

Phytochemicals and In Vitro Antioxidant Activities of Five Marine Red Algae Species of a Genus *Gracilaria* from Southeast Coast of Tamil Nadu, India

Dr. G. Subramanian^{*1}, A. Nagaraj², P. Sona³, J. Sasikala⁴, K. Ambiga⁵ & M. Manivannan⁶

^{*1}Assistant Professor & ²⁻⁶Research Scholars, PG and Research Department of Botany,

Arignar Anna Government Arts College, Namakkal – 637 002, Tamil Nadu, India.

* gobotanygs@gmail.com

Abstract

The present study focused on determining phytochemicals and antioxidant activity of ethanol extracts of five red algae species of a genus *Gracilaria*. The evaluation of phytochemicals such as total phenol, flavonoid, and carotenoid were estimated from the selected species of *Gracilaria*. Antioxidant properties were determined by using four standard methods namely 1,1-diphenyl-2-picrylhydrazyl (DPPH), Hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), and Superoxide anion (O_2^-). The results revealed that ethanol extract of *G. salicornia* species showed maximum phenolic, flavonoid and carotenoid content of 8.52 ± 0.43 mg GAE/g, 31.45 ± 0.35 mg RE/g and 48.02 ± 0.44 $\mu\text{g/g}$, respectively. Among the five species of *Gracilaria*, the maximum of 20.69 $\mu\text{g/ml}$ of IC_{50} value of DPPH in *G. verrucosa*, 11.33 $\mu\text{g/ml}$ of IC_{50} value of hydroxyl radical scavenging activity in *G. salicornia*, 12.37 $\mu\text{g/ml}$ of IC_{50} value of hydrogen peroxide scavenging activity in *G. verrucosa* and 5.62 $\mu\text{g/ml}$ of IC_{50} value of superoxide ion radical scavenging activity in *G. edulis*. The tested red algae also had antioxidant activity like other green and brown algae which are showing good antioxidant potential.

Keywords: *Gracilaria*, DPPH, Hydroxyl, Hydrogen Peroxide, Superoxide scavenging activity.

1. Introduction

Marine algae play an inevitable for their biochemical versatility and biological diversity, and therefore they are considered as extraordinary resources for the discovery of new biochemical for antibacterial, antifungal, antiviral, and anticancer drugs production. Patra and Muthuraman^[1] reported that the recent developments in the therapeutic action of natural products are useful to examine their potential activity. Seaweeds offer a rich source of bioactive molecules^[2]. The seaweeds are a large group of macroalgae, based on their photosynthetic pigments, which are broadly classified into three major types, that is, Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae).

Seaweeds are widely used in the diet, and it is also used as a traditional medicine in Japan and China. They have the most essential bioactive metabolites. *Gracilaria* species can be used to treat life-threatening diseases such as acquired immunodeficiency syndrome and cancer due it bioactive constituents^[3]. In Asian countries, seaweed is commonly used as diet foodstuff^[4]. The algal secondary metabolites are highly relevant compounds that are biologically active against human pathologies^[5]. Especially, antioxidants are the primary natural product known to be inhibitors of reactive oxygen species (ROS) accumulative and can prevent skin oxidative stress, skin cancer, and premature skin aging^[6]. As such, the antioxidants have been often applied in the pharmaceutical, nutraceutical, and cosmetic industries^[6].

Phenolic compounds are the major substances responsible for antioxidant activity in land plants^[7]. In seaweeds, they are primarily involved in UV protection, anti-herbivore defense, pathogen resistance, and epiphyte growth defense^[8]. Besides, carotenoids, a pigment class common in plants and algae are also protecting cells against ROS^[9]. Thus, this study was to assess a few phytochemicals and antioxidant activities of *Gracilaria* species.

2. Materials and Methods

2.1. Collection of sample

The sample *Gracilaria* species namely *Gracilaria corticata* (J.Agardh) J.Agardh, *Gracilaria edulis* (S.G.Gmelin) P.C.Silva, *Gracilaria crassa* (Forsskål) Børgesen, *Gracilaria salicornia* (C.Agardh) E.Y.Dawson, and *Gracilaria verrucosa* (Hudson) Papenfuss were collected from the intertidal zone of Southeast coast of Tamil Nadu, India. The collected samples were cleaned with seawater to remove the epiphytes and sand particles. The samples had been packed in a polythene bag and brought to the laboratory. Then, the samples were washed with fresh water and shade dried. The shade dried sample was stored and preserved for further use.

