

ORIGINAL RESEARCH ARTICLE

## Pharmacological Effect of *Gracilaria corticata* Solvent Extracts against Human Pathogenic Bacteria and Fungi

K.Kolanjinathan\* and D.Stella

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002, Tamil Nadu, India.

Received 19 Sep 2011; Revised 29 Nov 2011; Accepted 08 Dec 2011

### ABSTRACT

The present study was conducted to screen the pharmacological activity of *Gracilaria corticata* solvent extracts against human pathogenic bacteria and fungi. The seaweed, *Gracilaria corticata* was collected and powdered. The powdered material was extracted using the organic solvents viz., Methanol, Acetone, Chloroform, Hexane and Ethyl acetate. Antimicrobial activity of *Gracilaria corticata* solvent extract was determined by Disc diffusion method. Among the solvents tested, methanol extract of *Gracilaria corticata* showed maximum inhibitory activity than other solvents. The zone of inhibition of *Gracilaria corticata* methanol extract against bacteria was maximum against Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus epidermis* and *Bacillus cereus*. For Gram negative bacteria, the maximum zone of inhibition was recorded in methanol extract of *Gracilaria corticata* against *Klebsiella pneumoniae* followed by *Enterobacter aerogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Vibrio cholerae*. The minimum inhibitory concentration (MIC) values of *Gracilaria corticata* against bacteria was ranged between 1.25 to 80 mg/ml. For fungi, the zone of inhibition was maximum against *Aspergillus flavus* followed by *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. The minimum inhibitory concentration (MIC) value of *Gracilaria corticata* against fungi was ranged between 2 mg/ml to 16mg/ml

**Key words:** *Gracilaria corticata*, Solvent extracts, Antimicrobial activity and MIC.

### 1. INTRODUCTION

In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities. Seaweeds are the eukaryotic organisms that live in salty water and are recognized as a potential source of bioactive natural products. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine today seaweeds are the raw materials for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries<sup>[1]</sup>. In the sea, 3 types of plants occur and they are phytoplanktons, seaweeds or marine algae and seagrasses. Seaweeds or marine algae are macroscopic, attached or free floating plants. They form one of the important marine living renewable resources. They are primitive plants without any true root, stem and leaves. Seaweeds contribute to primary production of the sea and hence seaweed beds are considered to be highly

productive and dynamic ecosystem. Seaweeds vegetation provides an ideal habitat, food and shelter to various marine animals. They act as breeding, nursery and feeding grounds for many epiphytic fauna. The hapteron or holdfast of marine algae binds the sediments together and prevents coastal erosion.

Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. An understanding of the wide range of behavioral relationships that exist among organisms would provide us with clues to substances of biomedical interest. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds and other marine organisms. The host organisms biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment.

\*Corresponding Author: K.Kolanjinathan, Email: [kolanjinathan.micro@gmail.com](mailto:kolanjinathan.micro@gmail.com)

Some of these secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Nearly 50 lakhs species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation [2] apart from their potential ecological significances such as controlling reproduction, settlement and feeding deterrents [3].

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antifungal, antibacterial and antifungal activities. The extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria. The production of antimicrobial activities was considered to be an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites [4]. There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibacterial, antifungal, antiviral, antitumoral, anticoagulant and antifouling. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae. Also, considering their great taxonomic diversity, investigations related to the search of new biologically active compounds from algae can be seen as an almost unlimited field [5].

Tamil Nadu has a geographical extent of 1, 30,058 m<sup>2</sup>. It can be divided into two divisions namely the Eastern coastal plains and hills of north and east, which is endowed with the varied coastal habitat like mangroves, corals, seaweeds, sea grass beds, salt marshes, mud flats, sand dunes etc. The coast of Tamil Nadu bears luxuriant growth of seaweeds. More than two hundred species of seaweeds have been found in this area. Indian seaweed industries depend on this coastline for raw materials for the production of agar and sodium alginate. They are consumed in the form of soups as well as salads. The intake of seaweeds in the diet is said to prevent hair loss in men and women. It is also consumed by pregnant and lactating mothers because of their rich in iron content. They are called the medical food of the 21<sup>st</sup> century [6].

