Antibacterial activity of four *Gracilaria* species of red seaweeds collected from Mandapam Coast, Gulf of Mannar Marine Biosphere Reserve, India

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1. Introduction

Bacterial infection causes severe effect on the human population and aquatic organisms and the disease was prevented from treating with drugs or chemicals[1]. Recently, the use of antibiotics has increased due to heavy infections and pathogenic bacteria have resistant to drugs, so the use of antibiotics has indiscriminately increased. The decreased efficiency and resistant of pathogen to antibiotics has necessitated the development of new alteration[2,3]. Approximately 2,500 new metabolites were reported from different types of marine organisms including seaweeds during the years of 1977–1987[4]. There have been a number of reports on antimicrobial activity of the known antibiotics such as chloramphenicol, streptomycin, kanamycin and ampicillin was determined by testing them against different test organisms.

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Objective: To study the antibacterial activities of diethyl ether, toluene, ethanol and methanol extracts of red seaweeds such as *Gracilaria crassa* (*G. crassa*), *Gracilaria folifera* (*G. folifera*), *Gracilaria debilis* (*G. debilis*) and *Gracilaria corticata*.

Methods: The crude extracts were tested against different types of Gram-positive and -negative bacterial strains and all the seaweed extracts were tested a broad spectrum of antibacterial activity. Antibacterial activity was made using paper disc diffusion method. Four organic solvents (diethyl ether, toluene, methanol and ethanol) were used separately in a Soxhlet apparatus for seven bacterial strains. Antibacterial activity of the known antibiotics such as chloramphenicol, streptomycin, kanamycin and ampicillin was determined by testing them against different test organisms.

Results: The high antibacterial activity was noted in the extracts of *G. crassa*, *G. folifera* and *G. debilis*. However, *G. crassa* and *G. debilis* have good antibacterial activity. Pathogens like *Bacillus subtilis* and *Escherichia coli* were less susceptible to the methanol and diethyl ether extracts of *G. folifera*. The comparative study on the antibacterial activity was also made by using 200 μg concentration of solvent extracts (diethyl ether, ethanol, toluene and methanol) and different five antibiotics such as chloramphenicol, streptomycin, kanamycin, amoxicillin and ampicillin. The bacterial strains tested were more sensitive to chloramphenicol, streptomycin, kanamycin, and ampicillin when compared to algal extracts.

Conclusions: The present study proved that the extracts of *G. crassa*, *G. folifera* and *G. debilis* have high antibacterial activity. Although *G. crassa* and *G. debilis* showed good antibacterial activity, many known antibiotics are active against a few organisms individually. Hence, the extracts of seaweeds were active against all test organisms used and the activities were comparable to that of antibiotics and the seaweeds offer a feasible alternative for the development of new antibiotics. The results also suggest the need for a more dynamic search for pharmaceutically interesting substances from Indian seaweeds.

ABSTRACT

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and Sri Lankan waters and some marine algal species have also screened[14-16]. Recently, infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a growing global problem. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. The new therapeutic agents should be effective and have a novel mode of action that renders them impervious to existing resistance mechanisms[17]. The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produced. These limitations demand for improved pharmacokinetic properties which necessitate the continued research for new antimicrobial compounds for the development of drugs[18]. The season and age of collection of marine algae have an important role on their metabolic activity, nature and levels of proximate compositions[19]. Antimicrobial activity of organic solvents always provides a higher efficacy in extracting compounds[20]. Screening of organic extracts from marine algae and other marine organisms is a common approach to identify compounds of biomedical importance. The discovery and development of new antibiotics are the most significant and successful achievements of modern science and technology for the control of diseases[21]. Hence, the present work was aimed to screen and evaluate the efficiency of different solvent extracts selected from marine red algae as antibacterial agents and to select the most active species against the common pathogenic bacteria. The test organisms used and the activities were comparable with some common antibiotics and the seaweeds offer a probable alternative for the antibiotics.