2.2. Preparation of extract

Solvent Extraction

The seaweed was collected and dried it for 1 day, after the completion of drying; 50 g of seaweed was measured accurately and pulverized it gently. After pulverizing, added the seaweed in a conical flask and added 150 ml of ethanol to the seaweed and placed them in the orbital shaker for 24 h at room temperature. After that, the solvent was taken out, and the extraction liquid was kept ready for the filtration process. The extraction liquid was filtered by using Whatman filter paper. The extracted sample was condensed using Soxhlet extractor at 50°C and stored for further use^[10].

2.3. Total Phenolic Content

The estimation of the total phenolic content of the samples was done by Folin-Ciocalteu (FC) assay with minor modification^[11]. 200 µl of extracting sample was mixed with 200 µl FC reagent (0.5 N) followed by 1.6 ml 7.5% Na₂CO₃ and incubated for 2 hours in the dark at room temperature. The absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as standard and the phenolic content was expressed as a unit of Gallic Acid Equivalents (mg GAE/g) of macroalgae extract.

2.4. Total Flavonoid Content

Flavonoid content of the macroalgae extracts was determined by the spectrophotometric method of Zishen *et al.*,^[12] with slight modification. 0.5 ml of sample was mixed with 0.3 ml 15% sodium nitrite, 0.6 ml 10% ammonium chloride hexahydrate, and 3 ml of 1N sodium hydroxide, after 5 minutes, its absorbance was immediately measured at 510 nm. Flavonoid content value was expressed as a unit of Rutin equivalents of extract (mg RE/g).

2.5. Total Carotenoid Content

The carotenoid content of the acetone extracted samples was determined by the spectrophotometric method of Ranganna^[13].

2.6. DPPH free radical assay

The assay for DPPH scavenging activity was described by Ratty *et al.*^[14]. The sample was reacted with the stable DDPH radical in an ethanol solution. The reaction mixture consisted of a different concentration of sample and 2 ml of DPPH radical solution (0.4 mM). While DPPH reacts with antioxidant compounds that can donate hydrogen, it is reduced. The reaction mixture was incubated at 20 min in dark condition. After the incubation period, It was read absorbance at 517 nm by using a UV-vis spectrophotometer. The mixture of ethanol and the sample serves as blank^[10]. The control solution was prepared by mixing ethanol and DPPH radicals. The scavenging activity percentage [AA%] was determined according to the formula:- (Antioxidant Activity % = [control-sample]/control *100) (1)

2.7. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by a slightly modified off the 2-deoxyribose oxidation method [15]. Hydroxyl radical was generated from H_2O_2 by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. A reaction mixture containing every 0.2 ml of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA, and 10 mM 2-deoxyribose was mixed with 0.2 ml of the extract solution and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture until the total volume reached to 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 incubation, each 1 ml of 2.8% TCA (trichloroacetic acid) and 1.0% TBA (thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. The absorbance was measured at 532 nm.

2.8. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide radical scavenging assay was performed with ethanol extract of the seaweed sample [10]. The ability of the seaweed extracts to scavenge H_2O_2 was revealed, according to the method of Ruch *et al.* [16] with slight modification. About 40mM H_2O_2 was prepared in phosphate buffer (pH 7.4) and the H_2O_2 concentration was determined spectrophotometrically. Ethanol extracts with different concentrations in distilled water and ascorbic acid (20 – 100µg/ml) were added to 0.6 ml of 40 M H_2O_2 solution. It was kept for 10 min incubation against a blank solution containing phosphate buffer without H_2O_2 and then the absorbance of H_2O_2 was determined at 230 nm. The percentage of scavenging of H_2O_2 was calculated using the following formula: H_2O_2 radical scavenging activity (%) = $[(A_0 - A_1 / A_0) \times 100]$. (2)

