and scavenging activity from *E. compressa* and *C. fulvescens* were the most efficient DPPH scavengers.[45]

The ABTS radical scavenging assay is a tool for assessing the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants.[46] The results of the scavenging activity of the algal extracts against ABTS+ radical scavenging activities of *C. racemosa* compared with BHT at 20-100 µg/ml concentrations (Figure 2). ABTS+ radical scavenging activity of all extracts increased as the concentrations increased (P<0.05). It was observed that the ethanol extract showed higher activity than methanol, acetone and aqueous extracts. The percentage inhibition of ABTS+ radicals by ethanol extract reached up to 80.33±0.57 % at the concentration of 100 µg/ml, whereas the methanol (73.28±1.54), acetone (70.14±1.21) and aqueous (58.52±0.5) extracts showed at the same concentration respectively. Previously reported to the $ABTS^{\cdot+}$ scavenging capacities of the EtOAc fraction of *H. musciformis* was stronger than other two red seaweeds *H. valentiae* and *J. rubens* MeOH extracts and fractions.[47] The green macroalgae extracts could provide valuable and promising source of natural antioxidants, which could decrease or retard the risk of cancer, inflammation, obesity, etc. and can replace synthetic antioxidants. The high content of these phytochemicals explain its high radical scavenging activity.[48]

Hydroxyl radical scavenging activity assay was employed to understand the scavenging potential of different solvent fractions from seaweed extracts against short-lived radicals, viz., HO. radicals. HO. radicals were reported to abstract H- atoms from lipid membranes, and thus bring about peroxide reactions of lipids. Hydroxyl radical scavenging activity of the various solvent extracts of *C. racemosa* was analyzed using Fenton reaction (Fe^{2+}+ H_2O_2 —›Fe^{3+} +OH^- + OH) and these outcome were expressed as the inhibition rate. Hydroxyl radical scavenging activities of the various solvent extracts are shown in Figure 3 and these activities were compared to that of BHT. The higher level of inhibitory activity was recorded in ethanol extract (77.13 %) followed by methanol extract (72.23 %), acetone extract (67.24 %) and aqueous extract (57.88 %) extract compared to BHT (84.71%) at 100µg/ml concentration respectively**.** Cho *et al*.[49] reported that the ethanol extract of *E. prolifera* had profound DPPH and hydroxyl radical scavenging activity as well as reducing power compared to the commercial antioxidants such as BHA and α-

tocopherol. Many other species of seaweeds were also reported in literature to possess potential H_2O_2 scavenging activity.[50] these natural antioxidants are also found in marine algae, such as seaweeds. The present study indicates the high capacity of seaweed extracts as effective sources for antimicrobial and antioxidative potential.

Table 1: Antibacterial activity of *Caulerpa racemosa* extracts against human and fish pathogens (Mean ± SD, n = 3).

Solvents	Concentrations (µg/ml)	Zone of inhibition (mm)				
		Human Pathogens			*Fish Pathogens*	
		E. coli	*P. aeruginosa*	*S. aureus*	*A. hydrophila*	*V. vulnificus*
Aqueous	50	9.5±0.5	8±0.5	7±0.5	7.00±0.0	8.0±0.5
	100	10.5±0.5	9±0.5	10±0.5	11.0±0.5	9.0.5±0.5
	250	11.0±0.5	11±1.0	12±1.0	12.0±0.5	12.5±0.5
	500	13±0.5	14.66±0.5	18.0±0.5	14.0±1.5	13.5±1.1
	C	23.0±1.0	22.0±0.5	19.0±0.5	22.5±0.5	23.0±1.0
Ethanol	50	7.0±0.5	7.0±0.0	7.0±0.5	11±1.5	7.0±0.5
	100	9.5±0.5	8.5±0.5	13.0±0.5	15.0±1.0	12.6±0.5
	250	16.0±0.5	15.0±0.5	18.0±0.5	17.5±0.5	15.6±1.5
	500	18.0±0.5	20±0.5	22.0±1.0	20.0±0.5	16.0±1.0
	C	23.0±0.5	24.0±1.0	22.33±2.0	24.6±0.5	24.0±1.0
Methanol	50	8±0.0	10.66±0.5	6.0±0.0	7±0.5	6.0±0.5
	100	9±0.5	13.66±0.5	9±0.5	8.0±0.5	9.0±0.5
	250	11±1.0	13.66±0.5	11±1.0	12.6±0.5[c]	10.00±1.0
	500	17.66±0.5	15.0±1.1	12.0±0.5	17.6±0.5	13.66±0.5
	C	20.0±1.0	22.0±0.5	21.0±1.0	23.00±1.0	24.00±1.0
Acetone	50	9.0±1.0	10.5±0.5	6.0±0.0	10.33±0.5	9.0±0.5
	100	12.5±0.5	13.0±0.5	9.0±0.5	13.6±0.5	10.6±0.5
	250	14.0±0.5	18.0±0.5	10.0±0.5	16.66±0.5	13.6±0.5
	500	18.0±0.5	20.0±0.5	12.0±0.5	21.66±0.5	17.66±0.5
	C	22.5±0.5	23.0±0.5	22.0±1.0	25.00±1.0	20.66±0.5

