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Evaluation of antibacterial potential of seaweeds occurring along the coast of Mandapam, India against human pathogenic bacteria

Gopalraj Karthikai Devi[1*], Karunamoorthy Manivannan[2], Perumal Anantharaman[3]

[1]*Department of Zoology, D.G.G. Arts College for Women, Mayiladuthurai, Tamil Nadu-609001, India*

[2]*Division of Microbial Biodiversity and Bioenergy, Department of Microbiology, Bharathidasan University,Tiruchirapalli, Tamilnadu-620024, India*

[3]*Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu-608502, India*

PEER REVIEW

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Dr. Humberto J. Morris, Center for Studies on Industrial Biotechnology (CEBI), Faculty of Natural Sciences, University of Oriente, Avenida Patricio Lumumba s/n, Santiago de Cuba 5, CP 90500, Cuba.
Tel: 53-022-632095
E-mail: hmorris@cebi.uo.edu.cu; morrishumberto@gmail.com

Comments

This is a valuable research work in which authors have demonstrated the antimicrobial activities of seaweeds from India. The activity of extracts was assessed based on standard microbiological methods. The authors' findings will have immense potential on the control of clinical pathogens, since the strains used were collected from hospital sources and most of the strains appeared as multidrug resistant.
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ABSTRACT

Objective: To investigate the antibacterial activity of six seaweeds against twelve human bacterial pathogens using seven different solvents.
Methods: Antibacterial activity was evaluated using the disc diffusion technique on Mueller Hinton agar. Broth dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents.
Results: Among the six seaweeds tested, the antibacterial activity was higher in the brown seaweeds when compared to red and green seaweeds. *Padina gymnospora* was found exerting a strong antibacterial activity with a high range inhibition [(6.00±0.82) to (22.67±1.25) mm]. Broth dilution assay was conducted against *Salmonella* sp. with the chloroform extract of *Padina gymnospora* and the growth inhibition was found in the range of 74.26% to 82.56%.
Conclusions: Further research studies are being carried out on other species of seaweeds of different habitats in order to provide complete data of the antimicrobial potential of these algae. It is also essential to study the principle compound present in the seaweeds which is responsible for antimicrobial activity. It can be achieved by using advanced separation techniques.

KEYWORDS
Antibacterial, Seaweeds, Mandapam, Human pathogenic bacterium, Different extracts

1. Introduction

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. Due to the increase in bacterial resistance against commercial antibiotics, there is a growing need for new antibacterial compounds that are active against pathogenic bacteria. In addition, most of the existing antibiotics are occasionally associated with adverse effects to the host, including hypersensitivity, immune-suppression and allergic reactions. These developments demand that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One possible strategy is the rational localization of bioactive products, with the hope that systematic screening of these will result in the discovery of novel effective compounds with potent and useful activities against microbes.

*Corresponding author: Dr. Gopalraj Karthikai Devi, Guest Lecturer, Department of Zoology, D.G.G. Arts College for Women, Mayiladuthurai, Tamil Nadu-609001, India.
Tel: +91-4364-223393
Fax: +91-4364 243577
E-mail: mkarthikaidevi@gmail.com

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There is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and in most cases, easily available at affordable prices; they also have no side effects. Plant secondary metabolites are economically important in the field of food additives, nutraceutical and drugs. During the last decade, market for medicinal herbs has grown at an unprecedented rate. This growth is consumer-driven as people focus on health maintenance using natural products. An demand for biodiversity in the screening programs seeking therapeutic drugs from natural products is increasing, and there is now greater interest in marine organisms, especially algae.

Seaweeds are considered as such a source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumorals, antioxidant and antiinflammatories[1-7]. Extracts of marine algae were reported to exhibit antibacterial activity[8]. Therefore the present study was undertaken to investigate the antibacterial activity of six seaweeds against twelve human bacterial pathogens using seven different solvents.

2. Materials and methods

2.1. Preparation of extracts

A total of six seaweeds were used for the study, such as *Ulva reticulata (U. reticulata)*, *Codium tomentosum (C. tomentosum)*, *Turbinaria conoides (T. conoides)*, *Padina gymnospora (P. gymnospora)*, *Acanthophora spicifera (A. spicifera)* and *Gelidiella acerosa (G. acerosa)*. The dried seaweed materials were blended into coarse powder. Before extraction portions of the powdered samples (5 g) were packed in Soxhlet apparatus and extracted successively with methanol, acetone, chloroform, ethanol, ethyl acetate, diethyl ether and petroleum ether for 10 h[9]. The crude extracts were weighed and deep frozen (-20 °C) until tested.