*Gracilaria corticata* belongs to the family Rhodophyceae (Red algae). These are highly evolved multicellular forms with well-developed

branched thalli. Except for few species they are exclusively marine and vary in size and shape. They are epiphytes, growing as crust on the rocks or shells as a large fleshy, branched or blade like thalli. The thallus is basically filamentous, simple or branched, free or compacted to form pseudoparenchyma with uni or multiaxial construction. They inhabit intertidal to subtidal zones of coastal areas [7]. The present study was aimed to screen the pharmacological activity of *Gracilaria corticata* solvent extracts against human pathogenic bacteria and fungi.

## 2. MATERIALS AND METHODS

### 2.1. Collection of seaweeds

The seaweeds *Gracilaria corticata* was collected from East coast of Mandapam, Tamil Nadu, India. The seaweed was taxonomically identified at the CAS in Marine Biology, Annamalai University and voucher specimens were deposited at the Department of Zoology, Annamalai University.

### 2.2. Preparation of seaweed extracts

The collected *Gracilaria corticata* sample was cleaned and the necrotic parts were removed. The seaweeds washed with tap water to remove any associated debris and shade dried at room temperature (28±2°C) for 5-8 days or until they are brittle easily by hand. After completely drying, the seaweed materials (1.0 kg) were ground to a fine powder using Electrical blender. Forty gram of powdered sea weeds were extracted successively with 200 mL of solvents (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) 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.

### 2.3. Disc preparation

#### 2.3.1. Preparation of algal disc for antibacterial activity

5 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The solvent (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) extracts of seaweed *Gracilaria corticata* were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of *Gracilaria corticata* at two different concentrations ranging 2.5mg/ml and 5mg/ml to check their antibacterial activity. The Ampicillin (5mg/ml) was used as positive control and the 5% DMSO was used as a blind control.

### 2.3.2. Preparation of algal disc for antifungal activity

5 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The solvent (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) extracts of *Gracilaria corticata* were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of *Gracilaria corticata* at 10 mg/ml to check their antifungal activity. The Flucanazole (100 units/ml) was used as positive control and the 5% DMSO was used as a blind control.

### 2.4. Collection of test microbial cultures

Eleven different bacterial cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *Staphylococcus aureus* (MTCC 3160), *Streptococcus epidermis* (MTCC 889), *Streptococcus pyogenes* (MTCC 1926), *Bacillus subtilis* (MTCC 1427), *Bacillus cereus* (MTCC 7417), *Escherichia coli* (MTCC 1195), *Pseudomonas aeruginosa* (MTCC 7093), *Vibrio cholerae* (MTCC 3904), *Salmonella typhi* (MTCC 3215), *Klebsiella pneumoniae* (MTCC 4032) and *Enterobacter aerogenes* (MTCC 6804). Six different fungal isolates were used in this present study. The fungal cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *Aspergillus flavus* (MTCC 1883), *Aspergillus niger* (MTCC 4285), *Aspergillus fumigatus* (MTCC 4964), *Saccharomyces cerevisiae* (MTCC 2627), *Candida albicans* (MTCC 7315) and *Candida glabrata* (MTCC 3983).

### 2.5. Determination of Antibacterial activity of *Gracilaria corticata*

#### 2.5.1. Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful 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 Mc Farland standards and then used for the determination of antibacterial activity.

#### 2.5.2. Disc diffusion method

The antibacterial activity of *Gracilaria corticata* extracts were determined by Disc diffusion method proposed by Bauer *et al.* (1966)<sup>8</sup>. Petri plates were prepared by pouring 20 mL of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 mL of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates

allowed drying for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The Ampicillin (5mg/disc) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

#### 2.5.3. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the *Gracilaria corticata* extracts against bacterial isolates were tested in Mueller Hinton broth by Broth macro dilution method. The seaweed 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 Muller Hinton broth for bacteria to get a concentration of 80, 40, 20, 10, 5, 2.50 and 1.25 mg/ml for *Gracilaria corticata* extracts and 50µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of *Gracilaria corticata* extracts. The culture tubes were incubated at 37°C for 24 hours. The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

### 2.6. Determination of Antifungal activity of *Gracilaria corticata*

#### 2.6.1. Disc diffusion method

The antifungal activity of *Gracilaria corticata* extracts were determined by Disc diffusion method proposed by Bauer *et al.* (1966)<sup>8</sup>. Petri plates were prepared by pouring 20 mL of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 mL of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates allowed drying for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The flucanazole (100 units/ml) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 28°C for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