2.9. Superoxide ion radical scavenging activity

The superoxide anion scavenging activity was assayed according to a method described earlier [17] with slight modifications in the nitroblue tetrazolium (NBT) and phenazine methosulphate (PMS) concentrations. The reaction mixture containing 25µL of NBT solution (150µM NBT in 100mM phosphate buffer, pH 7.4), 2µL of PMS solution (60µM PMS in 100mM phosphate buffer, pH 7.4) and 20µL of NADH solution (468µM in 100mM phosphate buffer, pH 7.4) was added to different concentrations of extracts. The mixture was incubated in the dark for 10 min at 25 °C and the absorbance read at 560 nm. Ascorbic acid was used as a positive control. All experiments were done in triplicate and results were expressed as percentage inhibition of superoxide anion radical using the following equation.

$$\% \text{ of superoxide anion radical scavenging (\%)} = (A_{\text{blank}} - A_{\text{samples}} / \text{Positive Control}) / A_{\text{blank}} \times 100. \quad (3)$$

3. Results and Discussion

The total phenol content of the selected five red macroalgae was shown in Table 1. Total phenolic contents were 6.75 ± 0.17 mg GAE/g, 6.81 ± 0.18 mg GAE/g, 7.77 ± 0.15 mg GAE/g, 7.89 ± 0.05 mg GAE/g and 8.52 ± 0.43 mg GAE/g with *G. edulis*, *G. corticata*, *G. crassa*, *G. verrucosa* and *G. salicornia* respectively (Table 1). In this study, the highest phenolic content was more than 6 times lower as per a report by Ghannam *et al.*, [18]. The reason might be the difference in the growth period, geographic location, storage type, genetic diversity, etc., the solvent used (ethanol) may not be efficient to extract the phenolic compounds or due to the loss of some phenolic compounds during sample preparation from the tissue matrix causing low phenolic content. Phenolic compounds are very important constituents because of their scavenging ability due to their hydroxyl groups. Ruberto *et al.*, [19] reported that phenols are particularly effective antioxidants for polyunsaturated fatty acid; they easily transfer a hydrogen atom to lipid peroxy radicals and form the aryloxy, which is being incapable of acting as a chain carrier, couples with another radical thus quenching the radical process.

A similar trend was found in flavonoid content which slightly varied among the five species of *Gracilaria* species with 26.49 ± 0.05 mg RE/g, 28.18 ± 1.01 mg RE/g, 29.45 ± 1.25 mg RE/g, 31.45 ± 0.35 mg RE/g, and 27.39 ± 0.12 mg RE/g in *G. corticata*, *G. edulis*, *G. crassa*, *G. salicornia*, and *G. verrucosa* respectively (Table 1). The highest content of flavonoids may be due to the high content of total phenolics in *Gracilaria* sp. Similarly, the flavonoid content ranging from 7.66 to 42.5 mg QE/g was recorded in different species of brown algae by Ghannam *et al.* [18]. Yoshie *et al.*, [20] reported that the flavonoid content of the two *Halimeda* sp., collected from the same location was significantly different, but similar results in a green alga *Ulva* sp. was recorded.

Carotenoid content was also found to be highest in *G. salicornia* ($48.02 \pm 0.44 \mu\text{g/g}$) and lowest in *G. corticata* ($44.02 \pm 0.21 \mu\text{g/g}$) (Table-1). On the contrary, $1.38 \mu\text{g/g}$ of carotenoid content in a green alga *Ulva reticulata* [21]. Similar results were observed in *Enteromorpha* sp. ($47.78 \pm 0.46 \mu\text{g/g}$) by Sivaramakrishnan *et al.*, [22], and *Codium adharens* ($38.5 \mu\text{g/g}$) by Seenivasan *et al.* [23]. The high content of these phytochemicals could explain its high radical scavenging activity.

Table 1. Phenol, Flavonoid and Carotenoid Content of *Gracilaria* Species

Phytochemicals	<i>G. corticata</i>	<i>G. edulis</i>	<i>G. crassa</i>	<i>G. salicornia</i>	<i>G. verrucosa</i>
Phenol (mg GAE g-1)	6.81 ± 0.18	6.75 ± 0.17	7.77 ± 0.15	8.52 ± 0.43	7.89 ± 0.05
Flavonoid (mg RE g-1)	26.49 ± 0.05	28.18 ± 1.01	29.45 ± 1.25	31.45 ± 0.35	27.39 ± 0.12
Carotenoid ($\mu\text{g g-1}$)	44.02 ± 0.21	47.12 ± 1.00	45.97 ± 0.42	48.02 ± 0.44	47.99 ± 0.25

The antioxidant activity of the *G. corticata*, *G. edulis*, *G. crassa*, *G. salicornia*, and *G. verrucosa* ethanol extracts was measured based on the scavenging activity of the stable DPPH free radical, Hydroxyl radical scavenging activity, Hydrogen peroxide scavenging assay, and Superoxide ion radical scavenging activity. They show the active site of antioxidants in crude ethanolic extraction by increasing inhibition by increasing concentration. As in the crude ethanolic extracts, the antioxidant activities showed the best result in antioxidant activity and the following table mentioned as Tables 2-5. The major role of antioxidant activity is free radical scavenging.

