EVALUATION OF ANTIBACTERIAL, ANTIOXIDANT, AND ANTICANCER POTENTIALS FROM MARINE RED ALGAE *GRACILARIA CORTICATA*

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INTRODUCTION

In recent years, natural products have been playing a major role in search of novel drugs against many infectious diseases, inflammation, cancer, and many other complex illnesses. They are considered as treasure for researchers due to their enormous structural diversity and complexity. The marine algae or seaweed represents a largely untapped source for the isolation of novel bioactive compounds [1].

Seaweeds are the primitive angiosperm that has invariable mineral source, particularly marine red and brown algae. They are used as commercial products; stabilizers, thickeners, emulsifiers, foods, etc. In recent years, phylogenics focus the bioactive substances of marine plants because of the presence of macro and trace elements and their cell wall composition. Seaweeds or marine algae are classified into three main categories: Brown algae (Phaeophyta), green algae (Chlorophyta), and red algae (Rhodophyta) [2]. Thus, macroalgae have been recognized as a promising ultimate source of bioactive secondary metabolites with antitumor [3], antibacterial, antioxidant, anti-inflammatory, anticancer, anticholesterolemic activity, anti-diabetic activity, and hepatoprotective activity [4]. Several red algae contain agar as a water-soluble sulfated galactan located in the intercellular spaces. Agar is a mixture of polysaccharide, which can be composed of agarose and agaropectin with similar structural and functional properties as carrageenan of red algae [5]. Thus, the study was to assess antibacterial, antioxidative, and anticancer potentialities of *G. corticata* settled along the Mandapam coast of Tamil Nadu.

METHODS

Collection of sample

The sample *G. corticata* (Red algae) was collected from intertidal zone of Mandapam coast (Lat. 9°17’N; Lon. 79°19’E) of Gulf of Mannar, southeast coast of Tamil Nadu, India. The collected sample was cleaned with seawater to remove the epiphytes and sand particle, and the sample has been packed in polythene bag and brought to laboratory. Then, the sample was washed with freshwater and shade dried. The shade dried sample is stored.

Sample identification

The seaweeds were identified and authenticated by Dr. Ganesan, Senior Scientist, Central Salt and Marine Research Institute, Mandapam Camp, Ramanathapuram, Tamil Nadu, India (Fig. 1).

Preparation of extract

Methanol extraction

The acetone extract of *G. corticata* was extracted using 50 g of the power sample with 150 ml of acetone. The mixture was placed in the orbital shaker for 24 h at 32°C in room temperature. After squeezing, the solvent was taken out and extraction liquid was filtered using Whatman filter paper. The extracted sample was condensed using Soxhlet extractor at 50°C.

Aqueous extraction

The aqueous extract of seaweed *G. corticata* was dried. After drying, 3 g of seaweed is measured and pulverize it gently. Then, add 50 ml of distilled water to the added seaweed in the conical flask. The solution was filtered using Whatman filter paper and the filtered solution was condensed using Soxhlet extractor. The solution was stored in a refrigerator for further use as crude extract of aqueous.

Antibacterial activity

The *G. corticata* tested against various Gram-positive and Gram-negative strains using agar disc diffusion technique with *Escherichia coli* and *Bacillus subtilis* both bacterial culture were smeared in the agar disc...
is used to see the antibacterial activity. The antibacterial activity was carried out using a standard disc diffusion technique with concentration of 25 µL, 50 µL, 75 µL, and 100 µL crude extract of *Gracilaria corticata* with control of 21 µL zetamycin was used. These were allowed to dry under aseptic condition and incubated at 37°C for 24 h. The diameter of clear zone around the discs was measured as antibacterial activity (Table 1 and Fig 2) [6].

**Antioxidant activity**  
**DPPH free radical assay**  
The assay for DPPH scavenging activity was described by Ratty et al. [6]. The sample was reacted with the stable DPPH radical in a methanol solution. The reaction mixture consisted of different concentrations of sample and 2 mL of DPPH radical solution (0.4 mM). When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The reaction mixture was incubated at 20 min in dark condition. The changes in color (from deep violet to light yellow) were read absorbance at 517 nm using UV-vis spectrophotometer (Tables 2 and 3). The mixture of methanol and sample serves as blank. The control solution was prepared by mixing methanol and DPPH radicals [7]. The scavenging activity percentage (AA%) was determined according to the formula:

\[
(AA\%) = \frac{\text{Absorbance of untreated (control)}}{\text{Absorbance of treated sample}} \times 100
\]