### 2.6.2. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the *Gracilaria corticata* extracts against fungal isolates were tested in Sabouraud's dextrose broth by Broth macro dilution method. The *Gracilaria corticata* 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 Sabouraud's dextrose broth for fungi to get a concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for *Gracilaria corticata* extracts and 50µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 28°C for 48 hours (yeasts) and 72 hours (moulds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

### 3. RESULTS AND DISCUSSION

Seaweeds are eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products<sup>[9]</sup>. They contain compounds ranging from sterols, terpenoids to brominated phenolic, which shows bioactivity against microorganisms<sup>[10]</sup>. Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents. 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, antitumor, cytotoxic and antimutagenic activities<sup>[11]</sup>. 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<sup>[12]</sup>.

It is a real fact that the importance of marine organisms as a source of new substances is growing. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. A very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive, and aggressive surrounding very different in many aspects from the terrestrial

environment, a situation that demands the production of quite specific and potent active molecules. Although terrestrial biodiversity is the foundation of the pharmaceutical industry, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value<sup>[13]</sup>.

In the present study, antibacterial activity of five different solvents *viz.*, methanol, acetone, chloroform, hexane and ethyl acetate extracts of *Gracilaria corticata* was evaluated against pathogenic bacteria. Among five 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 Vlachos *et al.* (1996)<sup>[14]</sup>, Gonzalez *et al.* (2001)<sup>[15]</sup>, Ozdemir *et al.* (2004)<sup>[16]</sup>, Karabay-Yavasoglu *et al.* (2007)<sup>[17]</sup>, Taskin *et al.* (2007)<sup>[18]</sup> and Kandhasamy and Arunachalam (2008)<sup>[19]</sup>, who reported that extracts prepared with methanol showed the best activity. 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.

The methanol extracts of *Gracilaria corticata* (5.0mg/ml) showed highest mean zone of inhibition ( $18 \pm 0.4$ mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis* ( $17 \pm 0.5$ mm), *Staphylococcus aureus* ( $17 \pm 0.3$ mm), *Streptococcus epidermis* ( $16 \pm 0.6$ mm) and *Bacillus cereus* ( $16 \pm 0.2$ mm). For Gram negative bacteria, the maximum zone of inhibition was recorded in methanol extract of *Gracilaria corticata* against *Klebsiella pneumoniae* ( $17 \pm 0.5$ mm) followed by *Enterobacter aerogenes* ( $17 \pm 0.3$ mm), *Salmonella typhi* ( $16 \pm 0.6$ mm), *Pseudomonas aeruginosa* ( $16 \pm 0.5$ mm), *Escherichia coli* ( $16 \pm 0.3$ mm) and *Vibrio cholerae* ( $11 \pm 0.4$ mm). The zone of inhibition obtained from the hexane crude extracts of seaweed *Gracilaria corticata* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin showed zone of inhibition ranging from  $14 \pm 0.8$  mm to  $20 \pm 0.8$ mm against the test bacterial pathogens (**Table 1**). The Minimum inhibitory concentration (MIC)

values of *Gracilaria corticata* against bacteria was ranged between 1.25 to 80mg/ml. The lowest MIC (1.25 mg/ml) value value of methanol extract was recorded against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus epidermis*, *Bacillus subtilis* and *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* (Table 2).

Invariably, seaweeds have been proven to be potent source of antimicrobial compounds. Ethanolic extract of eight species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad-spectrum antibacterial and antifungal activities<sup>[20]</sup>. The algal extracts such as *Enteromorpha compressa*, *Cladophorosis zoolingeri*, *Padina gymnospora*, *Sargassum wightii* and *Gracilaria corticata* were active against gram positive and gram negative bacteria<sup>[21]</sup>. Based on the present findings, it could be inferred that the bioassay guided fractionation and purification may come up with potent antibacterial compounds.