DPPH- free radical scavenging activity of ethanol extract of *Gracilaria crassa* had the maximum and minimum of 65.20 ± 1.52 % and 57.33 ± 0.64 % in $500 \mu\text{g/ml}$ and $100 \mu\text{g/ml}$ than the remaining four *Gracilaria* species respectively (Table -2). The ethanol extracts of five species of *Gracilaria* exhibited significant concentration-dependent inhibition of DPPH activity. IC_{50} values in DPPH free radical scavenging activity of *G. corticata*, *G. crassa*, *G. edulis*, *G. salicornia*, and *G. verrucosa* were $27.89 \mu\text{g/ml}$, $26.4 \mu\text{g/ml}$, $25.89 \mu\text{g/ml}$, $21.37 \mu\text{g/ml}$, and $20.69 \mu\text{g/ml}$ respectively which were lesser behavior than standard ascorbic acid ($16.04 \mu\text{g/ml}$ of IC_{50}) (Fig. 1). Cotelte *et al.*, [24] reported that the DPPH has been used enormously as a free radical chemical to evaluate reducing substances. The salient feature of DPPH is the purple color, usually disappeared while an antioxidant compound is present in the medium. Thus, molecules with antioxidant can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm. So, the more rapidly the absorbance decreases the more potent antioxidant activity of the extract.

Antioxidant activity by hydroxyl radical scavenging with ethanol extract of *G. edulis* had the maximum and minimum of 85.50 ± 1.42 % and 69.87 ± 0.22 % in $500 \mu\text{g/ml}$ and $100 \mu\text{g/ml}$ than the remaining four *Gracilaria* species respectively (Table - 3). The ethanol extracts of five species of *Gracilaria* exhibited significant concentration-dependent inhibition of hydroxyl radical scavenging activity. IC_{50} values in hydroxyl radical scavenging activity of *G. corticata*,

G. crassa, *G. edulis*, *G. verrucosa*, and *G. salicornia* were 26.19 µg/ml, 22.48 µg/ml, 21.21 µg/ml, 14.48 µg/ml, and 11.33 µg/ml respectively which were lesser behavior than standard ascorbic acid (8.33µg/ml of IC₅₀) (Fig. 1). Subashini and Prasanth^[25] reported that hydroxyl radical iron is the major active oxygen and is causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to macromolecules like DNA, lipid, and protein.

Table 2: Antioxidant Activity by DPPH Free Radical Scavenging with Ethanol Extract of *Gracilaria* Species.

Concentration (µg/ml)	% of Inhibition [Mean ± Standard Deviation (n = 3)]					
	Ascorbic Acid (Standard)	<i>G. corticata</i>	<i>G. edulis</i>	<i>G. crassa</i>	<i>G. salicornia</i>	<i>G. verrucosa</i>
100	60.15 ± 0.22	55.23 ± 0.12	56.80 ± 0.14	57.33 ± 0.64	56.18 ± 1.21	54.26 ± 0.26
200	63.16 ± 0.31	57.12 ± 0.23	58.14 ± 0.43	59.31 ± 0.73	56.41 ± 1.22	55.55 ± 1.44
300	65.29 ± 0.72	58.30 ± 0.32	59.48 ± 1.22	61.46 ± 0.74	58.22 ± 1.51	56.81 ± 2.35
400	67.25 ± 1.12	59.85 ± 1.12	61.31 ± 1.51	63.52 ± 1.21	60.59 ± 2.63	57.33 ± 2.62
500	68.25 ± 2.11	61.50 ± 1.32	64.36 ± 1.63	65.20 ± 1.52	61.58 ± 2.83	58.52 ± 3.22