2. Materials and methods

2.1. Collection and processing of seaweeds

Fresh and healthy samples of some marine red algae such as Gracilaria crassa (G. crassa), G. debilis, Gracilaria folifera (G. folifera) and Gracilaria corticata (G. corticata) were collected during low tide period in the rocky shores of Mandapam coastal region, south-east coast of India during monsoon season of 2014–2015 (Figure 1A–D). The samples were cleaned with seawater to remove the epiphytes, sand particles, necrotic parts, pebbles and shells and brought to the laboratory in the sterile polythene bags. The samples were then thoroughly washed with tap water followed by sterile distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were cut into small pieces and powdered in a mixer grinder. The powdered samples were then stored in refrigerator for further use.

2.2. Extract preparation

In solvent preparation, 25 g of powder sample was transferred into 250 mL of four organic solvents viz. diethyl ether, toluene, methanol and ethanol separately in a Soxhlet apparatus at 50–55 °C. The extracts obtained were concentrated by air-drying. The residue was re-dissolved in small quantity of respective solvents and used to screen the antibacterial activity. Antibacterial activities of the known antibiotics such as chloramphenicol, streptomycin, kanamycin and ampicillin were determined by testing them against different test organisms.

2.3. Antibacterial assay

Antibacterial activity was made using paper disc diffusion method. Seven bacterial strains were tested viz., Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Shigella sp., Vibrio cholerae (V. cholerae), Proteus sp. and Pseudomonas fluorescens (P. fluorescens). Whatman No.1 filter paper disc of 6 mm in diameter was prepared. The antibacterial assay of Gram-positive and Gram-negative bacteria was carried out using the agar plate method. Different concentrations (100 μg, 200 μg, 300 μg) of diethyl ether, ethanol, toluene and methanol solvent extracts were applied to separate discs and dried. The discs were placed into the bacterial inoculated plates. Control plates were carried out using this containing ethanol as well as the respective solvents used in the extraction. Based on the preliminary result, one concentration (200 μg) of algal extract was taken and compared with known antibiotic concentration. The plates were kept in incubator at 37 °C for 24 h. After incubation period, the relative susceptibility of the pathogenic organism obtained from the algal extract was demonstrated by the clear zone formed around each disc. The zone of inhibition was measured by using the millimeter-scale.

3. Results

The present study showed that the extracts prepared from G.
Table 3
Antibacterial activity (mm of clear zone) of different solvent extracts prepared from G. crassa, G. folifera, G. debilis and G. corticata had inhibitory effect against the different pathogenic bacteria tested, which included Gram-positive and Gram-negative bacterial members. In G. crassa (Table 1), the four different solvents viz., toluene, diethyl ether, methanol and ethanol were tested. Toluene showed the maximum inhibition of growth against E. coli, Shigella sp., S. aureus, V. cholerae and Proteus sp. than against B. subtilis and P. fluroscens. Toluene extract showed a comparatively greater zone of inhibition than the successive extracts obtained from ethanol, diethyl ether and methanol. The diethyl ether extracts showed the maximum inhibition of growth against the Gram-negative bacteria like P. fluroscens, E. coli and Shigella at the concentration of 300 μg/disc. The ethanol extract inhibited the growth of Gram-negative bacteria like Proteus (20 mm) at the concentration of 300 μg/disc.

The highest range of inhibition was observed in methanol extracts against the Gram-negative bacteria like E. coli (12, 13 and 15 mm) at the concentration of 100, 200 and 300 μg/disc respectively. But methanol extracts showed trace activity against the Gram-negative bacteria like P. fluroscens at the concentration of 100 μg/disc.

Toluene showed the highest range of inhibition of the growth against the Gram-negative bacteria like Proteus sp. (11, 13 and 18 mm) at the concentrations of 100, 200 and 300 μg/disc respectively which was recorded in G. folifera (Table 2) but the methanol extract did not inhibit the growth of Gram-negative bacteria like Shigella at the concentration of 100 μg/disc.

