

Antioxidant and brine shrimp cytotoxic activities of ethanolic extract of red alga *Gracilaria corticata* (J. Agardh) J. Agardh

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Marine algae are one of the natural resources in the marine ecosystem, which contain various biologically active compounds and have been used as source of food, feed and medicine. Recently much attention has been paid on the antioxidant, anti-tumor and anti-cholesterolemic activity of seaweed constituents. In the present study, ethanolic extract of *Gracilaria corticata* (J. Agardh) J. Agardh was tested for its antioxidant and cytotoxic activities. *In vitro* antioxidant assay using DPPH radical and reducing power showed significant free radical scavenging property of the extract. The IC₅₀ value of ethanol extract for DPPH was found to be 1.93 mg/mL. The extract also proved toxic to brine shrimps with an LC₅₀ value of 1.081 mg/mL. The whole study shown that *G. corticata* has appreciable free radical scavenging activity along with significant cytotoxic property with a scope of further bioassay guided screening of the active components.

Keywords: *Gracilaria corticata*, Antioxidant activity, Cytotoxic activity, Seaweed, DPPH

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Introduction

Marine algal community signifies a huge source of compounds endowed with ingenious structures and potent biological activities. Seaweeds have been used as a novel food with potential nutritional benefits in industry and medicine for various purposes¹. Till now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations². Recent findings evidenced that seaweeds contained antiviral, antibacterial, antifungal and antitumoral potentials among numerous others³⁻⁶.

Marine algae are considered to be a rich source of natural antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides⁷⁻¹¹. Like all photosynthesizing plants, marine algae are also exposed to a combination of light and high oxygen concentrations, which leads to the formation of free radicals and other strong agents¹². The absence of such damage in seaweeds suggests that their cells have some protective antioxidative mechanisms and compounds¹³. Seaweeds also produce various types of antioxidants to counteract environmental stresses¹⁴. Hence, they can be considered as a potential source of novel antioxidants.

Now-a-days antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-ageing and anti-carcinogenic natural bioactive compounds, which possess health benefits. Moreover, antioxidants or ingredients having antioxidative properties are used extensively for the improvement of food stability. With the focus is being shifting towards finding alternatives for synthetic food ingredients, natural substances having antioxidative properties are in huge demand. Natural antioxidants are considered safe for use as ingredients in medicine, dietary supplements, nutraceuticals and cosmetics with the objective of improving consumer health, reducing the effects of harmful diseases and other broader aspects of immune system function¹⁵.

India ranks first among all countries bordering the Indian Ocean ahead of Australia and South Africa in the number of recorded specific and intraspecific seaweed taxa¹². These vast varieties of seaweeds were found to possess useful untapped biochemical compounds, which might be a potential source of drug leads in the future¹⁷. Several studies have investigated the antioxidant activity of marine algae¹⁸⁻²³ and some reports contribute to their cytotoxic potential too²⁴⁻²⁷. Further information on the bio-utilization of Indian seaweeds is limited as not much has been done to systemically study their therapeutic potential²⁵. This

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work was therefore, conducted to screen the antioxidant as well as cytotoxic potential of the seaweed *Gracilaria corticata* (J. Agardh) J. Agardh which is a common species on Indian coast.

Materials and Methods

Collection and extraction

The algae *G. corticata* were collected from Thirumullavaram (Kollam Dist) and identified at Dept. of Botany, S. N. College, Kollam. Epiphytic and extraneous matters from the collected algal material were removed by washing in sea water followed by fresh water. The algae were transported to the laboratory carefully packed in polyethylene bags with ice pack.

Extraction

Algae were shade dried and powdered in a blender. The dried algal powder (25 g) was macerated well with 100 mL ethanol and kept overnight for extraction. The supernatant was decanted out and stored in freezer at 0°C. The residue was again extracted with ethanol (100 mL) and the process was repeated twice. The final supernatant was evaporated to dryness at 50°C in a water bath to get the crude extract, which was weighed and stored in a glass vial.

In vitro antioxidant studies

Antioxidant property of the ethanol extract of *G. corticata* was evaluated using two *In vitro* assays. The assays were carried out in three sample replications and values were represented as the average of three replicates.