Ethanol extract of *Gracilaria* species showed that there was an increase in concentration with an increase in absorbance value at 517nm

Table 3: Antioxidant Activity by Hydroxyl Radical Scavenging with Ethanol Extract of *Gracilaria* Species

Concentration (µg/ml)	% of Inhibition [Mean ± Standard Deviation (n = 3)]					
	Ascorbic Acid (Standard)	<i>G. corticata</i>	<i>G. edulis</i>	<i>G. crassa</i>	<i>G. salicornia</i>	<i>G. verrucosa</i>
100	79.65 ± 0.27	61.52 ± 0.24	69.87 ± 0.22	62.80 ± 0.12	69.33 ± 0.65	67.41 ± 1.22
200	84.42 ± 1.42	67.44 ± 0.33	74.12 ± 0.33	63.14 ± 0.41	71.31 ± 0.74	71.41 ± 1.23
300	86.54 ± 2.34	69.25 ± 0.71	77.30 ± 0.52	64.48 ± 1.20	72.46 ± 0.75	75.10 ± 1.52
400	89.22 ± 2.64	70.30 ± 1.11	80.85 ± 1.22	70.31 ± 1.52	75.52 ± 1.24	77.14 ± 2.64
500	91.93 ± 3.23	75.51 ± 2.12	85.50 ± 1.42	73.36 ± 1.64	82.20 ± 1.54	79.32 ± 2.85

Ethanol extract of *Gracilaria* species showed that there was an increase in concentration with an increase in absorbance value at 532nm

Antioxidant activity by hydrogen peroxide scavenging with ethanol extract of *Gracilaria edulis* had the maximum and minimum of 87.52 ± 1.43 % and 70.27 ± 0.24% in 500µg/ml and 100µg/ml than the remaining four *Gracilaria* species respectively (Table -4). The ethanol extracts of five species of *Gracilaria* exhibited significant concentration-dependent inhibition of hydrogen peroxide scavenging activity. IC₅₀ values in hydrogen peroxide scavenging of *G. corticata*, *G. edulis*, *G. crassa*, *G. salicornia*, *G. corticata*, and *G. verrucosa* were 23.73 µg/ml, 21.39 µg/ml, 14.37µg/ml, 12.64 µg/ml, and 12.37 µg/ml respectively which were lesser behavior than standard ascorbic acid (7.21 µg/ml of IC₅₀) (Fig. 1). Srikanth *et al.*,^[26] reported that many species of seaweeds possess scavenging ability of hydrogen peroxide. It can cross membranes and may slowly oxidize many compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because

of the rise in the hydroxyl radicals in the cells. The H₂O₂ radical scavenging assay was also performed with the ethanol crude extract of the seaweed samples.

Table 4: Antioxidant Activity by Hydrogen Peroxide Scavenging with Ethanol Extract of *Gracilaria* Species

Concentration (µg/ml)	% of Inhibition [Mean ± Standard Deviation (n = 3)]					
	Ascorbic Acid (Standard)	<i>G. corticata</i>	<i>G. edulis</i>	<i>G. crassa</i>	<i>G. salicornia</i>	<i>G. verrucosa</i>
100	81.67 ± 0.29	67.51 ± 0.25	70.27 ± 0.24	63.82 ± 0.13	32.34 ± 0.67	69.42 ± 1.23
200	86.45 ± 1.43	69.46 ± 0.35	76.14 ± 0.33	65.12 ± 0.42	42.32 ± 0.72	73.51 ± 1.27
300	88.54 ± 2.36	71.27 ± 0.72	79.32 ± 0.53	67.52 ± 1.22	45.48 ± 0.77	77.12 ± 1.52
400	91.23 ± 2.65	74.32 ± 1.12	82.87 ± 1.22	72.33 ± 1.52	47.54 ± 1.22	79.24 ± 2.66
500	92.95 ± 3.22	79.53 ± 2.15	87.52 ± 1.43	75.38 ± 1.62	50.22 ± 1.55	81.34 ± 2.86

Ethanol extract of *Gracilaria* species showed that there was an increase in concentration with an increase in absorbance value at 230nm