Trace activity have performed against the Gram-positive bacteria like B. subtilis and the Gram-negative bacteria E. coli at the concentration of 100 μg/disc. The diethyl ether extract showed trace inhibition of growth against the Gram-negative bacteria like E. coli and Gram-positive bacteria like Bacillus at the concentration of 100 μg/disc. The ethanol extract did not affect the growth of Gram-positive bacteria like B. subtilis. But the growth of Gram-positive bacteria like S. aureus and Gram-negative bacteria like P. fluroscens was not inhibited at the concentration of 100 μg/disc. In G. debilis (Table 3), the extracts of diethyl ether and toluene were active against both Gram-positive and Gram-negative bacteria. But the toluene showed the highest range of inhibition of growth against the Gram-negative bacteria like Shigella sp. (13, 18 and 20 mm) at the concentrations of 100, 200 and 300 μg/disc respectively. The ethanol extract showed the trace activity of zone of inhibition of growth against the Gram-positive bacteria like B. subtilis and S. aureus at the concentration of 100 μg/disc. The methanol extract showed the maximum inhibition against both Gram-negative and Gram-positive bacteria, but showed the trace activity against the Gram-positive like B. subtilis at 100 μg/disc concentration. In G. corticata (Table 4), methanol showed the maximum inhibition (19 and 1 mm of growth at the concentration of 100 μg) against S. aureus followed by toluene which showed the highest inhibition (17 mm) of growth.

Table 1
Antibacterial activity (mm of clear zone) of different solvent extracts prepared from G. crassa.

| Bacterial strains | Diethyl ether | Ethanol | Toluene | Methanol |
|-------------------|--------------|---------|---------|----------|
|                   | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg |
| Bacillus sp.      | -            | 9       | 14       | -        | 8           | 10     | -          | 8        | 14     |
| S. aureus         | -            | 11      | 13       | -        | 9           | 11     | -          | 9        | 16     |
| E. coli           | -            | 10      | 15       | -        | 8           | 14     | -          | 9        | 17     |
| Shigella sp.      | -            | 10      | 15       | -        | 8           | 17     | -          | 12       | 20     |
| V. cholerae       | -            | 9       | 13       | -        | 9           | 12     | -          | 12       | 16     |
| Proteus sp.       | -            | 8       | 13       | -        | 9           | 20     | -          | 10       | 15     |
| P. fluroscens     | -            | 8       | 16       | -        | 11          | 15     | -          | 8        | 14     |

Table 2
Antibacterial activity (mm of clear zone) of different solvent extracts prepared from G. folifera.

| Bacterial strains | Diethyl ether | Ethanol | Toluene | Methanol |
|-------------------|--------------|---------|---------|----------|
|                   | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg |
| Bacillus sp.      | -            | Trace resistant | 14       | -        | Resistant | 9       | -          | Trace resistant | 16     |
| S. aureus         | -            | 8       | 12       | -        | 9           | 13     | -          | 8         | 12     |
| E. coli           | -            | Trace resistant | 17       | -        | Trace resistant | 10     | -          | 7         | 11     |
| Shigella sp.      | -            | 8       | 13       | -        | 7           | 11     | -          | 7         | 14     |
| V. cholerae       | -            | 10      | 13       | -        | 8           | 12     | -          | 7         | 14     |
| Proteus sp.       | -            | 10      | 15       | -        | 8           | 10     | -          | 11        | 18     |
| P. fluroscens     | -            | 8       | 14       | -        | -           | 10     | -          | 11        | 15     |

Table 3
Antibacterial activity (mm of clear zone) of different solvent extracts prepared from G. debilis.

| Bacterial strains | Diethyl ether | Ethanol | Toluene | Methanol |
|-------------------|--------------|---------|---------|----------|
|                   | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg | Control 100 μg | 300 μg |
| Bacillus sp.      | -            | 9       | 13       | -        | Trace resistant | 8       | -          | 7        | 11     |
| S. aureus         | -            | 8       | 13       | -        | Trace resistant | 9       | -          | 10       | 16     |
| E. coli           | -            | 9       | 15       | -        | 8           | 13     | -          | 9        | 12     |
| Shigella sp.      | -            | 8       | 15       | -        | 8           | 11     | -          | 13       | 20     |
| V. cholerae       | -            | 9       | 16       | -        | 8           | 14     | -          | 11       | 16     |
| Proteus sp.       | -            | 8       | 15       | -        | 9           | 11     | -          | 9        | 16     |
| P. fluroscens     | -            | 9       | 15       | -        | Trace resistant | 11     | -          | 9        | 11     |
Table 4
Antibacterial activity (mm of clear zone) of different solvent extracts prepared from G. corticata.