2.2. Bacterial strains

In the present study human pathogens were used, such as *Klebsiella pneumoniae (K. pneumoniae)*, *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Enterococci* sp., *Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa (P. aeruginosa)*, *Vibrio parahaemolyticus (V. parahaemolyticus)*, *Salmonella* sp., *Shewanella* sp., *Vibrio fluvialis (V. fluvialis)* and *Vibrio splendidus (V. splendidus)*. These strains were obtained from the department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamilnadu, India. The bacterial stock cultures were maintained on nutrient agar medium at 4 °C.

2.3. Antibacterial activity

Antibacterial activity was evaluated using the disc diffusion technique on Mueller Hinton agar[10]. Briefly, sterile filter paper discs 6 mm in diameters (Whatmann # 1) were loaded with 25 μL of different extracts (100 mg/mL) and air dried. Discs containing solvents alone were used as negative control and streptomycin was used as a positive control. The discs were placed on Mueller Hinton agar (Himedia, India). The plates were inoculated with each of the previously mentioned pathogens in triplicate and incubated for 24 h at 37 °C. The zone of inhibition was measured.

2.4. Broth dilution method

Dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents[11]. The test extracts or compounds are mixed with suitable media that has been inoculated with the test organism. About 5 mL of the nutrient broth, 0.1 mL of the 24 h growing bacterial culture of *Salmonella* sp. and the different concentration (25-100 μg/mL) of the chloroform extract of *P. gymnospora* were inoculated. About 0.1 mL of the 24 h growing *Salmonella* sp. was inoculated in 5 mL of the nutrient broth with standard antibiotic streptomycin (25-100 μg/mL). The tubes were incubated at 37 °C for 24 h. After the incubation, the optical densities of bacterial culture in each tube were measured in UV-Vis spectrophotometer at 600 nm. The percentage of viable cells was calculated using the following formula:

$$\% \text{ Viable cells} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Where OD is optical density.

3. Results

3.1. Antibacterial activity-disc diffusion assay

The antibacterial activity of specific concentration (25 μL of 100 mg/mL extract) of different extracts of *U. reticulata*, *C. tomentosum*, *T. conoides*, *P. gymnospora*, *A. spicifera* and *G. acerosa* against 12 human bacterial pathogens *viz.*, *K. pneumoniae*, *E. coli*, *S. aureus*, *Enterococci* sp., *Proteus* sp., *Streptococcus* sp., *P. aeruginosa*, *V. parahaemolyticus*, *Salmonella* sp., *Shewanella* sp., *V. fluvialis* and *V. splendidus* determined by disc diffusion method were presented in Table 1-6. The control antibiotic streptomycin inhibited all the tested pathogens.

3.2. C. tomentosum

The maximum inhibition zone [(11.33±0.47) mm] was observed in ethanol extract against *S. aureus*, followed by acetone, diethylether and methanol extracts which showed moderate activity against some pathogens. Minimum activity [(1.33±0.47) mm] was observed in the methanol extract against the same pathogen. Most of the pathogens were resistant to petroleum ether and ethyl acetate extracts and some pathogens showed least activity and *V. fluvialis* is sensitive to all the extracts except ethanol (Table 1).

3.3. U. reticulata

The highest inhibition zone was recorded in ethanol extract against *S. aureus* [(13.00±0.82) mm] and lowest [(2.33±0.47) mm] in the diethylether extract against *K. pneumoniae*. Among

the extracts ethanol was active against all the pathogens and most of the pathogens were resistant to methanol and petroleum ether extract. Among the pathogens *Enterococci* sp. and *Proteus* sp. were sensitive to all the extracts (Table 2).

3.4. T. conoides

All the extracts of *T. conoides* were active against all the tested pathogens. The maximum [(14.67±1.25) mm] zone of inhibition was noticed from the methanol extract against *Enterococci* sp. and minimum inhibition [(2.67±0.94) mm] was observed from the ethanol extract against *K. pneumoniae* (Table 3).