**Cell culture**  
The extracts were tested on McCoy cell. The cancer cell line was collected and grown in Dulbecco’s modified eagle modified medium (DMEM) with fetal bovine serum. Cells were seeded in 96-well microplates (1×10³ cells/well) and incubated at 37°C, with CO₂ in an incubator and allowed to grow 90% confluence. Then, the medium was replaced, and the cells were treated with drug at different concentrations such as 20, 40, 60, 80, and 100 µg/mL and incubated for 24 h. The cells were then washed with phosphate-buffer saline (PBS, pH - 7.4) and MTT solution (5 mg/mL) was added to each well. The plates were then stored at 37°C in the dark for 4 h. The formazan crystals were dissolved in 100 µL DMSO, and the absorbance was read spectrometrically at 570 nm. The percentage of cell viability was expressed as cell viabiliy.

\[
\text{Cell viabiliy} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100
\]

**RESULTS AND DISCUSSION**  
The antibacterial activity of *G. corticata* was determined in both Gram-positive and Gram-negative bacteria. The crude methanol extract. (a) Gram-positive (*Bacillus subtilis*). (b) Gram-negative (*Escherichia coli*)

**Table 1: Antibacterial activity of *Gracilaria corticata***

| Organism         | Concentration of the extract (µL) | Antibacterial activity (mm) |
|------------------|-----------------------------------|----------------------------|
|                  | 25 µL                             | 50 µL                      | 75 µL                      | 100 µL                      |
| *Escherichia coli* | -                                 | 18 mm                      | 20 mm                      |
| *Bacillus subtilis* | -                                 | 14 mm                      | 19 mm                      |

Methanol extract of *Gracilaria corticata* observed the inhibition zone of diameter and no activity of antibacterial against *Escherichia coli* and *Bacillus subtilis*.

**Table 2: Antioxidant activity by DPPH assay for crude methanol extract**

| Concentration (µL) | Wavelength (nm) | Absorbance (%) |
|--------------------|----------------|----------------|
| 100                | 517            | 14.52          |
| 200                | 517            | 20.25          |
| 300                | 517            | 32.76          |
| 400                | 517            | 36.20          |
| 500                | 517            | 44.15          |

Crude methanol extract of *Gracilaria corticata* interprets with increase in concentration with increase in absorbance at 517 nm.

**Table 3: Antioxidant activity by DPPH assay for crude aqueous extract**

| Concentration (µL) | Wavelength (nm) | Absorbance (%) |
|--------------------|----------------|----------------|
| 100                | 517            | 8.16           |
| 200                | 517            | 17.81          |
| 300                | 517            | 21.20          |
| 400                | 517            | 31.60          |
| 500                | 517            | 37.11          |

Crude aqueous extract of *Gracilaria corticata* interprets with increase in concentration with increase in absorbance at 517 nm.
The anticancer activity against cancer cell line was inhibited with increased concentration of solvent crude extract. In breast cancer cell line (MDA-MB 231), more cytotoxic effect was observed in methanol extract in 24 h treatment. It showed that the increased concentration of drug presents good toxicity over cancer cell line. It had a maximum of 95.67% cell viability for 20 μg of crude methanolic extract. Similarly, the drug showed its minimum of 39.9% cell viability of methanol extract. It represents that the increased concentration of drug presents good toxicity over breast cancer cell line (MDA-MB 231) (Table 5) [15,16].

CONCLUSION

Marine seaweeds have numerous bioactive compounds such as pathogens against antibacterial activity, DPPH assay activity, and anticancer activity. In this study, they are more significant and thus it suggests that the active components are responsible for antibacterial and antioxidant metabolites in seaweeds, and the results are found to be interesting. Thus, exploration of such biological agents might be a probable resource of an array of biologically active compounds, and the present results will ensure a starting point for exploiting natural bioactive substances present in the extracts of algae [15,16]. Seaweed plays a key role in reducing the breast cancer and other types of cancer. A mechanism in which cancer could be reduced or retard its rate of growth [17]. Further, work is in progress which aimed at the investigation of detailed studies on purification and evaluation of such compounds can take this to a large-scale application in pharmaceutical industries.

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AUTHOR'S CONTRIBUTION

Jayashree. P, Thiruchelvi. R, and Balashanmugam. P conceived and designed the experiment. Jayashree. P performed the experiment. Jayashree. P, Thiruchelvi. R, and Balashanmugam. P worked together on manuscript writing.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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