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Original Article

Antimicrobial activity of red seaweed *Gracilaria corticata* against human pathogenic bacterial strains

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ABSTRACT

Recent microorganisms have developed resistance to existing available antibiotics, thereby thriving to an emerging need for new generation of antibiotics. Since seaweeds provide a rich source of bioactive molecules, the present study aimed to investigate its antibacterial potential against clinically important microorganism. Red seaweed namely *Gracilaria corticata* collected from different coastal regions of Gulf Of Mannar Sea shore, Thoothukudi, India were used. For microbiological testing of the seaweed extracts, agar disc diffusion method was used. The zone of inhibition was measured for all the different crude algal extracts against eight strains of microorganisms including four Gram positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumonia*, *Enterococcus faecalis* and four Gram negative bacteria including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Salmonella typhi* that cause diseases in human beings, animals and plants. Crude extracts prepared from Acetone, Chloroform, Ethanol and Methanol extraction procedures revealed that methanol extraction procedure have a wide range of antibacterial activity against all the test pathogens. The overall antibacterial activity assessed from the above results indicates the presence of active constituents in the extractions of seaweeds which can be explored for the production of significant molecules which could be used in pharmaceutical industry.

Keywords: *Gracilaria corticata*, Red seaweed, Solvent Extracts, Antimicrobial activity

INTRODUCTION

The sea, covering 70% of the Earth's surface, offers a considerably broader spectrum of biological diversity than terra firma. Containing approximately 75% of all living organisms, the marine environment offers a rich source of natural products with potential therapeutic application [1]. A report suggests that marine organisms are source material for structurally unique natural products with pharmacological and biological activities [2, 3, 4].

Among the marine organisms, the macro algae (seaweeds) occupy an important place as a source of biomedical compounds [5, 6]. Seaweeds are the eukaryotic organism that lives in salty water in the ocean and it is found to be a potential source of bioactive natural products [7]. They contain compounds including sterols, terpenoids, tobrominated phenolic compounds which show bioactive against microorganisms. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, anti-tumoric, cytotoxic and antimitotic activities [8, 9]. About 2400 natural products have been isolated from macro algae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae [2]. Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations [10].

In addition, seaweeds provide many vitamins and are rich in iodine, potassium, iron, magnesium and calcium. Many bioactive compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been elucidated in green, brown and red algae. In contrast to the brown and green algae, the red algae are more known to synthesis halogenated metabolites, particularly bromine and iodine [11]. Thus this study aims to evaluate the antibacterial activity of red algae *Gracilaria corticata* against eight bacterial strains.

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MATERIALS AND METHODS

Collection of Marine Algae Samples: For screening of antibacterial activity of marine algae the study area considered was the Gulf Of Mannar Sea shore, Tuticorin of Tamil Nadu. Live and healthy marine algae were collected in the month of September 2013. The collected algae samples were identified morphologically and were rinsed with water to remove epiphytes and necrotic parts. The seaweeds were again washed with tap water to remove any associated debris and shade dried at room temperature (28±2ºC) for 5-8 days or until they are brittle. After completely drying, the seaweed materials were ground to a fine powder using electrical blender and then immediately subjected to extraction.

Preparation of Extracts: The algae after drying were weighed and then chopped. The chopped samples were finely powdered using mixer grinder. 40 g of powdered seaweeds were extracted sequentially with 200 ml of solvents (Acetone, Chloroform, Ethanol and Methanol) in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

Antimicrobial Activity:

Preparation of algal disc for antibacterial activity: Disc of 5 mm diameter were pre-treated by using Whatman filter paper No.1. These were sterilized in the hot air oven at 160°C for 1 hour. The solvent extracts of *Gracilaria corticata* (Acetone, Chloroform, Ethanol and Methanol) were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of sea weeds at two different concentrations ranging 2 mg /ml and 4 mg /ml to check their antibacterial activity. The paper discs which contain 5% DMSO were act as a coded control and the paper discs containing Ampicillin (5mg/disc) act as a positive control.