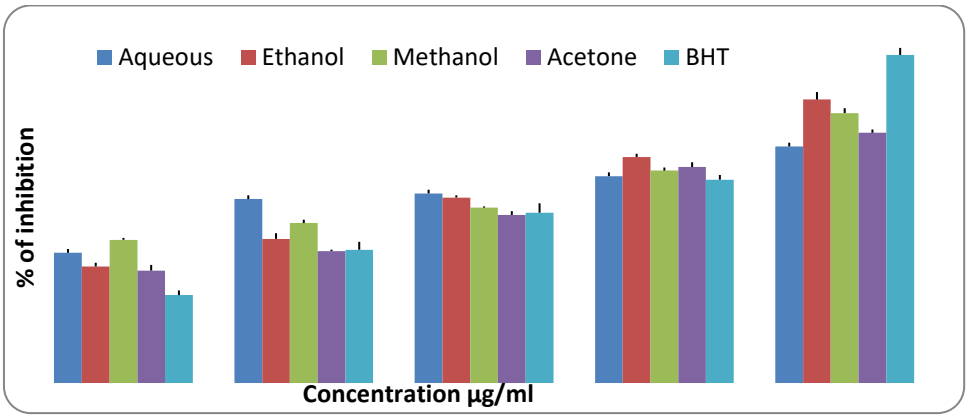

Figure 1: 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *Caulerpa racemosa* extracts (Mean ± SD, n = 3).

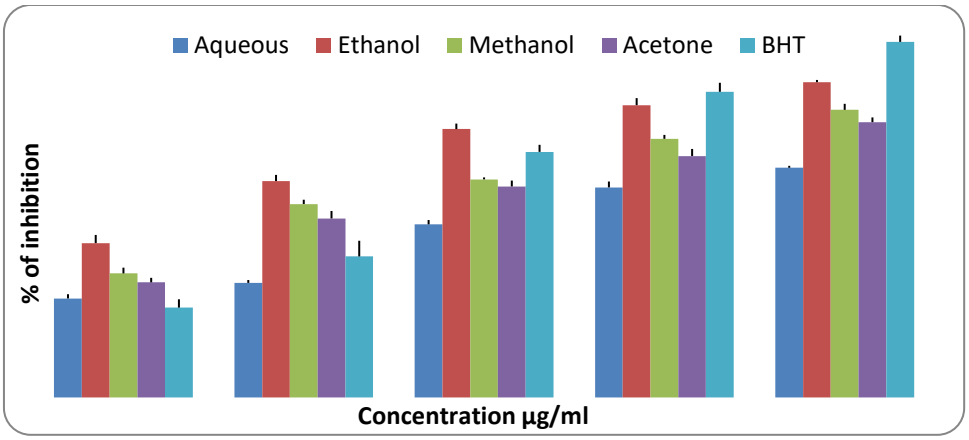

Figure 2: 2, 2 -azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS) radical scavenging activity of *Caulerpa racemosa* extracts (Mean ± SD, n = 3).

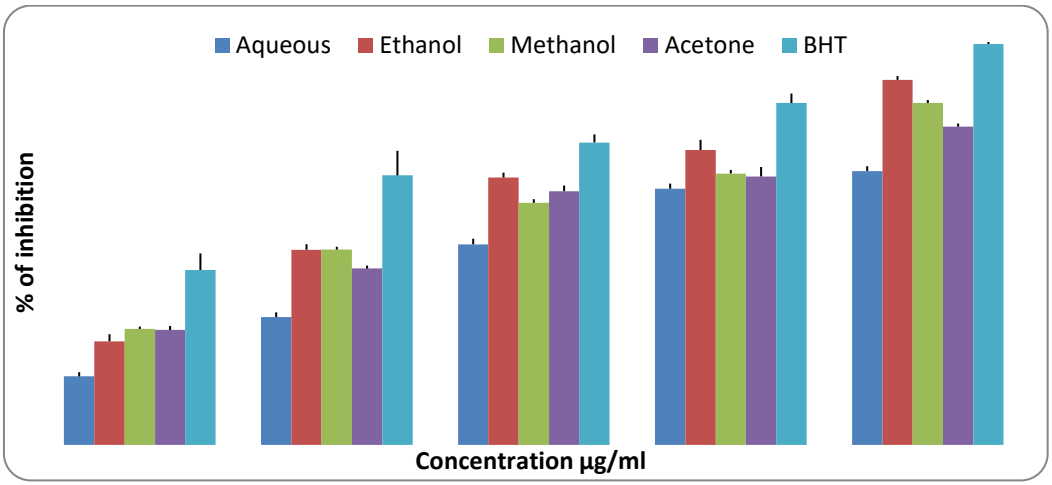

Figure 3: Hydroxyl radical scavenging activity of *Caulerpa racemosa* extracts (Mean ± SD, n = 3).

CONCLUSION

Algae are a rich source of natural antimicrobials and antioxidants. In the present study ethanol fractions of *C. racemosa* realized higher activities thereby signifying the importance of using ethanol to isolate potential antioxidant molecules. Finally, it can be concluded from the study that extracts of algal species used in the present investigation showed better antibacterial activity against pathogens used. They are potential sources of bioactive compounds and should be investigated for natural antibiotics. The results of the present study confirmed that macroalgae are a rich source of phytoconstituents which can be isolated and further screened for various biological activities like disease treatment for mankind. As there is a growing trend of disease and an increased requirement for medicine or drugs, this study recommends that macroalgae being an underutilized bioresource could be exploited sustainably for the welfare of mankind.

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