Santhanam Shanmugapriya *et al.* (2008)<sup>[22]</sup> collected fourteen seaweeds and tested against ten human pathogenic bacteria using the well diffusion test in the Casitone agar medium. Of these, seven species were determined to be highly bioactive and screened on the multiresistant pathogens. They found that drying process has eliminated the active principles in the seaweeds. In the present study, methanol:toluene (3:1) was found to be the best solvent for extracting the antimicrobial principles from fresh algae. However, the ethanolic extract showed no antibacterial activity. The extract from *Gracilaria corticata* was highly active against *Proteus mirabilis*, a Gram negative pathogenic bacterium. The findings of Santhanam Shanmugapriya *et al.* (2008)<sup>[22]</sup> revealed that the tested seaweed *Gracilaria corticata* was highly active against Gram negative bacteria than Gram positive bacteria but the findings of the present study showed that the seaweed *Gracilaria corticata* was highly active against Gram positive bacteria than

Gram negative bacteria. The results of the present study were in contrast with their results.

The methanol crude extracts of *Gracilaria corticata* (10mg/ml) showed highest mean zone of inhibition (14 ± 0.6mm) against *Aspergillus flavus* followed by *Aspergillus fumigatus* (14 ± 0.5mm), *Aspergillus niger* (13 ± 0.7mm), *Candida albicans* (12 ± 0.8mm), *Candida glabrata* (12 ± 0.5mm) and *Saccharomyces cerevisiae* (11 ± 0.6mm). The zone of inhibition obtained from the hexane crude extractsof seaweed *Gracilaria corticata* against fungal pathogens were comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Flucanazole showed zone of inhibition ranging from 10 ± 0.6mm to 15 ± 0.6mm against the test fungal pathogens (Table 3). The Minimum inhibitory concentration (MIC) value of *Gracilaria corticata* against fungi was ranged between 2 mg/ml to 16mg/ml. The lowest MIC (1mg/ml) value of methanolic extract recorded against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* (Table 4).

Subba Rangaiah *et al.* (2010)<sup>[23]</sup> showed that the seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium*, *Padina tetrastrumatica*, of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol crude extracts and minimum with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. Antifungal activity of all the crude extractions of *Gracilaria corticata* showed maximum activity against *Rhizopus stolonifer*. The crude extracts exhibited mild activity against *Mucor racemosus* and *Rhizoctonia solani* and no activity against *Candida albicans*. The results of the present findings showed that the seaweed crude extracts of *Gracilaria edulis* has the inhibitory activity against *Candida albicans* but their research was in contrast with the present study because their study did not showed inhibitory activity against *Candida albicans*.

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

Microorganisms	Zone of inhibition (mm) mg/ml											
	Methanol		Acetone		Chloroform		Hexane		Ethyl acetate		Positive control*	
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	5mg	
<i>Staphylococcus aureus</i>	13±0.5	17±0.3	13±0.3	16±0.3	7±0.5	11±0.4	7±0.5	10±0.4	8±0.3	10±0.6	16±0.5	
<i>Streptococcus pyogenes</i>	13±0.3	18±0.4	11±0.5	14±0.5	7±0.4	10±0.3	6±0.3	9±0.5	7±0.2	10±0.5	18±0.3	
<i>Streptococcus epidermis</i>	12±0.4	16±0.6	12±0.3	15±0.3	8±0.3	12±0.5	7±0.5	10±0.6	9±0.5	12±0.3	14±0.8	
<i>Bacillus subtilis</i>	13±0.5	17±0.5	13±0.4	16±0.3	10±0.2	12±0.3	9±0.4	11±0.5	12±0.3	14±0.4	19±0.6	
<i>Bacillus cereus</i>	12±0.4	16±0.2	12±0.3	15±0.8	10±0.6	13±0.4	10±0.5	12±0.3	11±0.6	14±0.5	17±0.5	
<i>Escherichia coli</i>	13±0.5	16±0.3	12±0.6	14±0.6	12±0.5	14±0.6	9±0.3	11±0.3	12±0.5	14±0.3	17±0.3	
<i>Pseudomonas aeruginosa</i>	13±0.3	16±0.5	12±0.2	14±0.3	10±0.3	13±0.3	9±0.6	12±0.4	12±0.3	14±0.3	18±0.7	

## Fungi

<i>Vibrio cholerae</i>	8±0.3	11±0.4	8±0.3	10±0.4	7±0.4	9±0.4	6±0.5	8±0.6	7±0.5	10±0.4	16±0.5
<i>Salmonella typhi</i>	13±0.4	16±0.6	12±0.4	15±0.4	10±0.2	12±0.5	9±0.3	12±0.4	11±0.3	13±0.6	19±0.6
<i>Klebsiella pneumoniae</i>	13±0.5	17±0.5	12±0.5	15±0.6	10±0.5	12±0.4	9±0.2	11±0.5	12±0.2	14±0.5	20±0.8
<i>Enterobacter aerogenes</i>	13±0.6	17±0.3	13±0.3	15±0.7	11±0.6	13±0.5	9±0.4	12±0.4	12±0.3	14±0.6	17±0.4