3.5. P. gymnospora

Like *T. conoides*, all the extracts of *P. gymnospora* were active to most of the pathogens with the highest inhibition [(22.67±1.25) mm] in chloroform extract against *Salmonella* sp., and lowest [(6.00±0.82) mm] in the ethyl acetate extract against *Proteus* sp. No activity was found for diethyl ether, petroleum ether and acetone extracts against *Proteus* sp. Among the solvents, chloroform and ethanol extracts were sensitive to all the pathogens (Table 4).

3.6. A. spicifera

The maximum activity was recorded from chloroform extract against *K. pneumoniae* [(15.33±1.25) mm] and lowest [(2.67±0.47) mm] in ethyl acetate against *Salmonella* sp. Among the extracts chloroform was active against all the pathogens and most of the pathogens were resistant to diethyl ether and petroleum ether extract. Among the pathogens *E. coli* and *Salmonella* sp. were sensitive to all the extracts (Table 5).

3.7. G. acerosa

The maximum inhibition zone [(17.33±0.47) mm] was observed in diethylether extract against *K. pneumoniae*, followed by diethylether, petroleum ether and acetone extracts, which showed moderate activity against some pathogens. Minimum activity [(2.67±0.47) mm] was observed in ethyl acetate extract against *Salmonella* sp. Most of the pathogens are resistant to petroleum ether extracts and some pathogens showed least activity (Table 6).

Table 1

Antibacterial activity of *C. tomentosum* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control (streptomycin 50 μg/mL)
K. pneumoniae	5.00±2.16	–	3.67±1.25	–	3.67±0.94	–	4.00±0.82	12.00±1.63
E. coli	2.67±0.47	3.33±0.47	3.00±0.82	5.00±0.82	–	–	–	13.67±0.94
S. aureus	1.33±0.47	3.33±0.47	11.33±0.47	–	–	3.67±0.94	3.33±0.94	24.67±0.94
Enterococci sp.	5.67±0.47	4.00±0.82	4.33±1.25	3.33±0.94	2.67±0.47	3.33±0.94	–	12.33±1.25
Proteus sp.	–	2.33±0.47	3.33±0.47	3.67±0.47	–	–	5.67±1.25	13.67±1.25
Streptococcus sp.	2.33±0.47	2.67±0.47	2.33±0.47	2.67±0.47	3.33±0.47	–	3.67±0.94	7.00±0.82
P. aeruginosa	3.33±0.47	5.33±0.94	2.67±0.94	2.33±0.47	–	3.33±0.47	–	10.33±1.25
V. parahaemolyticus	–	–	2.67±0.47	2.66±0.94	–	–	7.33±1.25	12.67±2.05
Salmonella sp.	2.67±0.94	–	5.33±0.47	–	4.67±0.47	3.33±0.47	7.67±0.47	13.33±0.94
Shewanella sp.	2.67±0.47	–	–	3.33±0.47	–	–	2.33±0.47	10.67±1.70
V. flurialis	2.67±0.94	3.33±0.47	–	4.00±0.82	2.67±0.47	3.67±1.25	3.33±0.94	11.00±2.16
V. splendidus	–	4.67±0.47	2.67±0.47	7.00±0.82	2.33±0.47	–	2.67±0.94	11.67±1.70

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

Table 2

Antibacterial activity of *U. reticulata* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control (streptomycin 50 μg/mL)
K. pneumoniae	–	2.33±0.47	4.66±0.94	2.33±0.47	–	–	3.67±1.25	12.00±1.63
E. coli	6.33±0.47	5.33±0.47	3.33±0.94	4.67±0.47	–	–	–	13.67±0.94
S. aureus	–	–	13.00±0.82	3.33±1.25	–	2.67±0.47	4.00±0.82	24.67±0.94
Enterococci sp.	5.00±0.82	3.33±0.94	4.33±0.47	3.66±0.94	2.33±0.47	3.67±0.94	9.33±1.25	12.33±1.25
Proteus sp.	3.33±0.47	4.67±0.47	7.33±1.70	2.67±0.47	3.33±0.94	3.67±0.47	5.33±0.47	13.67±1.25
Streptococcus sp.	–	7.33±0.47	8.00±0.82	4.00±0.82	–	2.66±0.94	2.67±0.47	7.00±0.82
P. aeruginosa	–	3.33±0.47	7.33±0.47	2.67±0.47	–	–	–	10.33±1.25
V. parahaemolyticus	3.33±0.47	5.33±0.47	3.33±0.47	3.67±1.25	1.67±0.47	2.33±0.47	–	12.67±2.05
Salmonella sp.	3.33±0.47	3.67±0.47	3.67±0.47	–	–	3.67±0.47	5.66±0.94	13.33±0.94
Shewanella sp.	–	–	4.33±0.47	–	5.33±0.47	2.67±0.47	2.67±0.47	10.67±1.70
V. flurialis	–	–	8.33±0.47	–	–	–	–	11.00±2.16
V. splendidus	–	7.33±0.47	3.67±0.94	–	2.67±0.94	–	–	11.67±1.70

