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

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ABSTRACT

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.

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 2500 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 red seaweeds against several pathogens[5-11]. The extracts and activities of various seaweeds have good antibacterial activity against various Gram-positive and Gram-negative bacteria[12,13]. The antibacterial activities of the red seaweed *Gracilaria debilis* (*G. debilis*) associated with epiphytic bacteria against human bacterial pathogens reported from Indian waters, west coast of India waters

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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.

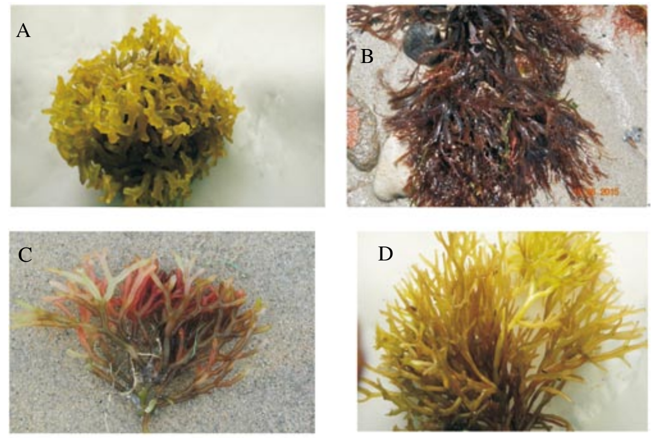

Figure 1. Collected seaweeds for antibacterial study.
A: *G. crassa*; B: *G. folifera*; C: *G. debilis*; D: *G. corticata.*

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 fluroscens (P. fluroscens)*. 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.*

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	-	8	10
S. aureus	-	11	13	-	9	11	-	9	16	-	9	12
E. coli	-	10	15	-	8	14	-	9	17	-	12	15
Shigella sp.	-	10	15	-	8	17	-	12	20	-	9	12
V. cholerae	-	9	13	-	9	12	-	12	16	-	8	11
Proteus sp.	-	8	13	-	9	20	-	10	15	-	8	13
P. fluroscens	-	8	16	-	11	15	-	8	14	-	Trace resistant	10

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	16	-	Trace resistant	11
S. aureus	-	8	12	-	-	9	-	9	13	-	8	12
E. coli	-	Trace resistant	17	-	Trace resistant	10	-	7	11	-	Trace resistant	14
Shigella sp.	-	8	13	-	7	11	-	7	14	-	-	11
V. cholerae	-	10	13	-	8	12	-	7	14	-	9	15
Proteus sp.	-	10	15	-	8	10	-	11	18	-	9	12
P. fluroscens	-	8	14	-	-	10	-	11	15	-	9	13

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	-	Trace resistant	10
S. aureus	-	8	13	-	Trace resistant	9	-	10	16	-	9	15
E. coli	-	9	15	-	8	13	-	9	12	-	10	17
Shigella sp.	-	8	15	-	8	11	-	13	20	-	8	12
V. cholerae	-	9	16	-	8	14	-	11	16	-	9	12
Proteus sp.	-	8	15	-	9	11	-	9	16	-	9	11
P. fluroscens	-	9	15	-	Trace resistant	11	-	9	11	-	8	11

Table 4

Antibacterial activity (mm of clear zone) of different solvent extracts prepared from *G. corticata.*

Bacterial strains	Diethyl ether			Ethanol			Toluene			Methanol		
	C	100 µg	300 µg	C	100 µg	300 µg	C	100 µg	300 µg	C	100 µg	300 µg
Bacillus sp.	-	5	11	-	Trace resistant	12	-	17	17	-	5	16
S. aureus	-	8	10	-	Trace resistant	15	-	16	16	-	19	16
E. coli	-	11	12	-	9	16	-	11	15	-	11	14
Shigella sp.	-	8	12	-	8	17	-	13	10	-	18	15
V. cholerae	-	9	13	-	8	14	-	11	15	-	15	14
Proteus sp.	-	5	14	-	7	11	-	12	16	-	13	12
P. fluroscens	-	10	15	-	9	12	-	12	11	-	18	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	12	14	12	12	10	12
	Ethanol	9	10	10	13	10	11	14
	Toluene	10	11	13	15	14	13	11
	Methanol	9	10	13	11	9	10	9
G. folifera	Diethyl ether	11	11	11	9	12	12	11
	Ethanol	Resistant	Trace resistant	8	9	9	9	8
	Toluene	12	10	10	9	12	13	14
	Methanol	10	10	10	9	12	10	10
G. debilis	Diethyl ether	10	10	11	10	12	12	11
	Ethanol	7	8	10	10	10	10	10
	Toluene	10	12	10	18	13	11	10
	Methanol	8	11	14	11	11	10	10
G. corticata	Diethyl ether	11	15	11	17	16	16	11
	Ethanol	6	18	13	12	12	16	14
	Toluene	12	11	14	14	12	11	13
	Methanol	18	11	16	15	14	13	16
Antibiotics	Chloramphenicol	25	33	32	32	28	30	25
	Kanamycin	32	31	32	30	30	30	26
	Amphicillin	27	30	11	14	9	14	17
	Amoxicillin	9	9	8	8	9	10	9
	Streptomycin	33	38	32	32	25	28	32

to against *Bacillus* sp. at the concentration of 100 and 300 µg and ethanol with 300 µg concentration. The minimum inhibition (5 mm) was observed by diethyl ether against *Bacillus* and *Proteus* sp. and methanol against *Bacillus* sp. The antibacterial study was compared by using different antibiotics such as chloramphenicol, streptomycin, kanamycin and amphicillin at 200 µg concentration (Table 5).

4. Discussion

The results were in accordance with the study conducted by Rao who found that the crude extracts in the brown algae *Sargassum merrifiedii* and *Sargassum cinctum* had greater antibacterial activity against *S. aureus*, *Sargassum citrate*, *Bacillus* and *Pseudomonas aeroginosa*[22]. 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. cholerare, 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[23,24]. 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 amphicillin at 200 µg concentration. In general, the bacterial strains tested were more sensitive to chloramphenicol, streptomycin, kanamycin and amphicillin 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[25]. 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 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 chloramphenicol, streptomycin, kanamycin and ampicillin when compared 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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