Bacterial Strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* stock cultures were obtained from Department of Microbiology, S.V.N College of Arts & Science, Madurai, Tamil Nadu, India.

Bacterial Inoculum Preparation: Bacterial inoculum was prepared by inoculating a loop full of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antibacterial activity.

Disc diffusion method: The antibacterial activity of *Gracilaria corticata* extracts was determined by Disc diffusion method. A bacterial suspension (number 0.5 in McFarland scale about 1.5 x 10^8 bacteria ml^{-1}) was spread on Mueller-Hinton (pH 7.4) agar using a cotton swab. The Mueller Hinton agar plates were prepared and inoculated with test bacterial organisms by spreading the bacterial inoculum on the surface of the media. The discs containing *Gracilaria corticata* extracts (Acetone, Chloroform, Ethanol and Methanol) at two different concentrations (2 mg/ml and 4 mg/ml) were placed on the surface of the Mueller Hinton agar plates. The paper discs which contain 5% DMSO were act as a coded control and the paper discs containing Ampicillin (5mg/disc) act as a positive control. The plates were incubated at 37^0C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm). Each assay in these experiments was repeated several times for concordance.

Minimum inhibitory concentration: Minimum inhibitory concentration (MIC) of the *Gracilaria corticata* extracts against bacterial isolates was tested in Mueller Hinton broth by Broth macro dilution method. The seaweeds extracts were dissolved in 5%DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller Hinton broth for bacteria to get a concentration of 2 and 4 mg/ml (for *Gracilaria corticata* extracts). 50µl of standardized suspension of the test organism and devoid of seaweeds extracts/FAME active principle. The culture tubes were incubated at 37ºC for 24 hours. The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determined as Minimum inhibitory concentration.

RESULTS AND DISCUSSIONS

Seaweeds are the eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products [7]. They contain compounds ranging from sterols, terpenoids, tobrominated phenolic, which shows bioactive against microorganisms [10]. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumoric, cytotoxic and antimitotic activities [11]. Presently seaweeds constitute commercially important marine

renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations [12]. In the present study, antibacterial activity of four different solvents *viz*., Acetone, Chloroform, Ethanol and Methanol extracts of *Gracilaria corticata* was evaluated against pathogenic Gram positive and Gram negative bacteria. Among four solvent extracts tested, the methanol extract showed the greatest inhibition diameters against Gram positive and Gram negative bacterial isolates. These results are in agreement with the observations of many reports [12, 13, 14, 15, 16, 17], who reported that extracts prepared with methanol showed the best activity. The higher frequency of activity against Gram-positive bacteria has also been observed in most of the surveys on antimicrobial activities from seaweeds reported in literature [17, 18]. The present investigation revealed that *Staphylococcus aureus* was more sensitive than all other organisms, with the largest inhibition diameter of 13mm with an inhibiting minimal concentration of 1.25µg/ml. Minor differences between the results of the present investigation and those of other studies may be due to the organic solvents used for the extraction of bioactive compounds and the differences in the assay methods, the geographical zone and the seasonal production of bioactive compounds. Salvador *et al*., (2007) studied the antimicrobial activities of 82 marine algae in fresh and lyophilized forms and according to a seasonal variation; they reported that red algae had both the highest values and the broadest spectrum of bioactivity. The results from the present study showed that the Gram positive bacteria are more susceptible than Gram negative bacteria on seaweeds extracts which was also supported from earlier works with different species of seaweeds indicating that the more susceptibility of Gram-positive bacteria to the algal extracts was due to the differences in their cell wall structure and their composition [16].