Mean± S.D. \*Ampicillin

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

Microorganisms	Minimum inhibitory concentration (mg/ml)					
	Hexane	Methanol	Acetone	Chloroform	Ethyl acetate	Positive Control*
<i>Staphylococcus aureus</i>	10	1.25	1.25	5	2.50	5
<i>Streptococcus pyogenes</i>	10	1.25	2.50	10	5	10
<i>Streptococcus epidermis</i>	20	1.25	2.50	10	5	10
<i>Bacillus subtilis</i>	5	1.25	1.25	5	2.50	10
<i>Bacillus cereus</i>	10	1.25	1.25	5	2.50	10
<i>Escherichia coli</i>	10	2.50	2.50	10	5	5
<i>Pseudomonas aeruginosa</i>	20	2.50	5	10	10	5
<i>Vibrio cholerae</i>	80	5	10	40	20	20
<i>Salmonella typhi</i>	40	5	5	20	10	20
<i>Klebsiella pneumoniae</i>	10	1.25	2.50	5	2.50	10
<i>Enterobacter aerogens</i>	5	1.25	1.25	5	2.50	10

\*Ampicillin

Table 3: Antifungal activity of solvent extracts of *Gracilaria corticata*

Microorganisms	Zone of inhibition (mm) /10mg/ml					
	Hexane	Ethyl Acetate	Acetone	Methanol	Chloroform	Positive Control*
<i>Aspergillus flavus</i>	9±0.7	11±0.4	13±0.5	14±0.6	10±0.6	15±0.6
<i>Aspergillus niger</i>	10±0.8	12±0.6	13±0.4	13±0.7	11±0.7	13±0.4
<i>Aspergillus fumigatus</i>	11±0.6	12±0.7	12±0.6	14±0.5	12±0.6	14±0.7
<i>Saccharomyces cerevisiae</i>	9±0.7	16±0.3	15±0.3	11±0.6	13±0.5	10±0.6
<i>Candida albicans</i>	9±0.7	10±0.4	12±0.4	12±0.8	10±0.7	12±0.7
<i>Candida glabrata</i>	8±0.4	9±0.6	11±0.6	12±0.5	8±0.3	13±0.6

Mean± S.D. \* Flucanazole

Table 4: Minimum inhibitory concentration of solvent extracts of *Gracilaria corticata*

Microorganisms	Minimum inhibitory concentration (mg/ml)					
	Hexane	Methanol	Acetone	Chloroform	Ethyl acetate	Positive control*
<i>Aspergillus flavus</i>	32	8	16	32	16	8
<i>Aspergillus niger</i>	16	4	8	16	8	8
<i>Aspergillus fumigatus</i>	32	8	8	16	16	8
<i>Saccharomyces cerevisiae</i>	64	16	32	64	64	32
<i>Candida albicans</i>	16	2	4	8	8	4
<i>Candida glabrata</i>	32	4	8	16	8	4

\* Flucanazole

## 4. CONCLUSION

The present research concluded that the organic solvent extraction was suitable to verify the antimicrobial properties of *Gracilaria corticata* and they are supported by many investigations. The investigation on antimicrobial activity of crude extracts of *Gracilaria corticata* showed that the methanol crude extracts showed promising antimicrobial activity when compared to other solvent extracts. The results also indicated that scientific studies carried out on seaweed extracts having traditional claims of effectiveness might warrant fruitful results. These seaweeds could serve as useful source of new antimicrobial agents. The present study justifies the claimed uses of seaweeds in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable seaweeds in large scale to increase the economic status of the cultivators in the country. The obtained results may provide a support to use of the seaweeds in traditional medicine.

