

**ANTIOXIDANT, ANTIBACTERIAL, AND ANTICANCER ACTIVITY FROM MARINE RED ALGAE  
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**ABSTRACT**

**Objective:** The marine seaweed *Gracilaria edulis* was collected from Rameshwaram Mandapam coast. The main objective of *G. edulis* was to check about the antioxidant, antibacterial, and anticancer activity. The potential use of seaweeds is to prevent colon cancer and leukemia. The crude extract of antioxidant activity was done in methanol and aqueous extract in various concentrations using DPPH assay. The antibacterial activity was analyzed in methanol extract. The antibacterial activity was tested in Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas*) bacteria with various concentration. The anticancer activity was tested against using the breast cancer cell line (MDA-MB231)

**Methods:** The antibacterial activity was checked by crude methanol extract using Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*Pseudomonas*) with different concentrations. The antioxidant activity was determined using DPPH radical scavenging assay in various concentrations. The anticancer activity was done using 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against breast cancer cell line (MDA-MB231).

**Results:** The antioxidant activity of marine red algae was checked using the stable DPPH radical scavenging assay. The antibacterial activity was analyzed and shows the activity of two different bacteria strain against methanol extract. The anticancer activity was done against breast cancer cell line using MTT assay.

**Conclusion:** Thus, the result shows that red algae *G. edulis* has good biological activity, and further it can be carried out in pharmaceutical, food preservatives, and cosmetic purpose.

**Keywords:** *Gracilaria edulis*, Antioxidant, Antibacterial, Anticancer.

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**INTRODUCTION**

Marine environment plays an inevitable for their chemical and biological diversity, and therefore it is considered as an extraordinary resource for the discovery of new anticancer drugs. Recent developments in the elucidation of therapeutic action of natural products help us to evaluate for their various potential activity [1]. Seaweeds offer a rich source of bioactive molecules [2]. The seaweed is a diverse and large group of macroalgae. Based on the presence of photosynthetic pigments the aquatic macroalgae organisms which are broadly classified into various types, that is, rhodophyta (red algae), chlorophyta (green algae), and phaeophyta (brown algae). They are grouped according to their unique photosynthetic pigments, which give them their characteristics color and unique properties [3].

There are about 4000 species of red algae found in nature. In Japan and China, the seaweeds are widely used in diet, and it is also used as traditional medicine. Seaweeds are one of the most promising and richest sources of bioactive primary and secondary metabolites. In Asian centuries, the seaweed is used as diet foodstuff commonly [4]. Marine seaweeds or other plants the food with the help of light and oxidizing agent that leads to the formation of free radical and other strong oxidizing agents. Seaweeds are considered as an essential source in the form of bioactive metabolites, in which constituents from the *Gracilaria* species can be used to treat life-threatening diseases such as acquired immunodeficiency syndrome and cancer [5]. Thus, the study was to assess antibacterial, antioxidative, and anticancer potentialities of *G. edulis* settled along the Mandapam coast of Tamil Nadu.

**METHODS****Collection of sample**

The sample *Gracilaria edulis* (Red algae) were 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 particles. The sample has been packed in a polythene bag and brought to laboratory. Then, the sample was washed with fresh water and shade dried. The shade dried sample is stored and preserved for further use.

**Sample identification**

The seaweed was identified and authenticated by Dr. Ganesan, senior scientist, CSIR-Central salts and Marine Chemical Research Institute, Mandapam camp, Ramanathapuram, Tamil Nadu, India [Fig. 1].

**Preparation of extract****Methanol extraction**

The seaweed was collected and dried it for 1 day, after the completion of drying; 50 g of seaweed is measured accurately and pulverize it gently. After pulverizing, add the seaweed in a conical flask and add 150 ml of methanol to the added seaweed and place it in the orbital shaker for 24 h at 32°C in room temperature. After squeezing, the solvent was taken out, and the extraction liquid is 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 [6].

### Aqueous extraction

The seaweed of aqueous extract 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 [6].