DPPH radical scavenging assay

The scavenging activity of ethanol extract of *G. corticata* against DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical was measured according to the method of Hou *et al*²⁸. The extracts at different concentrations (0.1-4 mg/mL) were added to 0.1 mL of 1 M Tris-HCl (pH 7.9) and then mixed with 0.6 mL of DPPH (100 µM) in methanol for 20 min at room temperature under protection from light. Butylated Hydroxyl Toluene (BHT) was used as the reference antioxidant. Absorbance of the mixture was read at 517 nm against the blank. The percentage inhibition of test concentrations were calculated as:

$$\% \text{ Inhibition} = (A_{\text{Control}} - A_{\text{Test}}) / A_{\text{Control}} \times 100$$

where A_{Control} is the absorbance of the control (without extract) and A_{Test} is the absorbance of the sample of extract.

Evaluation of reducing power

Reducing power of ethanol extract of *G. corticata* was investigated using the method developed by Oyaizu²⁹. Different concentrations of the extract were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was placed in a water bath for 20 min at 50° C. The resultant solution was cooled rapidly to room temperature, mixed with 2.5 mL of 10% trichloroacetic acid and centrifuged at 3,000 rpm for 10 min. The supernatant (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. Absorbance of the test samples were measured at 700 nm after 10 min. BHT was used as the standard antioxidant. The higher absorbance indicates the stronger reducing power of the respective extract.

Brine shrimp lethality bioassay

Dried cysts of brine shrimps were hatched in a shallow rectangular glass dish, filled with filtered sea water. After hatching, the phototrophic nauplii were collected using a pipette from the lighted side. Ten nauplii each were transferred to vials containing different concentrations (0.4-2 mg/mL) of ethanol extract of *G. corticata* and the total volume was made up to 5 mL using sea water of 30 ppt. A control vial without extract was also maintained. A drop of dry yeast suspension was added as food to each vials and maintained under illumination. The experiments were done in triplicate. The number of survivors was counted after 12 h and 24 h and the percentage of death at each dose and control were determined³⁰. Larvae were considered dead if no movement of the appendage was observed within 10 sec.

Statistical analysis

The IC₅₀ and LC₅₀ values, respectively for DPPH radical scavenging activity and brine shrimp lethality bioassay were found out by using Linear regression Probit analysis using SPSS version 14.

Results and Discussion

Extraction of *G. corticata* with ethanol resulted in a green dry extract weighing 1.45 g. percentage yield of the extract was found to be 5.8 % of the dried algal powder.

DPPH scavenging property of ethanol extract of *G. corticata* is shown in Fig. 1. In the range of concentrations (0.1-4 mg/mL) tested, the extract showed a dose dependent pattern in DPPH radical scavenging indicated by the decrease in purple colour formation. IC₅₀ value of the extract was found to be 1.93 mg/mL whereas it was 0.343 mg/mL for the standard BHT. Recent work done on methanolic extract of *G. corticata* showed a percentage inhibition of 67.9% against DPPH radical¹⁸. Similar work on the methanolic extract of *G. edulis*, showed a percentage inhibition of 14.84% for 100 µg/mL of extract²³.

This method is based on the reduction of a stable free radical, DPPH to yellow coloured diphenyl-picrylhydrazine. Any reducing agents that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption at 517 nm. The absorption strength is decreased and the resulting decolorization is stoichiometric with respect to the number of electron captured³¹. Thus from the results it can be attributed that the antioxidant property of ethanol extract of *G. corticata* could be due to the presence of reducing agents.

Fig.2 illustrate the reducing properties of *G. corticata* extract at different concentrations. The extract showed minimum absorbance of 0.097±0.006 nm at 1mg/mL and maximum of 0.760±0.036 nm at 5 mg/mL. From the result it is evident that, the extract in the range of concentrations tested exhibited a dose dependent increase in Perl's Prussian blue formation at 700 nm. The reference antioxidant BHT showed much higher reducing property than that of *G. corticata* extract at the concentrations tested.

