

## Pharmacological Efficacy of Marine Seaweed *Gracilaria edulis* Extracts Against Clinical Pathogens

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**Abstract:** Seaweeds are the eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products. The present study was conducted to screen the pharmacological activity of *Gracilaria edulis* solvent extracts against human pathogenic bacteria and fungi. The seaweed *Gracilaria edulis* was extracted using the organic solvents viz., methanol, acetone, petroleum ether, hexane and ethanol. Antimicrobial activity of *Gracilaria edulis* solvent extract was determined by Disc diffusion method. Among the solvents tested, methanol extract of *Gracilaria edulis* showed maximum inhibitory activity than other solvents. The zone of inhibition of *Gracilaria edulis* 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 edulis* against *Klebsiella pneumoniae* followed by *Enterobacter aerogens*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Vibrio cholerae*. For fungi, the zone of inhibition was maximum against *Aspergillus flavus* followed by *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*.

**Key words:** *Gracilaria edulis* • Solvent Extracts • Antimicrobial Activity • Bacteria And Fungi

### INTRODUCTION

Marine organisms are source material for structurally unique natural products with pharmacological and biological activities. Among the marine organisms, the macroalgae occupy an important place as a source of biomedical compounds [1]. About 2400 natural products have been isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae. The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macroalgae for its synthesis of bioactive secondary metabolites. The compounds derived from macroalgae are reported to have broad range of biological activities such as antibacterial [2], anticoagulant [3] and antifouling activity [4]. The antimicrobial agents such as Chlorellin derivatives, acrylin acid, halogenated aliphatic compounds, phenolic inhibitors and more recently Guaiane sesquiterpenes and labdane diterpenoids were also detected from macroalgae [2].

Seaweeds have been traditionally used in human and animal nutrition. Seaweeds are rich source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals [5]. Important polysaccharides such as agar, alginates and carrageenans obtained from seaweeds are used in pharmaceutical as well as in the food industries. Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens chemical defense mechanisms against herbivore; for example, grazer-induced mechanical damage triggers the production of chemicals that acts as feeding deterrents or toxins in seaweeds [6].

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique

compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals. The genus *Gracilaria* is an important component of the flora on most tropical to cool temperate shores. Many of the species, however, are poorly defined, minimally described and inadequately illustrated. Fuelled by the economic interest in phycocolloids, the study of the Gracilariaceae, an important source of agar, has spread rapidly throughout the world in the last decade, resulting in meticulously detailed generic descriptions [7] and numerous regional floristic accounts of the genus *Gracilaria* [8] mainly focusing on the representatives of the Pacific ocean.

## MATERIALS AND METHODS

**Collection of Seaweeds:** The seaweeds *Gracilaria edulis* 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.

**Preparation of Seaweed Extracts:** The collected *Gracilaria edulis* was cleaned and necrotic parts were removed. The seaweed was washed with tap water to remove any associated debris and shade dried at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 5-8 days or until they are brittle easily by hand. After completely drying, the seaweed material (1.0 kg) was ground to a fine powder using Electrical blender. 40 g of powdered sea weeds were extracted successively with 200 ml of solvents (Methanol, Acetone, Petroleum ether, Hexane and Ethanol) 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  $4^\circ\text{C}$  for future use.

**Disc Preparation:** Disc of 5 mm diameter were pretreated using Whatman filter paper No.1. These were sterilized in the hot air oven at  $160^\circ\text{C}$  for 1 hour. The solvent extracts of *Gracilaria edulis* (Methanol, Acetone, Petroleum ether, Hexane and Ethanol) were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 $\mu\text{l}$  of different solvent extracts of sea weeds at two different concentrations ranging 2.5mg/ml and 5mg/ml to check their antibacterial activity and antifungal activity. The paper discs which contain 5% DMSO were act as a blind control and the paper discs containing Ampicillin

(5mg/disc) act as a positive control for bacteria and the paper discs containing Flucanazole (100 units/disc) act as a positive control for fungi.