### Antibacterial activity

The antibacterial activity of *G. edulis* was tested against various Gram-positive and Gram-negative strains using agar disc diffusion technique with *Staphylococcus* and *Pseudomonas* bacterial culture were smeared in the agar disc is used to see the antibacterial activity. The antibacterial activity was carried out using different concentrations such as 25 uL, 50 uL, 75 uL, and 100uL crude extract of *G. edulis* with the control value of 21 uL Zentamycin was used [6]. These were allowed to dry under aseptic condition and incubated at 37°C for 24 h [7]. The diameters of a clear zone around the discs were measured as antibacterial activity [8].

### Antioxidant activity

#### DPPH free radical assay

The assay for DPPH scavenging activity was described by Ratty *et al.* [9]. The sample was reacted with the stable DPPH radical in an methanol solution. The reaction mixture consisted of a different concentration 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. The mixture of methanol and sample serves as blank [10,11]. The control solution was prepared by mixing methanol and DPPH radicals. The scavenging activity percentage [AA%] was determined according to formula:-

$$(AA\% = [\text{control-sample}]/\text{control} * 100)$$

### Cell culture

The extracts were tested on McCOY cell. The cancer cell line was collected and grown in Dulbecco's Modified Eagle Modified Eagle Medium (D-MEM) with fetal bovine serum. Cells were seeded in 96-wells and allowed to adhere for 24 h at 37°C, with CO<sub>2</sub>, in an incubator. Then, 1 of it serially diluted samples in the medium were dispensed into the wells of the cell plates and incubated for a further 72 h. After removal of the sample medium, the cells were added with DMEM medium and incubated. After 72 h cells were fixed with cold 40% trichloroacetic acid at 4°C for 1 h and washed with water. The absorbance was measured at 492 nm using a microplate reader [12]. Percentage of dead cells was calculated in comparison to control. The concentration of the extract 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<sup>6</sup> cells/well) and incubated at 37°C for 24 h in 5% CO<sub>2</sub> incubator and allowed to grow 90% confluence. Then, the medium was replaced, and the cells were treated with the drug at a different concentration of 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 stand 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 IC<sub>50</sub> 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 [13-15].

$$\text{Cell viability}(\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

## RESULTS AND DISCUSSION

The antibacterial activity of *G. edulis* was determined in Gram-positive and Gram-negative bacteria. The crude methanol extract shows the good result in antibacterial activity. The zone of inhibition produced by these extracts against the pathogenic microorganisms, and it's measured in diameter (mm). The Gram-positive bacterium has the inhibition zone of 18mm in 100uL, and the Gram-negative bacteria have the inhibition zone of 19mm in 100uL. Both the Gram-positive and Gram-negative bacteria increased in the concentration of 100uL were showed in the showed in Table 1. The *in vitro* antibacterial activity of seaweeds extracted with methanol and aqueous was showed in various studies [7,10,16]. The reviewed articles reported that the antibacterial activities suggest the possibility of the presence of the therapeutic value of seaweed against the bacterial infection. The lowest antibacterial activity was due to the presence of only one of secondary metabolite compound (alkaloid), and the highest antibacterial activity was caused by the activity of any of three phytochemicals such as alkaloid, saponin, and steroids.

The antioxidant activity of the *G. edulis* extracts was measured on the basis of the scavenging activity of the stable DPPH free radical. It shows the active site of antioxidant in crude methanolic extraction by increasing of inhibition by increasing of concentration. As in the crude aqueous extract, the antioxidant activity that shows slight inhibition increase, with increase of concentration. The crude extract of methanol shows the best result in antioxidant activity and the following table mentioned as Tables 2 and 3. The major role of antioxidant activity is free radical scavenging. The effectiveness of an antioxidant is measured by monitoring the inhibition of oxidation of a suitable substrate. In a biological system, antioxidant effectiveness is classified into two groups that the evaluation of lipid peroxidation and measurement of free radical scavenging ability [17]. The phenolic compounds which have a centre of unsaturation and