Antioxidant activity by superoxide ion radical scavenging with ethanol extract of *Gracilaria salicornia* had the maximum and minimum of 89.22 ± 1.57 % and 77.36 ± 0.69% in 500µg/ml and 100µg/ml than the remaining four *Gracilaria* species respectively (Table - 5). The ethanol extracts of five species of *Gracilaria* exhibited significant concentration-dependent inhibition of superoxide ion radical scavenging activity. IC₅₀ values in superoxide ion radical scavenging activity of *G. verrucosa*, *G. crassa*, *G. corticata*, *G. salicornia*, and *G. edulis* were 12.33 µg/ml, 10.87 µg/ml, 6.98 µg/ml, 6.91µg/ml, and 6.71 µg/ml respectively which were lesser behavior than standard ascorbic acid (3.69 µg/ml of IC₅₀) (Fig. 1). Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated after the oxygen is taken into living cells. Srikanth *et al.*,^[26] stated that superoxide anion changes to other harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radicals, which induce oxidative damage. The decrease in the absorbance at 560 nm with the *G. verrucosa*, *G. crassa*, *G. corticata*, *G. salicornia*, and *G. edulis* thus indicate the consumption of superoxide anion in the reaction mixture. The antioxidant activity of the ethanol crude extract determined by superoxide anion radical assay varied as seen in Table 5 and Fig. 1.

Table 5: Antioxidant Activity by Superoxide Ion Radical Scavenging with Ethanol Extract of *Gracilaria* Species

Concentration (µg/ml)	% of Inhibition [Mean ± Standard Deviation (n = 3)]					
	Ascorbic Acid (Standard)	<i>G. corticata</i>	<i>G. edulis</i>	<i>G. crassa</i>	<i>G. salicornia</i>	<i>G. verrucosa</i>
100	83.69 ± 0.29	67.50 ± 0.05	70.26 ± 0.26	65.87 ± 0.14	77.36 ± 0.69	69.45 ± 1.25
200	86.47 ± 1.43	69.45 ± 0.45	73.15 ± 0.37	67.12 ± 0.45	81.34 ± 0.71	73.52 ± 1.29
300	88.52 ± 2.56	71.26 ± 0.71	75.36 ± 0.57	69.55 ± 1.23	83.51 ± 0.79	69.10 ± 1.54
400	91.23 ± 2.67	74.31 ± 1.02	77.88 ± 1.26	71.33 ± 1.55	85.56 ± 1.23	77.26 ± 2.64
500	92.97 ± 3.12	76.51 ± 2.14	79.50 ± 1.45	76.39 ± 1.64	89.22 ± 1.57	81.36 ± 2.88

Ethanol extract of *Gracilaria* species showed that there was an increase in concentration with an increase in absorbance value at 560nm

The effectiveness of an antioxidant is measured by monitoring the inhibition of oxidation of a suitable substrate. Arulkumar *et al.*,^[27] reported that in a biological system, antioxidant effectiveness is classified into two groups that the evaluation of lipid peroxidation and measurement of free radical scavenging ability. The phenolic compounds which have a center of unsaturation and multi OH group in their structural moieties, enable them to donate a proton to DPPH radical thereby neutralizing the later^[28]. Ganesan *et al.*,^[29] reported that *Gracilaria edulis* and its fractions with petroleum ether, ethyl acetate, dichloromethane, butanol, and water showed a degradation oxidative deoxyribose with percentages of inhibition between 57.87 and 98.36%, which was similarly agreed with this present finding.