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

Table 3

Antibacterial activity of *T. conoides* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control (streptomycin 50 μg/mL)
K. pneumoniae	7.33±0.47	12.33±1.25	2.67±0.94	11.33±0.94	10.67±1.25	9.33±1.25	6.67±0.47	12.00±1.63
E. coli	7.67±0.47	4.67±0.47	7.33±0.47	4.67±0.47	3.00±0.82	6.33±0.47	3.67±2.36	13.67±0.94
S. aureus	4.33±1.25	5.33±0.47	13.33±1.25	4.33±0.47	6.67±0.47	12.00±0.82	6.00±0.82	24.67±0.94
Enterococci sp.	14.67±1.25	7.67±0.47	7.67±0.94	7.67±0.47	6.33±0.47	6.67±0.47	7.67±0.47	12.33±1.25
Proteus sp.	7.33±0.47	7.00±0.82	9.00±0.82	6.33±0.47	8.33±0.47	3.67±0.94	13.33±1.25	13.67±1.25
Streptococcus sp.	4.33±0.47	7.67±0.47	5.33±0.47	4.33±0.47	5.33±0.47	4.67±0.94	13.33±1.89	7.00±0.82
P. aeruginosa	7.67±0.47	4.33±0.47	7.00±0.82	7.67±0.94	4.00±0.82	6.67±0.94	8.00±0.82	10.33±1.25
V. parahaemolyticus	7.67±0.47	5.67±0.47	7.33±0.94	5.00±0.82	6.67±0.47	4.67±0.47	6.00±1.41	12.67±2.05
Salmonella sp.	3.67±0.94	7.33±0.47	3.33±0.47	11.33±1.25	7.67±0.47	3.33±0.47	3.00±0.82	13.33±0.94
Shewanella sp.	5.33±0.94	4.33±0.47	6.67±0.94	7.33±0.47	4.33±0.94	3.67±0.94	4.67±0.94	10.67±1.70
V. flurialis	5.33±0.47	6.33±0.94	4.67±0.94	5.33±0.47	6.00±1.41	5.67±0.94	6.67±0.94	11.00±2.16
V. splendidus	8.67±0.47	7.33±0.47	5.33±0.47	4.33±0.47	6.00±0.82	4.33±0.47	5.00±0.82	11.67±1.70

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

Table 4

Antibacterial activity of *P. gymnospora* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control (streptomycin 50 μg/mL)
K. pneumoniae	14.67±1.25	15.67±1.25	15.00±0.82	15.33±0.94	15.00±0.82	14.33±1.25	14.67±1.25	12.00±1.63
E. coli	9.33±1.25	12.67±1.70	13.67±2.05	15.67±1.25	11.33±0.47	12.67±1.25	10.67±1.25	13.67±0.94
S. aureus	10.67±1.25	13.00±1.63	13.33±2.62	14.33±1.70	10.67±1.25	12.67±2.05	13.00±0.82	24.67±0.94
Enterococci sp.	–	7.67±0.94	14.00±2.16	10.67±1.25	13.33±1.25	12.67±0.94	12.67±1.70	12.33±1.25
Proteus sp.	12.33±1.25	13.33±0.94	10.67±2.05	–	–	6.00±0.82	–	13.67±1.25
Streptococcus sp.	16.00±0.82	12.33±1.25	8.00±0.82	9.67±4.11	12.33±1.25	20.00±0.82	7.33±0.47	7.00±0.82
P. aeruginosa	13.33±1.25	13.00±1.63	8.00±0.82	–	12.33±1.25	–	13.67±1.70	10.33±1.25
V. parahaemolyticus	15.33±1.70	13.00±1.63	8.67±1.70	21.00±1.63	17.67±0.47	17.33±1.25	12.33±1.25	12.67±2.05
Salmonella sp.	16.67±1.25	22.67±1.25	14.33±0.47	8.00±0.82	–	12.67±1.70	16.33±1.25	13.33±0.94
Shewanella sp.	9.33±1.25	12.33±1.25	11.33±1.25	15.33±1.70	16.67±1.25	14.00±0.82	16.00±1.63	10.67±1.70
V. flurialis	–	10.00±1.63	7.00±2.45	18.00±0.82	14.33±1.25	15.67±0.47	18.00±1.41	11.00±2.16
V. splendidus	9.33±1.25	7.67±1.25	15.00±1.63	8.33±0.47	9.00±1.63	6.33±0.94	–	11.67±1.70