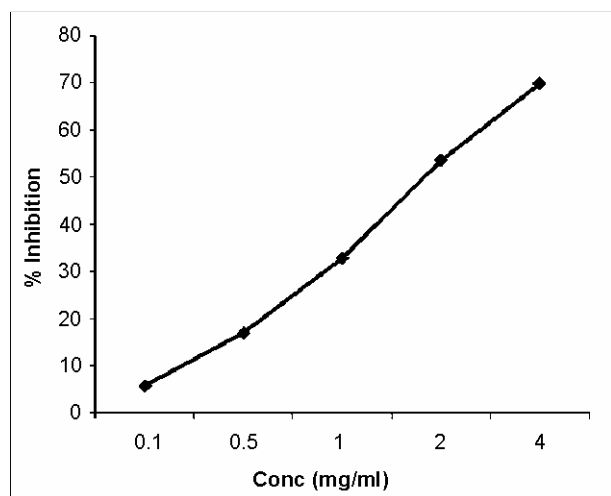


Fig.1—Effect of ethanolic extract of *G. corticata* on DPPH radical scavenging activity (values are expressed as mean±S.D, n=3)

Similar studies were also reported on the chloroform and ethyl acetate extracts of *G. edulis*²³.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity³². Different studies have indicated that the antioxidant effect is related to the presence of reductones, which are the terminators of free radical chain reaction. The result showed that the extract was able to reduce the ferric ions to ferrous ions, which is a measure of antioxidant activity. Increase in the absorbance at 700 nm indicates increase in reductive ability of the ethanol extract.

The brine shrimp cytotoxic assay is considered to be a convenient probe for preliminary assessment of toxicity, detection of fungal toxins, heavy metals, pesticides³⁰. It can also be extrapolated for cell-line toxicity and anti-tumor activity³³. Cytotoxicity assay

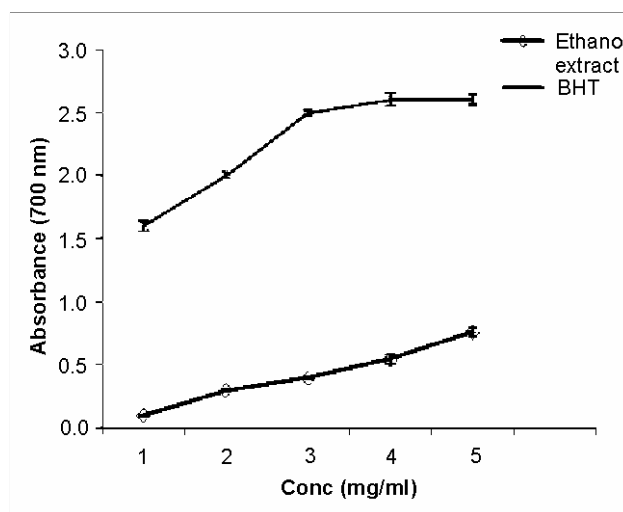


Fig. 2—Effect of ethanolic extract of *G. corticata* and BHT on reducing power (values are expressed as mean±S.D, n=3)

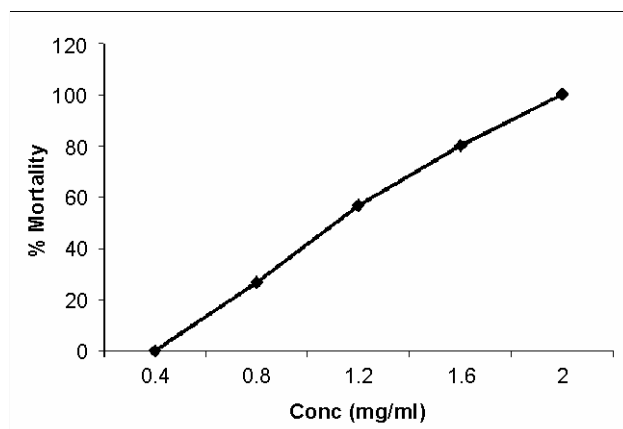


Fig. 3—Effect of ethanolic extract of *G. corticata* on brine shrimp lethality (values are expressed as mean±S.D, n=3)

on brine shrimp showed that the mortality was directly related to the concentration of the ethanol extract of *G. corticata* tested. The LC₅₀ value was found to be 1.081mg/mL. The higher dose (2 mg/mL) of ethanol extract showed 100 % inhibition at 24 h incubation (Fig. 3). Related work on the ethyl acetate extract from *G. salicomia* was proved to be cytotoxic against brine shrimp nauplii with an LC₅₀ value of 3 µg/mL³⁴.