**Collection of Test Microbial Cultures:** Eleven different bacterial cultures were obtained from RMMC Hospital, Annamalai University, Chidambaram. *Staphylococcus aureus*, *Streptococcus epidermis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Enterobacter aerogens*. Six different fungal isolates were used in this present study. The fungal cultures were procured from RMMC Hospital, Annamalai University, Chidambaram. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*.

## Determination of Antibacterial Activity of *Gracilaria edulis*

**Bacterial Inoculum Preparation:** Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at  $37^\circ\text{C}$  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.

**Disc Diffusion Method:** The antibacterial activity of *Gracilaria edulis* extract was determined by Disc diffusion method proposed by Bauer *et al.* [9]. A bacterial suspension (number 0.5 in McFarland scale about  $1.5 \times 10^8$  bacteria  $\text{ml}^{-1}$ ) 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 edulis* extracts (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) at two different concentration (2.5mg/ml and 5mg/ml) was placed on the surface of the Mueller Hinton agar plates. The paper discs which contain 5% DMSO were act as a blind control and the paper discs containing Ampicillin (5mg/disc) act as a positive control. The plates were incubated at  $37^\circ\text{C}$  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 three times for concordance.

**Minimum Inhibitory Concentration:** Minimum inhibitory concentration (MIC) of the *Gracilaria edulis* 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 128 mg/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 1.25, 2.50, 5, 10, 20, 40 and 80 mg/ml (for *Gracilaria edulis* extracts). Fifty  $\mu$ l of standardized suspension of the test organism and devoid of seaweeds extracts/FAME active principle. The culture tubes were incubated at 37°C for 24 hours. The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determined as Minimum inhibitory concentration.

#### Determination of Antifungal Activity of *Gracilaria edulis*

**Disc Diffusion Method:** The antifungal activity of *Gracilaria edulis* extract was determined by Disc diffusion method proposed by Bauer *et al.* [9]. A fungal suspension (number 0.5 in McFarland scale about  $1.5 \times 10^8$  fungi  $\text{ml}^{-1}$ ) was spread on Mueller-Hinton (pH 7.4) agar using a cotton swab. The Mueller Hinton agar plates were prepared and inoculated with test fungal organisms by spreading the fungal inoculum on the surface of the media. The discs containing *Gracilaria edulis* extracts (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) at 10 mg/ml was placed on the surface of the Mueller Hinton agar plates. The paper discs which contain 5% DMSO were act as a blind control and the paper discs containing Flucanazole (100 units/disc) act as a positive control. The plates were incubated at 28°C for 2 days (yeasts) and 3 days (moulds). The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm). Each assay in these experiments was repeated three times for concordance.

**Minimum Inhibitory Concentration:** Minimum inhibitory concentration of *Gracilaria edulis* extracts was tested in Sabouraud's dextrose broth for fungi by Broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth to get a concentration of 2, 4, 8, 16, 32 and 64 mg/ml (for *Gracilaria edulis* extracts). Fifty  $\mu$ l of standardized suspension of the test organism and devoid of seaweed extracts/FAME active principle. The culture tubes were incubated at 28°C for 2 days (yeasts) and 3 days (moulds). The lowest concentrations which did not show any growth of tested organism after macroscopic valuation was determine the Minimum inhibitory concentration.

## RESULTS AND DISCUSSION

Marine organisms represent a valuable source of new compounds. The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of new active substances in the field of the development of bioactive products. More than 1, 50,000 macro algae or seaweed species are found in oceans of the globe, but only a few of them were identified. Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest for the pharmacological industry. Special attention has been reported for antiviral, anti bacterial and antifungal activities related to marine algae against several pathogens. The antimicrobial compounds derived from the marine flora consist of diverse groups of chemical compounds. The cell extracts and active constituents of various algae have been shown to have antibacterial activity against Gram positive and Gram negative bacteria. In most previous studies only one kind of solvent was used to screen seaweeds for their antimicrobial activities [4].