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

Table 5

Antibacterial activity of *A. spicifera* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control (streptomycin 50 μg/mL)
K. pneumoniae	11.67±1.25	15.33±1.25	–	–	11.00±1.63	13.67±1.25	13.00±1.63	12.00±1.63
E. coli	9.33±1.25	11.67±0.94	13.00±0.82	13.33±1.70	12.33±0.47	15.67±1.25	11.33±2.05	13.67±0.94
S. aureus	7.67±0.94	13.00±0.82	13.33±0.94	12.33±1.25	10.33±1.25	7.67±1.25	5.33±0.47	24.67±0.94
Enterococci sp.	11.67±1.25	12.33±1.25	–	9.33±1.25	8.33±0.94	8.33±0.47	5.67±1.25	12.33±1.25
Proteus sp.	13.67±1.25	13.00±1.63	–	7.33±0.47	4.33±0.47	8.00±0.82	3.67±0.47	13.67±1.25
Streptococcus sp.	8.00±0.82	8.00±0.82	10.67±1.25	8.00±0.82	6.33±0.47	5.67±0.94	8.00±0.82	7.00±0.82
P. aeruginosa	9.33±0.47	9.33±1.25	8.67±0.47	6.67±1.25	7.67±0.47	–	5.33±1.25	10.33±1.25
V. parahaemolyticus	9.00±0.82	11.67±1.25	12.67±1.25	8.67±0.47	6.67±1.89	6.00±0.82	10.67±1.25	12.67±2.05
Salmonella sp.	12.33±1.25	12.33±1.25	10.67±1.25	5.00±0.82	3.67±0.47	2.67±0.47	10.67±1.25	13.33±0.94
V. flurialis	12.33±0.47	14.33±0.47	13.67±0.94	3.00±0.82	7.00±0.82	4.33±0.47	–	10.67±1.70
V. splendidus	7.67±1.25	8.00±0.82	8.67±0.47	5.33±0.47	–	–	–	11.00±2.16

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

3.8. Broth dilution assay

The maximum zone of clearance was observed in chloroform extract of brown alga *P. gymnospora* against in *Salmonella* sp. in disc diffusion assay. Broth dilution assay was conducted for *Salmonella* sp. using the chloroform extract of brown alga *P. gymnospora* and standard antibiotic streptomycin ranging from 0.25 μg/mL to 100 μg/mL. Commercial antibiotics streptomycin showed a maximum activity against *Salmonella* sp. (77.25%) at concentration 75 μg/mL and minimum activity (68.65%) at 25 μg/mL. *P. gymnospora* showed a maximum activity against *Salmonella* sp. (82.56%) at concentration 50 μg/mL and minimum activity (74.26%) at 25μg/mL (Table 7).

Table 6

Antibacterial activity of *G. acerosa* against human pathogens.