The methanol extract of *Gracilaria corticata* (4.0 mg/ml) showed highest mean zone of inhibition (13 ± 0.2 mm) against the Gram positive bacteria *Staphylococcus aureus* followed by *Bacillus subtilis* (12± 0.5 mm), *Streptococcus pneumonia* (12±0.6 mm) and *Enterococcus faecalis* (9±0.7 mm). For Gram negative bacteria, the maximum zone of inhibition was recorded in methanol extract of *Gracilaria corticata* against *Pseudomonas aeruginosa* (13±0.8 mm) followed by *Salmonella typhi* (12±0.3 mm), *Klebsiella pneumoniae* (12±0.4 mm), and *Proteus mirabilis* (12±0.2 mm). Zone of inhibition was not seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from 9±0.8 mm to 15±0.8 mm against the test bacterial pathogens (Table-1). The Minimum inhibitory concentration (MIC) values of *Gracilaria corticata* against bacterial isolates was ranged between 1.25 to 20µg/ml. The lowest MIC (1.25 µg/ml) value was recorded against *Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi and Pseudomonas aeruginosa* (Table-2). According to these reports, and taking into account the results detailed in the present contribution, it appears that the seaweeds from our coasts possess significant bioactive capacities, and thus deserve a place in marine biotechnology programs to examine the properties of natural products. The methanol extracts of *Gracilaria* showed a real potential antibacterial activity with good yields. These results suggest the possibility of using marine algae extracts in therapy as natural alternatives to antibiotics currently in the market, and clearly show that seaweeds from the Gulf of Mannar coast of are valuable source of biologically active compounds. Further research is underway to determine the structure and nature of these antibacterial substances.

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

Seaweed	**Human Pathogens**	**Zone of Inhibition in (mm) mg/ml**								
		Acetone		**Chloroform**		**Ethanol**		**Methanol**		**Positive control (Ampicillin)**
		2	**4**	**2**	**4**	**2**	**4**	**2**	**4**	**5 mg**
Gracilaria corticata	*Staphylococcus aureus*	7± 0.4	11± 0.3	8± 0.2	12± 0.3	6± 0.6	12± 0.2	9± 0.6	13± 0.2	14± 0.3
	Bacillus subtilis	8± 0.3	10± 0.5	7± 0.3	11± 0.4	7± 0.4	11± 0.8	8± 0.4	12± 0.5	13± 0.2
	Streptococcus pneumoniae	8± 0.5	10± 0.6	8± 0.5	12± 0.2	8± 0.8	12± 0.4	8± 0.2	12± 0.6	14± 0.5
	Enterococcus faecalis	7± 0.2	8± 0.4	7± 0.2	8± 0.3	8± 0.2	10± 0.2	9± 0.8	9± 0.7	12± 0.6
	Pseudomonas aeroginosa	8± 0.5	10± 0.3	7± 0.5	11± 0.6	7± 0.3	11± 0.3	8± 0.3	13± 0.8	14± 0.3
	Klebsiella pneumoniae	8± 0.4	11± 0.5	8± 0.4	12± 0.8	8± 0.7	12± 0.5	8± 0.5	12± 0.4	11± 0.2
	Proteus mirabilis	9± 0.3	10± 0.2	7± 0.3	10± 0.4	8± 0.2	11± 0.7	9± 0.4	12± 0.2	13± 0.5
	Salmonella typhi	8± 0.2	10± 0.3	7± 0.4	12± 0.2	6± 0.9	12± 0.3	8± 0.3	12± 0.3	12± 0.7

Table 2: Minimum inhibitory concentration of solvent extracts of *Gracilaria corticata*

Seaweed	**Human Pathogens**	**Minimum inhibitory concentration in µg/ml**				
		Acetone	**Chloroform**	**Ethanol**	**Methanol**	**Positive control (Ampicillin)**
Gracilaria corticata	*Staphylococcus aureus*	2.5	2.5	5	1.25	5
	Bacillus subtilis	1.25	1.25	2.5	2.5	5
	Streptococcus pneumoniae	1.25	1.25	2.5	2.5	10
	Enterococcus faecalis	10	10	20	1.25	2.5
	Pseudomonas aeruginosa	10	20	20	1.25	20
	Klebsiella pneumoniae	10	10	10	2.5	10
	Proteus mirabilis	10	10	20	10	20
	Salmonella typhi	5	10	5	1.25	5