Recent study on the phytochemical analysis of *G. corticata* reported it a rich source of phytochemicals particularly flavonoids, triterpenes, steroids, tannins, alkaloids, phenol and glycosides, which are seemed to be the basis of various biological activities including antioxidant and cytotoxic activities³⁵. Thus it can be attributed that, the presence of these active components can be the basis of the aforesaid properties.

Conclusion

Considering the appreciable antioxidant and cytotoxic activities of the ethanol extract of *G. corticata*, it may be suggested that the extracts possessing antioxidant and cytotoxic activities make them good candidates for future investigation. Further work is necessary to isolate the active principles and elucidate the mode of action of these compounds.

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References

- Santoso J, Yumiko Y and Takeshi S, Antioxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model, *Fish Sci*, 2004, **70**, 183-188.
- Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi R and Panikkar MVN, Biopotentials of Seaweeds Collected From Southwest Coast Of India, *J Mar Sci Technol*, 2009, **17**, 67-73.
- Matsuhira B, Conte AF, Damonte EB, Kolender AA, Matulewicz MC, Mejías EG, Pujol CA and Zúñiga EA, Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales Rhodophyta), *Carbohydr Res*, 2005, **340**, 2392-2402.
- Xu N, Fan X, Yan XJ, Li X, Niu R and Tseng CK, Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*, *Phytochemistry*, 2003, **62**, 1221-1224.
- Li XC, Jacob MR, Ding Y, Agarwal AK, Smillie T J, Khan SI, Nagle DJ, Ferreira D and Clark AM, Capisterones A and B, which enhance fluconazole activity in *Saccharomyces cerevisiae*, from the marine green alga, *Penicillus capitatus*, *J Nat Prod*, 2006, **69**, 542-546.
- Harada H, Noro T and Kamei Y, Selective anti-tumor activity *in vitro* from marine algae from Japan coast, *Biol Pharm Bull*, 1997, **20**, 541-546.
- Morgan KC, Wright JLC and Simpson FJ, Review of chemical constituents of the red alga *Palmaria palmate* (dulse), *Econ Bot*, 1980, **34**, 27-50.
- Nakamura T, Nagayama K, Uchida K and Tanaka R, Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicyclis*, *Fisheries Sci*, 1996, **62** (6), 923-926.
- Yoshie Y, Wang W, Petillo D and Suzuki T, Distribution of catechins in Japanese seaweeds, *Fisheries Sci*, 2000, **66** (5), 998-1000.
- Nakano T, Watanabe M, Sato M and Takeuchi M, Characterization of catalase from the seaweed *Porphyra yezoensis*, *Plant Sci*, 1995, **104** (2), 127-133.
- Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry D A, Câmara RBG, Nobre LTDB, Costa MSSP, Almeida-Lima J, Farias EHC, Leite EL and Rocha HAO, Biological activities of sulfated polysaccharides from tropical seaweeds, *Biomed Pharmacother*, 2010, 13, article 005.
- Dykens JA, Shick JM, Benoit C, Buettner GR and Winston GW, Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae, *J Exp Biol*, 1992, **168**, 219-241.
- Matsukawa R, Dubinsky Z, Kishimoto E, Masaki K, Masuda Y, Takeuchi T, Chihara M, Yamamoto Y, Niki E and Karube I, A comparison of screening methods for antioxidant activity in seaweeds, *J App Phycol*, 1997, **9**, 29-35.
- Lesser P M, Oxidative stress in marine environment, biochemistry and physiological ecology, *Annu Rev Physiol*, 2006, **68**, 253-278.
- Shahidi F, Nutraceuticals and functional foods whole versus processed foods, *Trends Food Sci Tech*, 2009, **20**, 376-387.
- Sahoo D, Sahu N and Sahoo D, Seaweeds of Indian Coast, New Delhi, A. P. H. Publishing Corporation, 2001, 283.
- Huang X, Zhou H and Zhang H, The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp *Fenneropenaeus chinensis*, *Fish Shellfish Immun*, 2005, **20**, 750-757.
- Vadlapudi V and Naidu KC, *In vitro* bioevaluation of antioxidant activities of selected marine algae, *J Pharm Res*, 2010, **3**(2), 329-331.
- Ganesan P, Chandini S K and Bhaskar N, Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds, *Biores Technol* 2007, **99**, 2717-2723.
- Kumar KS, Ganesan K and Rao PVS, Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty—an edible seaweed, *Food Chem*, 2007 **107**, 289-295.
- Devi K P, Suganthi N, Kesika P and Pandian S K, Bioprotective properties of seaweeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food bore bacteria in relation to polyphenolic content, *BMC Complement Altern Med*, 2008, **8**, 38.

