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

JAYASREE P1, THIRUCHELVI R1*, BALASHANMUGAM P2

1Department of Bio-Engineering, School of Engineering, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai - 600 117, Tamil Nadu, India. 2Avanz Bio Pvt. Ltd., Chennai - 600 059, Tamil Nadu, India. Email: thiruchelvi.se@velsuniv.ac.in

Received: 28 March 2018, Revised and Accepted: 05 May 2018

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 inincompatible mineral source, particularly marine red and brown algae. They are used as commercial products; stabilizers, thickeners, emulsifiers, foods, etc. In recent years, phylogenists 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 agarapectin 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°17ʺN; Lon. 79°19ʺE) 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 pulverized it gently. Then, add 50 ml of distilled water to the added seaweed in the conical flask. The solution was filtered with 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:

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

Cell culture

The extracts were tested on McCoy cell. The cancer cell line was collected and grown in Dubbecco’s modified eagle modified eagle medium (DMEM) with fetal bovine serum. Cells were seeded in 96-well microplates (1×10^4 cells/well) and incubated at 37°C, 5% CO₂, and allowed to grow to 90% confluence. Then, the medium was replaced, and the cells were treated with different concentrations such as 20, 40, 60, 80, and 100 µg/mL and incubated for 24 h. The culture solution was 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 concentration of cell viability was expressed as in formula. The concentration of the extracts that inhibition of cells growth was determined in Table 4.

Determination of cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] assay)

The anticancer activity of drug tested against breast cancer cell line (MDA-MB 231) by MTT assay.

The MDA-MB 231 cells seeded in 96-well microplates (1×10^4 cells/well) and incubated at 37°C for 24 h in 5% CO₂ incubator and allowed to grow to 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 additional 2–4 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 in formula. The concentration that inhibited 50% of cell growth was referred as IC₅₀ value, which was used as a parameter for cytotoxicity study. The morphological changes of untreated (control) and the cells treated were observed under bright field microscope after 24 h (Table 5 and Fig. 3).

Cell viability (%)= \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-negarive (Escherichia coli)

Fig. 1: Gracilaria corticata

Fig. 2: Antibacterial activity of Gracilaria corticata by crude methanol extract. (a) Gram-positive (Bacillus subtilis). (b) Gram-negative (Escherichia coli)

Table 1: Antibacterial activity of Gracilaria corticata

<table>
<thead>
<tr>
<th>Organism</th>
<th>Concentration of the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µL</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (µL)</th>
<th>Wavelength (nm)</th>
<th>Absorbance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>517</td>
<td>14.52</td>
</tr>
<tr>
<td>200</td>
<td>517</td>
<td>20.25</td>
</tr>
<tr>
<td>300</td>
<td>517</td>
<td>32.76</td>
</tr>
<tr>
<td>400</td>
<td>517</td>
<td>36.26</td>
</tr>
<tr>
<td>500</td>
<td>517</td>
<td>44.15</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (µL)</th>
<th>Wavelength (nm)</th>
<th>Absorbance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>517</td>
<td>8.16</td>
</tr>
<tr>
<td>200</td>
<td>517</td>
<td>17.81</td>
</tr>
<tr>
<td>300</td>
<td>517</td>
<td>21.20</td>
</tr>
<tr>
<td>400</td>
<td>517</td>
<td>31.60</td>
</tr>
<tr>
<td>500</td>
<td>517</td>
<td>37.11</td>
</tr>
</tbody>
</table>

Crude aqueous extract of Gracilaria corticata interprets with increase in concentration with increase in absorbance at 517 nm
Anticancer activity of *Gracilaria corticata* was observed with the cell viability by decrease in concentration with increase in cell viability.

The antioxidant of the seaweed extracts was measured on the basis of scavenging the stable DPPH free radical. It shows active antioxidant activity that shows the slight inhibition increase, with increase of concentration. The best progress result shows that the antioxidant activity against pathogenic microorganism. The Gram-positive bacterial zone inhibition is 19 mm at 100 μL. In Gram-negative bacterial zone inhibition is 20 mm at 100 μL. The zone formation shows increased concentration at 75 μL and 100 μL in both Gram-positive and Gram-negative bacteria (Table 1 and Fig. 1). The *in vitro* antibacterial activity of seaweeds extracts with methanol and aqueous was showed in various studies. The reviewed articles reported that antibacterial compounds are more active against Gram-positive bacteria than Gram-negative bacteria. The resistance of Gram-negative bacteria toward antibacterial substance was related to hydrophilic surface which is rich in lipopolysaccharide molecules, presenting a barrier to penetrate numerous antibiotic molecules. However, Gram-positive bacteria do not possess outer membrane and cell wall structure [10-12].

The antioxidant of the seaweed extracts was measured on the basis of the scavenging activity of the stable DPPH free radical. It shows active site of antioxidant in crude methanolic extraction by increasing of inhibition by increasing of concentration. As in crude aqueous extract, the antioxidant activity that shows the slight inhibition increase, with increase of concentration. The best progress result shows that antioxidant activity of crude extract of methanol in *Gracilaria corticata* (Tables 2 and 3). DPPH is considered as a good kinetic model for peroxyl radicals [13]. The antioxidants have main roles in scavenging the free radicals, maintaining the cell integrity, slow down ageing, and prevent the development of various complications associated with oxidative stress-related disease and cancer [14].

The criteria used to categorize the activity of extracts against human breast (MCCoy) cell lines based on the values of cells (Table 4 and Fig. 2). 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.

**ACKNOWLEDGMENT**

The author acknowledge the support of Dr. Ganesan, Senior Scientist, Central Salt and Marine Research Institute, Ramanathapuram, Tamil Nadu, India, for authenticate the seaweed. Author also grateful for Dr. P. Balashanmugam, Principle, Scientist of Avunz Bio Pvt. Ltd., and would like to thank Mrs. R. Thiruchelvi, Faculty of Bioengineering, VISTAS, for the support and guidance.

**AUTHOR’S CONTRIBUTION**


**CONFLICTS OF INTEREST**

There are no conflicts of interest.

**REFERENCES**