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

K L Sreejamole* and P M Greeshma

P.G and Research Dept. of Zoology, Sree Narayana College, Cherthala, Alappuzha Dist, Kerala, India

Received 18 July 2012; Accepted 24 April 2013

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 IC$_{50}$ value of ethanol extract for DPPH was found to be 1.93 mg/mL. The extract also proved toxic to brine shrimps with an LC$_{50}$ 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

**IPC code; Int. cl. (2011.01)−A61K 36/00**

**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 purposes\(^1\). Till now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations\(^2\). Recent findings evidenced that seaweeds contained antiviral, antibacterial, antifungal and antitumoral potentials among numerous others\(^3-6\).

Marine algae are considered to be a rich source of natural antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides\(^7-11\). 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 agents\(^12\). The absence of such damage in seaweeds suggests that their cells have some protective antioxidative mechanisms and compounds\(^13\). Seaweeds also produce various types of antioxidants to counteract environmental stresses\(^14\). 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 function\(^15\).

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 taxa\(^12\). These vast varieties of seaweeds were found to possess useful untapped biochemical compounds, which might be a potential source of drug leads in the future\(^17\). Several studies have investigated the antioxidant activity of marine algae\(^18-23\) and some reports contribute to their cytotoxic potential too\(^24-27\). Further information on the bio-utilization of Indian seaweeds is limited as not much has been done to systemically study their therapeutic potential\(^25\). This
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 icepack.

**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-picryl hydrazyl) radical was measured according to the method of Hou *et al.*

\[
\% \text{ Inhibition} = \left( \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \right) \times 100
\]

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

\[
\text{Absorbance of the test samples were measured at 700 nm after 10 min. BHT was used as the standard antioxidant. The higher absorbance indicates the stronger reducing power of the respective extract.}
\]

**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 vial 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 determined.

Larvae were considered dead if no movement of the appendage was observed within 10 sec.

**Statistical analysis**
The IC\(_{50}\) and LC\(_{50}\) 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. \( IC_{50} \) 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 radical\(^ {18} \). Similar work on the methanolic extract of *G. edulis*, showed a percentage inhibition of 14.84% for 100 µg/mL of extract\(^ {23} \).

This method is based on the reduction of a stable free radical, DPPH to yellow coloured diphenylpicrylhydrazine. 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 stochiometric with respect to the number of electron captured\(^ {31} \). 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.

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 activity\(^ {32} \). 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, pesticides\(^ {30} \). It can also be extrapolated for cell-line toxicity and anti-tumor activity\(^ {33} \). Cytotoxicity assay
on brine shrimp showed that the mortality was directly related to the concentration of the ethanol extract of *G. corticata* tested. The LC$_{50}$ value was found to be 1.081 mg/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 LC$_{50}$ value of 3 µg/mL.¹⁴

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 alkaloids, phenol and glycosides, which are seemed to be the basis of various biological activities including antioxidant and cytotoxic activities.³⁵ 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.

**Acknowledgements**

We are grateful to Dr. M. B. Lissy, Associate Professor, Dept. of Botany, S. N. College, Kollam for elucidate the mode of action of these compounds.

**References**


Blois MS, Antioxidant determinations by the use of stable free radical, Nature, 1958, 26, 1199.