- 22 Sachindra NM, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M and Miyashita K, Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites, *J Agric Food Chem*, 2007, 8516-22.
- 23 Murugan K and Iyer VV, Antioxidant and antiproliferative activities of marine algae, *Gracilaria edulis* and *Enteromorpha lingulata*, from Chennai Coast, *Int J Cancer Res*, 2012, **8**, 15-26.
- 24 Manilal M, Sujith S, Kiran S G, Selvin J and Shakir C, Cytotoxic potentials of Red algae *Courenicia brandenii* collected from the Indian west coast, *J Pharm*, 2009, **3**, 90-94.
- 25 Vinayak RC, Sabu AS and Chatterji A, Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities, *Evid Based Complementary Altern Med*, 2011, Article ID neq 024, 1-9.
- 26 Sasidharan S, Darab I and Jain K, *In vivo* and *in vitro* toxicity study of *Gracillaria changii*, *Bio Sci*, 2008, **46**, 413-417.
- 27 Vinayak R C, Sabu A S and Chaterji A, Bio-screening of a few green seaweeds from India for their cytotoxic and antioxidant potential, *J Sci Food Agric*, 2011, **10**, 1002-4490.
- 28 Hou WC, Hsu FL and Lee MH, Yam (*Dioscorea batatas*) tuber mucilage exhibited antioxidant activities *in vitro*, *Planta Med*, 2002, **68**, 1072-1076.
- 29 Oyaizu, M, Studies on product of browning reaction prepared from glucose amine, *Jpn J Nutr*, 1986, **44**, 307-315.
- 30 Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB and Nichols DE, Brine shrimp a convenient general bioassay for active plant constituents, *Plant Med*, 1982, **45**, 31-34.
- 31 Blois MS, Antioxidant determinations by the use of stable free radical, *Nature*, 1958, **26**, 1199.
- 32 Kallithraka S, Bakker J and Clifford, MN, Correlations between saliva protein composition and some T-I parameters of astringency, *Food Qual Prefer*, 2001, **12**, 145-152.
- 33 Anderson JE, Goetz CM, Mc Laughlin JL, Suffness M, A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumour prescreens, *Phytochem Analysis*, 1991, **2**, 107-111.
- 34 Saeidnia S, Gohari A R, Shahverdi A R, Perme P, Nasiri M, Mollazadeh K and Farahani F, Biological activity of two red algae, *Gracilaria salicornia* and *Hypnea flagelliformis* from Persian Gulf, *Pharmacognosy Res*, 2009, **1**(6), 428-430.
- 35 Eahamban K and Antonisamy JM, Preliminary phytochemical, UV-VIS, HPLC and anti-bacterial studies on *Gracilaria corticata* J. Ag., *Asian Pac J Trop Biomed*, 2012, S568-S574. Eahamban K and Antonisamy JM, Preliminary phytochemical, UV-VIS, HPLC and anti-bacterial studies on *Gracilaria corticata* J. Ag, *Asian Pac J Trop Biomed*, 2012, S568-S574.