Pathogens	Zone of inhibition (mm)							
	ME	CH	ET	DEE	PE	EA	A	Positive control Streptomycin 50 μg/mL
K. pneumoniae	10.67±1.25	12.33±1.25	14.67±1.25	17.33±0.47	13.33±0.94	10.33±0.94	8.33±0.47	12.00±1.63
E. coli	8.00±0.82	14.33±0.94	8.00±0.82	8.00±0.82	–	4.00±0.82	5.33±0.47	13.67±0.94
S. aureus	8.00±0.82	10.00±0.82	8.33±0.94	5.67±0.47	–	5.00±0.82	5.00±0.82	24.67±0.94
Enterococci sp.	11.67±0.94	9.00±0.82	8.67±1.25	8.67±1.25	8.33±0.47	12.33±0.47	10.67±1.25	12.33±1.25
Proteus sp.	7.67±0.94	11.67±1.25	7.00±1.63	7.00±0.82	8.33±0.94	13.00±0.82	10.33±0.94	13.67±1.25
Streptococcus sp.	5.67±0.47	15.00±1.63	5.00±0.82	8.00±0.82	6.67±0.47	8.33±0.47	10.33±1.70	7.00±0.82
P. aeruginosa	2.33±0.47	7.67±1.25	3.33±0.47	11.67±0.47	3.00±0.82	8.67±0.94	14.67±1.25	10.33±1.25
V. parahaemolyticus	9.00±0.82	11.67±1.25	12.67±1.25	8.67±0.47	6.67±1.89	6.00±0.82	10.67±1.25	12.67±2.05
Salmonella sp.	11.00±1.63	7.67±0.47	13.67±1.25	7.67±0.47	–	3.67±0.47	7.00±0.82	13.33±0.94
Shewanella sp.	12.33±1.25	10.00±1.41	3.33±0.94	3.67±0.47	4.33±0.47	2.67±0.47	7.33±0.47	10.67±1.70
V. flurialis	8.00±0.82	5.67±0.47	5.33±0.47	4.67±0.47	7.67±0.47	–	–	11.00±2.16
V. splendidus	8.33±0.94	–	–	–	4.67±0.94	6.33±0.47	6.33±0.47	11.67±1.70

Each value representing mean±SD of 3 triplicates, –: No acivity.

ME–Methanol extract, CH–Chloroform extract, ET–Ethanol extract, DEE–Diethylether extract, PE–Petroleum ether extract, EA–Ethyl acetate extract, A–Acetone extract.

Table 7

Broth dilution assay percentage inhibition of chloroform extract of *P. gymnospora* and streptomycin against *Salmonella* sp.

Standard drug and Seaweed	Viability of cells (%) extracts with *Salmonella* sp. in various concentrations (μg/mL)				
	25	50	75	100	Control (OD) (*Salmonella* sp. alone)
Streptomycin	68.65	74.26	77.25	73.23	2.574
P. gymnospora	74.26	82.56	76.84	78.34	2.574

4. Discussion

There have been a number of reports that demonstrate the antimicrobial activity of mangroves and other marine forms, and only limited information were available from the seagrasses and seaweeds of the corners of the world and even very little information available from India. The aim of this study is to evaluate and compare the ability of different seaweed extracts to produce bioactive compounds of potential therapeutic interest. Antimicrobial activities found in seaweeds were considered to be an indication of synthesis of bioactive secondary metabolites. The marine macroalgae have an effective antibacterial activity against most of the human bacterial pathogens. It was reported that 151 species of macroalgal crude extracts showed inhibitory activity against pathogenic bacteria[12]. There have been a number of reports that demonstrate the antimicrobial activity of marine plants[13], marine algae or seaweeds[14–17], mangrove flora[18] and seagrass[19,20]. Still, in India only limited information is available on marine algae.

The antibacterial activity of different extracts of *C. tomentosum*, *U. reticulata*, *T. conoides*, *P. gymnospora*, *A. spicifera* and *G. acerosa* on twelve human pathogens were effective. Among them chloroform and methanol extracts were more effective than the others, which showed that chloroform and methanol is suitable for extracting active compounds from seaweeds. The result of the present investigation is consistent to some earlier reports.

Choi *et al*. noticed that methanol extracts of *Enteromorpha linza*, *Sargassum sagamianum* and *Ulva pertusa* showed strong inhibitory effects against both *Prevotella intermedia* and *Porphyromonas gingivalis*[21]. Rajauria *et al*. reported that water, methanol and mixtures (20%–80%) have been used to extract phenolic compounds from Irish brown seaweed *Himanthalia elongata* for studying its antimicrobial and antioxidant properties[22]. This is also true in the present study, here *T. conoides* were active against *Enterococci* sp. Sastry and Rao carried out a successive extraction using benzene, chloroform and methanol, and reported that the chloroform extract exhibited the strongest activity[23]. It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain. In the present study maximum activity were noted in chloroform extract of *P. gymnospora* against *Salmonella* sp.