## REFERENCES

- Mishra, V.K., F. Temelli, P.F. Ooraikul Shacklock and J.S. Craigie. 1993. Lipids of the red alga *Palmaria palmata*. *Botanica Marina*, 36 (2): 169-174.
- Elena, M., Francisco, Y., and Erickson, K.L. 2001. "Mailiohydrin, a Cytotoxic Chamigrene Dibromohydrin from a Phillipine *Laurencia* Species," *J. Nat. Prod.*, 64 (6): 790-791.
- Selvin, J and Lipton, A.P. 2004. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. *J. Marine Sci. Technol.*, 12: 1-6.
- Nair R, Chabhadiya R, Chanda S. 2007. Marine algae: Screening for a potent antibacterial agent. *Journal of Herbal Pharmacotherapy*, 7: 73-86.
- Athukorala, Y., Lee, K.W., Kim, S.K and Jeon, Y.J. 2006. Anticoagulant activity of

- marine green and brown algae collected from Jeju Island in Korea. *Bioresource Technology*, 98: 1711-1716.
6. Isnansetyo A and Y.Kamei. 2003. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* nov 0-BC30<sup>T</sup>, against Methicillin resistant *Staphylococcus aureus*. *Antimicrobial agents Ch.*, 47: 480-488.
  7. Jones W. E. 1959. The growth and fruiting of *Gracilaria verrucosa* (Hudson) Papenfuss ; *J. mar. boil. Ass.*, 38: 47-56.
  8. Bauer, A. W., W. M. M. Kirby, J. C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.*, 45 (4): 493 - 496.
  9. Michael, T.M., M.M. John and P. Jack. 2005. Brock Microbiology of Microorganisms. 11<sup>th</sup> Edition, New Jersey. ISBN: 13-978-0226701479.
  10. Perry N.B., J.W. Blunt, M.H. Munro, A. 1991. Cytotoxic and antifungal 1,4-naphthoquinone and related compounds from a New Zealand brown algae.. *Landsburgia quercifolia*. *J Nat Prod.*, 54 (4): 978-985.
  11. Harada, H., T. Naro and Y. Kamei. 1997. Selective antitumor activity *in vitro* from marine algae from Japan coasts. *Biol. Pharm. Bull.*, 20: 541- 546.
  12. Elena, M., Francisco, Y., and Erickson, K.L. 2003. "Mailiohydrin, a Cytotoxic Chamigrene Dibromohydrin from a Phillippine *Laurencia* Species," *J. Nat. Prod.*, 64 (6): 790-791.
  13. Smit, A.J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J Appl Phycol.*, 16: 245-262
  14. Vlachos, V., Critchley, A.T., Von, H.A. 1996. Establishment of a protocol for testing antimicrobial activity in southern African macroalgae. *Microbios*, 88: 115-123.
  15. Gonzalez Del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, Vicente F, Portillo E, Jiménez Del Rio M, Reina GG, Pelàez F. 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.* 4: 35-40.
  16. Ozdemir G, Karabay N.U., Dalay M.C and Pazarbasi B. 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytother. Res.* 18: 754-757. Paul NA, De Nys R, Steinberg PD.
  17. Karabay-Yavasoglu NU, Sukatar A, Ozdemir G and Horzum Z. 2007. Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. *Phytother. Res.*, 21: 153-156.
  18. Taskin E, M. Ozturk, E Taskin and Kurt. 2007. Antibacterial activities of some marine algae from the Aegean sea (Turkey). *African journal of Biotechnology*, 6 (24) : 2746-2751.
  19. Kandhasamy.M and K.D.Arunachalam. 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *African journal of Biotechnology*, 7(12) : 1958-1961.
  20. Taskin E, Ozturk M and Kurt O. 2001. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *Afr. J. Biotechnol.*, 6: 2746-2751.
  21. De-Campos, T.G.M., Diu, M.B.S., Koenig, M.L., and Periera, E.C. 1998. "Screening of Marine Algae from Brazilian Northeastern Coast for Antimicrobial Activity," *Bot. Mar.*, 31 (5): 375-377.
  22. Santhanam Shanmughapriya, Aseer Manilal, Sugathan Sujith, Joseph Selvin, George Seghal Kiran, Kalimuthusamy Nataraja Seenivasan. 2008. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology*, 58 (3): 535-541.
  23. Subba Rangaiah G, Lakshmi, P and Manjula E. 2010. Antimicrobial activity of seaweeds *Gracillaria*, *Padina* and *Sargassum* sp. on clinical and phytopathogens. *International Journal of Chemical and Analytical Science*, 1(6): 114-117.