Seaweeds are the eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products [10]. They contain compounds ranging from sterols, terpenoids to brominated phenolic, which shows bioactive against microorganisms [11]. In the present study, antibacterial activity of five different solvents *viz.*, methanol, acetone, Petroleum ether, hexane and ethanol extracts of *Gracilaria edulis* 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.* [12], Gonzalez *et al.* [13], Ozdemir *et al.* [14], Karabay-Yavasoglu *et al.* [15], Taskin *et al.* [16] and Kandhasamy and Arunachalam [17], 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 [17].

Different solvent systems were used earlier to extract bioactive principles from macroalgae with concomitant changes in the antibacterial activities [18]. The solvents such as acetone, benzene, butanol [19, 20], ethanol [21]

were used to extract antimicrobial compounds from macroalgae. The methanol extract of *Gracilaria edulis* (5.0 mg/ml) showed highest mean zone of inhibition ( $21 \pm 0.4$  mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis* ( $20 \pm 0.5$  mm), *Staphylococcus aureus* ( $20 \pm 0.3$  mm), *Streptococcus epidermis* ( $19 \pm 0.6$  mm) and *Bacillus cereus* ( $19 \pm 0.2$  mm). For Gram negative bacterium, the maximum zone of inhibition was recorded in methanol extract of *Gracilaria edulis* against *Klebsiella pneumoniae* ( $20 \pm 0.5$  mm) followed by *Enterobacter aerogens* ( $20 \pm 0.3$  mm), *Salmonella typhi* ( $19 \pm 0.6$  mm), *Pseudomonas aeruginosa* ( $19 \pm 0.5$  mm), *Escherichia coli* ( $19 \pm 0.3$  mm) and *Vibrio cholerae* ( $14 \pm 0.4$  mm). The zone of inhibition obtained from the Hexane extract of seaweed *Gracilaria edulis* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from  $16 \pm 0.8$  mm to  $23 \pm 0.8$  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 80 µg/ml. The lowest MIC (1.25 µg/ml) value was recorded against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus epidermis*, *Bacillus subtilis* and *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogens* (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 [22]. 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 [23]. 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.* [24] 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 edulis* was highly active against *Proteus mirabilis*, a Gram negative pathogenic bacterium. The findings of Santhanam Shanmugapriya *et al.* [24] revealed that the tested seaweed *Gracilaria edulis* was highly active against Gram negative bacteria than Gram positive bacteria but the findings of the present study showed that the seaweed *Gracilaria edulis* was highly active against Gram positive bacteria than Gram negative bacteria.

The methanol extract of *Gracilaria edulis* (10 mg/ml) showed highest mean zone of inhibition ( $18 \pm 0.6$  mm) against *Aspergillus flavus* followed by *Aspergillus fumigatus* ( $18 \pm 0.5$  mm), *Aspergillus niger* ( $17 \pm 0.7$  mm), *Candida albicans* ( $16 \pm 0.8$  mm), *Candida glabrata* ( $16 \pm 0.5$  mm) and *Saccharomyces cerevisiae* ( $15 \pm 0.6$  mm). The zone of inhibition obtained from the Hexane extract of seaweed *Gracilaria edulis* against fungal pathogens was 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  $9 \pm 0.6$  mm to  $14 \pm 0.6$  mm against the test fungal pathogens (Table 3). The Minimum inhibitory concentration (MIC) value of *Gracilaria corticata* against fungi was ranged between 1 µg/ml to 16 µg/ml. The lowest MIC (1 µg/ml) value was recorded against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* (Table 4).

Subba Rangaiah *et al.* [22] showed that the seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium*, *Padina tetrastrum*, of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol 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 extract *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*. Margret *et al.* [25] reported that methanol extract of *Acanthophora spicifera* was active against Gram negative bacterial pathogen *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*.