Table 1: Antibacterial activity of *G. edulis*

Organism	Concentration of the extract			
	20 µL	50 µL	75 µL	100 µL
Staphylococcus	NA	NA	15 mm	18 mm
Pseudomonas	NA	NA	10 mm	19 mm

The Gram-positive bacteria are *Staphylococcus aureus* and the Gram-negative bacteria are *Pseudomonas species*

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

Concentration (µL)	Wavelength (nm)	Absorbance (%)
100	517	13.361
200	517	19.08
300	517	23.43
400	517	33.93
500	517	41.99

Crude methanol extract of *Gracilaria edulis* shows that there is increase in concentration with increase in absorbance value at 517 nm

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

Concentration (µL)	Wavelength (nm)	Absorbance (%)
100	517	8.37
200	517	14.42
300	517	20.57
400	517	27.57
500	517	36.90

Crude aqueous extract of *Gracilaria edulis* shows that there is increase in concentration increases the absorbance value at 517 nm

multi OH group in their structural moieties, enable them to donate a proton to DPPH radical thereby neutralizing the later [11,18].

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

The anticancer activity was done and tested against the MDA-MB 231 by MTT assay [19]. The anticancer activity against cancer cell line was inhibited with increased concentration of solvent crude extract. In MDA-MB 231 more cytotoxic effect was observed in methanol extract in 24 h treatment. It showed that the increased concentration of drug present good toxicity over cancer cell line. It had a maximum of 94.06% cell viability for 20 µg of crude methanolic extract. Similarly, the drug showed its minimum of 37.75% cell viability of methanol extract. It represents that the increased concentration of drug present good toxicity over MDA-MB 231 [14,16,20] (showed in Table 5).



Fig. 1: *Gracilaria edulis*

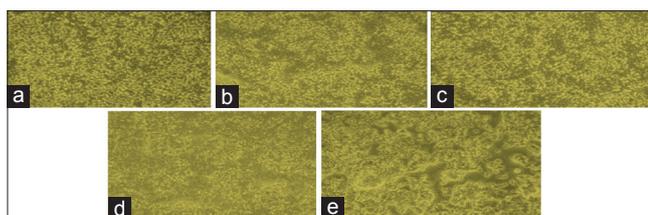


Fig 2: Anticancer activity of *G.corticata* crude methanol extract of different concentrations. (a) 20 µg, (b) 40 µg, (c) 60 µg, (d) 80 µg, (e) 100 µg

Table 4: Cytotoxicity activity of McCoy cancer cell line

Concentration	%Inhibition
20	95.39
40	89.06
60	82.04
80	72.15
100	62.71

Table 5: Anticancer activity of *Gracilaria edulis*

Concentrations	% cell viability
20 ug	94.06
40 ug	80.46
60 ug	69.55
80 ug	48.97
100 ug	37.75

## CONCLUSION

The methanol extract of marine seaweed red algae (*G. edulis*) shows a good result in various bioactive compounds such as antibacterial activity, anticancer activity, and antioxidant activity (DPPH scavenging of free radicals). It plays a key role in reducing of cancer activity against *in vitro* study of breast cancer. In previous studies, the red algae species of *Gracilaria corticata* reported that have numerous bioactive compounds such as pathogens against the antibacterial activity, DPPH assay activity, and anticancer activity [14]. Hence, the marine red algae might have the ability to fight against the cancer pathogen.

Further work is in progress which aimed at the investigation of detailed studies on purification and evaluation of such compounds can take this to large-scale application in pharmaceutical industries.

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## AUTHORS' CONTRIBUTION

Hemasudha.T.S, Thiruchelvi.R and Balashanmugam.P conceived and designed the experiment.

Hemasudha.T.S performed the experiment.

Hemasudha.T.S and Thiruchelvi.R worked together on manuscript writing.

## CONFLICTS OF INTEREST

There are no conflicts of interests

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