| Bacterial strains | Diethyl ether | Ethanol | Toluene | Methanol |
|-------------------|---------------|---------|---------|----------|
| Bacillus sp.       | C 100 μg 300 μg | C 100 μg 300 μg | C 100 μg 300 μg | C 100 μg 300 μg |
| S. aureus          | - 5 11 Trace resistant | 12 17 17 | - 5 16 | |
| E. coli           | - 8 10 Trace resistant | 15 16 16 | - 16 16 | |
| Shigella sp.      | - 8 12 16 | - 8 17 17 | - 13 15 15 | - 11 16 16 |
| V. cholerae       | - 9 13 15 | - 8 14 15 | - 11 15 15 | - 11 14 15 |
| Proteus sp.       | - 5 14 14 | - 7 11 11 | - 12 16 16 | - 13 12 12 |
| P. fluroscens     | - 10 15 14 | - 9 12 12 | - 12 11 11 | - 18 17 17 |

Table 5
Comparison of antibacterial activity of different solvent extracts (200 μg) with the activity of different antibiotics.

| Parameters | Bacillus sp. | S. aureus | E. coli | Shigella sp. | V. cholerae | Proteus sp. | P. fluroscens |
|------------|--------------|-----------|---------|-------------|-------------|-------------|--------------|
| G. crassa  | Diethyl ether | 12 14 12  | 12 14 12 | 10 12 12  | 12 12 12  | 12 12 12  | 12 12 12  |
| Ethanol    | 9 10 10 12  | 10 13 10  | 11 14 11  | 13 14 13  | 12 14 12  | 13 14 13  | 12 14 13  |
| Toluene    | 10 11 12 13 | 10 13 12  | 11 14 11  | 12 14 12  | 13 14 13  | 14 15 14  | 12 14 13  |
| Methanol   | 9 10 11 12  | 11 12 11  | 9 10 11  | Trace resistant | 10 11 12  | 10 11 12  | 10 11 12  |
| G. folifera| Diethyl ether | 11 12 11  | 11 11 12 | 11 12 11  | 12 11 12  | 11 12 11  | 12 11 12  |
| Ethanol    | Resistant 8 9 10 | Trace resistant | 9 12 12  | 13 14 13  | 11 12 11  | 13 14 13  | 12 11 12  |
| Toluene    | 12 10 10 11 | 10 12 10  | 11 12 11  | 12 11 12  | 13 14 13  | 14 15 14  | 13 14 13  |
| Methanol   | 10 10 10 11 | 10 10 10  | 9 10 10  | 9 10 10  | 10 10 10  | 10 10 10  | 10 10 10  |
| G. debilis | Diethyl ether | 10 11 11  | 10 12 11  | 10 12 11  | 12 11 12  | 11 12 11  | 12 11 12  |
| Ethanol    | 7 8 9 10 11 | 8 10 9 10  | 10 10 10  | 10 10 10  | 10 10 10  | 10 10 10  | 10 10 10  |
| Toluene    | 10 12 12 13 | 12 13 12  | 11 12 11  | 12 12 12  | 13 14 13  | 14 15 14  | 13 14 13  |
| Methanol   | 8 11 12 13 | 11 12 11  | 9 10 10  | 10 10 10  | 10 10 10  | 10 10 10  | 10 10 10  |
| G. corticata| Diethyl ether | 11 12 11  | 11 12 11  | 12 12 11  | 13 12 12  | 14 13 14  | 15 14 15  |
| Ethanol    | 6 18 18 19 | 13 14 13  | 12 12 12  | 13 12 14  | 14 15 14  | 15 14 15  | 16 16 16  |
| Toluene    | 12 12 12 13 | 14 14 14  | 13 14 13  | 14 15 14  | 15 14 15  | 16 16 16  | 16 16 16  |
| Methanol   | 18 18 18 19 | 16 16 16  | 15 15 15  | 16 16 16  | 17 17 17  | 18 18 18  | 18 18 18  |
| Antibiotics | Chloramphenicol | 25 33 32 | 32 28 28 | 30 25 25 | 26 26 26 | 27 27 27 | 28 28 28 |
| Kanamycin  | 32 31 32 32 | 30 30 30 30 | 30 30 30 30 | 30 30 30 30 | 30 30 30 30 | 30 30 30 30 | 30 30 30 30 |
| Ampicillin | 27 30 30 30 | 11 14 14 14 | 9 14 14 14 | 17 17 17 17 | 17 17 17 17 | 17 17 17 17 | 17 17 17 17 |
| Amoxicillin | 9 9 9 9 9 | 8 8 8 8 | 10 10 10 10 | 9 9 9 9 9 | 9 9 9 9 9 | 9 9 9 9 9 | 9 9 9 9 9 |
| Streptomycin| 33 38 38 38 | 32 32 32 32 | 25 25 25 25 | 28 28 28 28 | 29 29 29 29 | 29 29 29 29 | 29 29 29 29 |