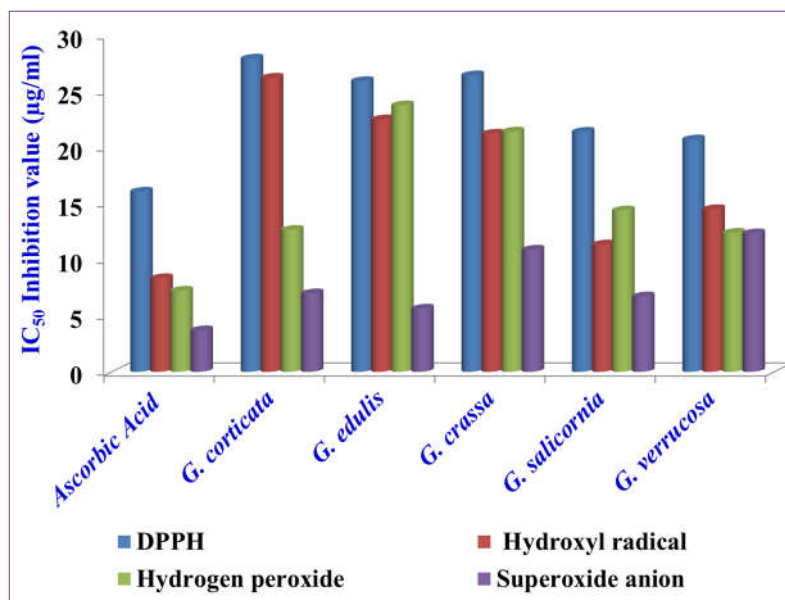


Fig. 1: IC₅₀ Inhibition value of Ethanolic Extract of Marine Red Algae *Gracilaria* Species and Ascorbic Acid (standard)

4. Conclusion

The higher antioxidant activity observed in *Gracilaria* species such as *G. corticata*, *G. crassa*, *G. edulis*, *G. salicornia*, and *G. verrucosa* seems to be related to the phenolic and carotenoid contents. Therefore, these preliminary results suggest that *Gracilaria* species may have a wide range of chemical constituents from secondary metabolism, mainly phenolic compounds and carotenoids, that can present antioxidant activities and that probably these contents are higher when compared to the same orders or family taxa collected in subtropical temperate due to adaptive routes. This contribution is the first report of antioxidant activities in *Gracilaria* species from the Southeast coast of Tamil Nadu, India. The high Antioxidant activity of *G. corticata*, *G. crassa*, *G. edulis*, *G. salicornia*, and *G. verrucosa* from high latitudes as possible new sources of natural products with potential uses in cosmetic, nutraceutical and pharmacological industries for their anti-oxidative stress effects. In conclusion, the ethanolic extracts of the examined red algae contain antioxidative compounds that can strongly scavenge ROS such as hydroxyl radical, superoxide anion, hydrogen peroxide, and DPPH free radical. These results indicate that the ethanolic extracts can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry. However, the responsible compounds related to the antioxidant activity of the algal extracts are not yet cleared. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant component in seaweeds.