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

Microorganisms	Zone of inhibition (mm) mg/ml										
	Methanol		Acetone		Petroleum ether		Hexane		Ethanol		Positive control*
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	5mg
<i>Staphylococcus aureus</i>	16±0.5	20±0.3	16±0.3	19±0.3	15±0.5	19±0.4	15±0.5	18±0.4	16±0.3	18±0.6	19±0.5
<i>Streptococcus pyogenes</i>	16±0.3	21±0.4	14±0.5	17±0.5	15±0.4	18±0.3	14±0.3	17±0.5	15±0.2	18±0.5	21±0.3
<i>Streptococcus epidermis</i>	15±0.4	19±0.6	15±0.3	18±0.3	16±0.3	20±0.5	15±0.5	18±0.6	17±0.5	19±0.3	17±0.8
<i>Bacillus subtilis</i>	16±0.5	20±0.5	16±0.4	19±0.3	18±0.2	20±0.3	17±0.4	19±0.5	20±0.3	21±0.4	21±0.6
<i>Bacillus cereus</i>	15±0.4	19±0.2	15±0.3	18±0.8	18±0.6	21±0.4	18±0.5	20±0.3	19±0.6	21±0.5	20±0.5
<i>Escherichia coli</i>	16±0.5	19±0.3	15±0.6	17±0.6	20±0.5	22±0.6	17±0.3	19±0.3	20±0.5	21±0.3	20±0.3
<i>Pseudomonas aeruginosa</i>	16±0.3	19±0.5	15±0.2	17±0.3	18±0.3	21±0.3	17±0.6	20±0.4	20±0.3	21±0.3	21±0.7
<i>Vibrio cholera</i>	14±0.3	16±0.4	14±0.3	13±0.4	15±0.4	17±0.4	14±0.5	16±0.6	15±0.5	18±0.4	19±0.5
<i>Salmonella typhi</i>	16±0.4	19±0.6	15±0.4	18±0.4	18±0.2	20±0.5	17±0.3	20±0.4	19±0.3	21±0.6	22±0.6
<i>Klebsiella pneumoniae</i>	16±0.5	20±0.5	15±0.5	18±0.6	18±0.5	20±0.4	17±0.2	19±0.5	20±0.2	22±0.5	20±0.8
<i>Enterobacter aerogens</i>	16±0.6	20±0.3	16±0.3	18±0.7	19±0.6	21±0.5	17±0.4	20±0.4	20±0.3	22±0.6	20±0.4

± - Standard deviation, \*Ampicillin

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

Microorganisms	Minimum inhibitory concentration (µg/ml)					
	Hexane	Methanol	Acetone	Petroleum ether	Ethanol	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 cholera</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 edulis*

Microorganisms	Zone of inhibition (mm) /10mg/ml					
	Hexane	Ethanol	Acetone	Methanol	Petroleum ether	Positive control*
<i>Aspergillus flavus</i>	13±0.7	19±0.4	17±0.5	18±0.6	14±0.6	19±0.6
<i>Aspergillus niger</i>	14±0.8	16±0.6	17±0.4	17±0.7	15±0.7	17±0.4
<i>Aspergillus fumigatus</i>	15±0.6	16±0.7	16±0.6	18±0.5	16±0.6	18±0.7
<i>Saccharomyces cerevisiae</i>	13±0.7	20±0.3	19±0.3	17±0.6	17±0.5	14±0.6
<i>Candida albicans</i>	13±0.7	14±0.4	16±0.4	16±0.8	14±0.7	16±0.7
<i>Candida glabrata</i>	12±0.4	13±0.6	15±0.6	16±0.5	13±0.3	17±0.6

± - Standard deviation, \* Flucanazole

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

Microorganisms	Minimum inhibitory concentration (µg/ml)					
	Hexane	Methanol	Acetone	Petroleum ether	Ethanol	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>	—	16	32	—	64	32
<i>Candida albicans</i>	16	2	4	8	8	4
<i>Candida glabrata</i>	32	4	8	16	8	4

\* Flucanazole

## CONCLUSION

The present research concluded that the organic solvent extraction was suitable to verify the antimicrobial properties of *Gracilaria edulis* and they supported by many investigation. The investigation on antimicrobial activity of extracts of *Gracilaria edulis* showed that the methanol extract shows 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.

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