Kandhasamy and Arunachalam had studied the Chlorophyceae members and it showed high antibacterial activity than other members[24]. The current study inferred that Phaeophyceae members showed higher antibacterial activity than Rhodophyceae and Chlorophyceae. The brown algae have naturally high secondary metabolites compared to red and green. The result shows that the methanol extract possesses a strong antimicrobial activity compared with streptomycin. Extracts of marine brown algae have been reported to exhibit antibacterial activity and antimicrobial activity[25–28].

The present results agreed with the findings of Rao and Parekh[29], and Padmakumar and Ayyakkannu[30] that organic extract of Indian seaweed exhibit antimicrobial activity against Gram negative and Gram positive biomedical pathogens. The present findings will have immense potential on the control of clinical pathogens, since the strains used in the study were collected from hospital sources and most of the strains appeared as multidrug resistant and cannot be controlled with commercially prescribed antibiotics. The Gram negative bacteria are more resistant to seaweed extract compared to Gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism[31].

Previous studies reported the screening of seaweeds on human and plant pathogenic virus, bacteria and fungi[32,33]. Hence, more studies pertaining to the use of seaweed as therapeutic agent should be emphasized, especially those related to the control of multidrug resistant microbes. Margret *et al*. reported that methanol extract of *A. spicifera* was active

against Gram negative bacterial pathogen *P. aeruginosa*, *K. pneumoniae* and *E. coli*[34]. Similarly in the present study, the same extract is active against the same pathogens.

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, *etc*., Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain that which kind of solvents is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons[22,35-42]. Several researchers make an effort using diverse pathogens for the antimicrobial assay[43-45].

There have been few reports on quantitative methods utilised for seaweed extracts such as the 96-well method which was applied in this study. Percentage inhibition of each seaweed extract was calculated over a 24 h period and the assays revealed different susceptibilities of the bacteria under investigation to the seaweed extracts. Observation in the broth dilution assay of the present study showed that *P. gymnospora* exerted a maximum activity (82.56%) against *Salmonella* sp. at concentrations 50 μg/mL. Further research studies are carried out on other species of seaweeds of different habitats in order to provide complete data of the antimicrobial potential of these algae. It is also essential to study the principle compound present in the seaweeds which is responsible for antimicrobial activity. It can be achieved by using advanced separation techniques.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

There is a growing need for new antibacterial compounds active against pathogenic bacteria because of the increase in bacterial resistance to commercial antibiotics and the adverse effects caused by most of the existing antibiotics. Therefore, renewed efforts are made to seek antibacterial agents from marine organisms, especially algae.

Research frontiers

The present research work depicts antibacterial potential of seaweeds occurring along the coast of Mandapam, India against twelve human pathogenic bacteria using standard methods (disc diffusion technique on Mueller Hinton agar and broth dilution assays) to compare the inhibition efficiency.

Related reports

Extracts of marine algae are reported as a source of bioactive molecules such as antibiotics which exhibit antibacterial activity. Antimicrobial activities found in seaweeds were considered to be an indication of synthesis of bioactive secondary metabolites.

Innovations and breakthroughs

Only limited information is available on the antimicrobial activities of seaweeds from India and only few reports on quantitative methods are available. In the present study authors have demonstrated that seaweed extracts exerted antimicrobial activities consistent with the synthesis of bioactive secondary metabolites of potential therapeutic interest.

Applications

Previous survey has found the screening of seaweeds on human and plant pathogenic virus, bacteria and fungi. This scientific study support and suggest the use of Indian seaweed extracts as a new class of antimicrobial therapeutic agents, especially for controlling multidrug resistant microbes.

Peer review

This is a valuable research work in which authors have demonstrated the antimicrobial activities of seaweeds from India. The activity of extracts was assessed based on standard microbiological methods. The authors' findings will have immense potential on the control of clinical pathogens, since the strains used were collected from hospital sources and most of the strains appeared as multidrug resistant.

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