References

- [1]. Patra, S, and Muthuraman, M. S. *Gracilaria edulis* extract induces apoptosis and inhibits tumor in *Ehrlich ascites* tumor cells *in vivo*. *BMC Complement Altern Med*, (2013);13:331.
- [2]. Nithya, P, and Dhanalakshmi, B. Antibacterial activity of methanol extracts from selected seaweed of southeast coast of India. *Int J Adv Res*. (2016) ;2:714-8.
- [3]. Deepa, S., Bhuvana, B., Hemamalini, S., Janet, C., and Kumar, S. Therapeutic potential and pharmacological significance of the marine algae *Gracilaria corticata*. *Pharm Biol Eval*. (2017);4: 68-72.
- [4]. Boobathy, S, Soundarapandian, P, Prithivraj, M, and Gunasundari V. Biochemical characterization of protein isolated from seaweed, *Gracilaria edulis*. *Curr Res J Biol Sci*. (2010); 2: 35-7.
- [5]. Maschek, J. A. and Baker, B.J. The chemistry of algal secondary metabolism. In: *Algal Chemical Ecology*. Springer-Verlag Berlin Heidelberg, Germany, (2008); 322 p.
- [6]. Guaratini, T., Lopes, N. P., Marinho-Soriano, E., Colepicolo, P. and Pinto, E. Antioxidant activity and chemical composition of the non-polar fraction of *Gracilaria domingensis* (Kützting) Sonder ex Dickie and *Gracilaria birdiae* (Plastino and Oliveira). *Brazilian Journal of Pharmacognosy*. (2012); 22: 724-729.
- [7]. Hayase, F. and Kato, H. Antioxidative components of sweet potatoes. *J. Nut. Sci. Vitaminology*, (1984); 30: 37-46.
- [8]. Amsler, C. D. and V. A. Fairhead. Defensive and sensory, chemical ecology of brown algae. *Adv. Bot. Res*. (2006); 43: 1-91.
- [9]. Gressler, V., Fujii, M.T., Martins, A.P., Colepicolo, P., Mancini, J. and Pinto, E. Biochemical composition of two red seaweed species grown on the Brazilian coast. *Journal of the Science of Food and Agriculture*, (2011); 91: 1687-1692.
- [10]. Subramanian, G., Ravi, P., Sona, P., Sasikala, J., and Manivannan, M. Evaluation of Antioxidant Activities of a Marine Brown Alga *Padina gymnospora* (Kutz.) Sond from the Pamban Coast of Rameswaram, Tamil Nadu, India, 2020; xii (vii): 205-211.
- [11]. Singleton, V.L. and Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Viticulture*, (1985); 16: 144-58.
- [12]. Zishen, J., Mengcheng, T. and Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effect on superoxide radicals. *Food Chemistry*, (1999); 64: 555-559.
- [13]. Ranganna, S. Manual analysis of fruits and vegetable products. New Delhi, Tata Macgrow-Hill Publishing Company Ltd., (1977); 634 pp.
- [14]. Ratty, A. K., Sunamoto, J, and Das, N. P. Interaction of flavonoids with 1, 1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. *Biochem Pharm* (1988); 37:989-95.
- [15]. Chung, S. K., Osawa, T. and Kawakishi, S. Hydroxyl radical scavenging effects of spices and scavengers from Brown Mustard (*Brassica nigra*). *Biosci. Biotech. Bioch.* (1997); 61: 118-123.
- [16]. Ruch, R.J., Cheng, S.J, and Klaunig, J. E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, (1989); 10(6): 1003-1008.
- [17]. Shaikhi, W., Shameel, M., Usmanghani, K., and Ahmad, V.U. Phycochemical examination of *Padina tetrestromatica* (Dictyotales, Phaeophyta). *Pakistan J Pharmaco Sci*. 4 (1991): 55–61.
- [18]. Ghannam, A. N., Cox, S. and Gupta, S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, (2010);17: 205-220.
- [19]. Ruberto, G., Baratta, M. T., Biondi, D. M, and Amico, V. Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. *J. Appl. Physiol*. (2001); 13: 403-407.
- [20]. Yoshie, Y., Wang, W., Hsieh, Y, and Suzuki, T. Compositional difference of phenolic compounds between two seaweeds, *Halimeda* spp. *J. Tokyo Univ. Fish*. (2001) 88: 21-24.
- [21]. Vimala, T. and Poonghuzhali, T.V. Estimation of pigments from seaweeds by using acetone and DMSO. *International Journal of Science and Research*, (2015); 4(10): 1850-1854.
- [22]. Sivaramakrishnan, Sachidananda Swain, K. Saravanan, Kiruba Sankar. R, S. Dam Roy, Lipika Biswas, and Baby Shalini. In Vitro Antioxidant and Free Radical Scavenging Activity and Chemometric Approach to Reveal Their Variability in Green Macroalgae from South Andaman Coast of India. *Turkish Journal of Fisheries and Aquatic Sciences*, (2017); 17: 639-648.

- [23]. Seenivasan, R., Rekha, M., Indu, H. and Geetha, S. (2013). Antibacterial activity and phytochemical analysis of selected seaweeds from Mandapam coast. *Indian Journal of Pharmaceutical Science*, (2013); 2(10): 159-169.
- [24]. Cotellet, N., Bemier, J. L., Catteau, J. P., Pommery, J., Wallet, J. C., and Gaydou, E. M. Antioxidant properties of hydroxyl-flavones. *Free Radical Biol MED*. (1996); 20: 35-43.
- [25]. Subashini, R., and Prasanth, R. Studies of free radicals scavenging potential of seaweed *Enteromorpha* sp. *Chem Sci Rev Lett*. (2014); 3(12): 931-940.
- [26]. Srikanth, G., Babu, S. M., Kavitha, C.H.N., Roa, M.E.B., Vijaykumar, N., and Pradeep, C.H. Studies on in-vitro antioxidant activities of *Carica papaya* aqueous leaf extract. *Res J Pharm Biol Chem Sci*.1 (2010): 59-65.
- [27]. Arulkumar, A, Rosemary T, Paramasivam S, and Rajendran, R. B. Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay, India. *Biocatal Agric Biotechnol*. (2018);15: 63-71.
- [28]. Chakraborty, K., Joseph, D, and Praveen, N. K. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of peninsular India. *J Food Sci Technol*. (2015); 52: 1924-35.
- [29]. Ganesan, P., Chandini, S. K, and Bhaskar, N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour. Technol*. (2008); 99:2717-2723.