Table 4 indicates that the crude extracts in the brown algae *Sargassum merrifedi*ii and *Sargassum cinctum* had greater antibacterial activity against *S. aureus, Sargassum citrate, Bacillus* and *Pseudomonas aeroginosa*. However, in the present study *Bacillus* was unaffected by the ethanol extract of *G. folifera*. While screening algal extracts against human pathogens (*Bacillus, S. aureus, E. coli, Shigella, V. cholerae, Proteus, P. fluroscens*), inhibition was observed with all the solvent extracts and this indicates that the inhibiting hydrophobic compound was observed on the cell surface of seaweed in all the solvents, namely, diethyl ether, toluene and methanol. Inhibitory effect was observed in many bacteria exposed to methanol and toluene extracts and response was less in ethanol and diethyl ether extracts. The comparative study on the antibacterial activity was also made by using the 200 μg concentration of solvent extracts like diethyl ether, ethanol, toluene and methanol and five different antibiotics such as chloramphenicol, streptomycin, kanamycin and ampicillin at 200 μg concentration. In general, the bacterial strains tested were more sensitive to chloramphenicol, streptomycin, kanamycin and ampicillin when compared to algal extracts. However, the bacterial strains tested were resistance to amoxicillin but were sensitive to algal extracts. No change was observed in any of the control plants against the pathogenic organisms. The present study proved that the extracts of *G. crassa, G. folifera* and *G. debilis* have high antibacterial activity. Although *G. crassa* and *G. debilis* showed good antibacterial activity, some pathogens like *Bacillus* and *E. coli* were less susceptible to the diethyl ether and methanol extracts of *G. folifera*. Many known antibiotics are active against a few organisms individually. Susceptible organisms are developing resistance due to the continuous exposure to these antibiotics. Hence, it is very important to develop and evaluate new drugs with a wider range and increased potency. Since the extracts from seaweeds were active against all the test organisms used and the activities were comparable to that of antibiotics, the seaweeds offer a probable alternative for the antibiotics. The results also suggest the need for a more vigorous search for pharmaceutically interesting substances from Indian seaweeds.

The algal extracts were tested for antibacterial activity against the Gram-negative bacteria like *V. cholerae, Proteus* and Gram-positive bacteria.
bacteria like *S. aureus*. However, ethanol and methanol extracts showed less inhibitory effects against both Gram-positive and Gram-negative bacteria than the diethyl ether and toluene extracts. The antibacterial potential of the seaweeds was in the following order: *G. crassa*, *G. debilis*, *G. folifera* and *G. corticata*. A comparative study on the antibacterial activity was also made by using the 200 μg concentration of solvent extracts like diethyl ether, ethanol, toluene and methanol and five different antibiotics such as chloramphenicol, streptomycin, kanamycin, amoxicillin and ampicillin at 200 μg concentration. In general, the bacterial strains tested were sensitive to algal extracts. However, the bacterial strains tested were resistant to amoxicillin but were sensitive to algal extracts. In general, the bacterial strains tested were sensitive to the 200 μg concentration. In general, the bacterial strains tested were sensitive to the marine organisms. A comparative study on the antibacterial activity was also made by using the 200 μg concentration of solvent extracts like diethyl ether, ethanol, toluene and methanol and five different antibiotics such as chloramphenicol, streptomycin, kanamycin, amoxicillin and ampicillin at 200 μg concentration. In general, the bacterial strains tested were sensitive to algal extracts. However, the bacterial strains tested were resistant to amoxicillin but were sensitive to algal extracts.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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