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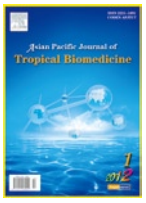

Document heading

Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity

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ABSTRACT

Objective: To screen the antibacterial efficacy of various solvent extracts of marine algae such as *Sargassum wightii (S. wightii)*, *Chaetomorpha linum (C. linum)* and *Padina gymnospora (P. gymnospora)* against some selected gram-positive and gram-negative human pathogenic bacteria. **Methods:** Crude extracts were prepared from the selected marine algae using different solvents namely, hexane, ethyl acetate, acetone and methanol and were tested for their antibacterial activity against human pathogenic bacteria using disc diffusion method. Minimum inhibitory concentration (MIC) was also performed for selected solvent extracts for all the bacterial species. A suitable positive control was also maintained. **Results:** Among the three marine algae screened *P. gymnospora* and *S. wightii* were found to be more active than *C. linum*. It was observed that the acetone extracts of all the three marine algae showed higher inhibitory activity for the selected bacterial species than other solvent extracts. The results revealed that the crude acetone extracts seem to be a good source material in identifying the effective pure antibacterial compound(s) in all the three marine algae and particularly, *S. wightii*. **Conclusions:** The present study showed that the acetone extracts of marine algae such as *S. wightii*, *C. linum* and *P. gymnospora* exhibited good antimicrobial activity. But the acetone extracts of *S. wightii* possessed highest antibacterial activity than others and so it could be useful in seeking active principles against human pathogenic bacteria.

1. Introduction

Despite the remarkable progress in the field of human medicine, the infectious diseases caused by bacteria, virus, fungi and parasites are still a major threat to public health and universal economies. They are caused by different types of infections such as drug-resistant infections, mostly involving bacteria, and many emerging pathogens are increasing significantly over time. These diseases are the most important cause of early death and killing of about 50 000 people worldwide every day[1,2]. The bacterial pathogens mainly cause severe problems to human beings by spreading various diseases, as they are found in multiple environmental habitats[3,4]. Bacterial pathogens like *Bacillus subtilis (B. subtilis)* are accountable for causing food borne gastroenteritis. *Escherichia coli (E. coli)*, *Staphylococcus aureus (S.aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)* are responsible for diseases like mastitis, endocarditis, meningitis and upper respiratory complications. Various species of *Salmonella* cause diarrhea, typhoid and enteric fever[5,6]. Enteric infections are major public health problems in developing countries and contribute to the death of 3.3-6.0 million children annually[7]. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* are the major etiological agents of sporadic and epidemic diarrhea both in children and adults[8]. People mostly use synthetic drugs to prevent or control the infectious diseases caused by microbes. Regular use of these drugs leads to development of resistance by the microbes against the drugs[9-11]. It is not only the resistance but also the cost of synthetic chemicals lead to search for alternate medicine such as antimicrobial compounds from natural sources. Plant derived natural products and antibiotics are found to be the effective alternative recognized from natural environmental resources. At this

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time, essential pharmacological and therapeutic products are being obtained and actively sought from the ocean[12-18]. One of the potential groups of natural resource is algae which are known to possess promising novel bioactive substances[19-21].

Amongst approximately 50 000 known plant species[22] and 30 000 species of algae only a small percentage is known to possess potential bioactive compounds[23]. The chemical forms of these compounds include haloforms, halogenated alkenes, alkenes, alcohols, aldehydes, hydroquinones and ketones that are used in the treatment of most of the diseases as antibiotic materials[24]. According to a survey by National Cancer Institute, USA, about 64% of the 974 small-molecule new chemical entities identified from natural resources in the past 25 years were introduced as drugs in the market worldwide during 1981 and 2006[25]. Especially, marine algal species serve as a rich source of several novel biologically active compounds but a very few species have been investigated for their medicinal properties. Likewise, certain marine algae like *Ulva lactuca (U. lactuca)*, *Sargassum wightii (S. wightii)* and *Gracillaria edulis (G. edulis)* are known to be active against certain pathogenic and non-pathogenic bacterial strains[26]. Thus, there is an interest in phytomedicine from marine algae and therefore many marine algal species are now examined for their pharmacological properties. Marine algae or seaweeds are potential renewable source of marine environment and also known to produce a variety of secondary metabolites with broad spectrum biological activities. There are numerous reports with reference to several pathogen inhibitory compounds from marine macroalgae against human viral, microbial, fungi and yeast pathogens. The secondary metabolites with cytostatic[27,28], antiviral[28,29], HIV antiviral agents[30-32], antihelminthic[33-36], antiproliferative[34], antimycobacterial[37,38], antifungal[39,40] and antibacterial[41-45], antimicrobial[46-51], antileishmanial and anti-trichomonal[52,53], anticoagulant[54], antitumor[55], antiprotozoal[56], nematicidal and fungicidal activities[57] have been detected in marine algae. In the present study, antibacterial efficacy of various organic solvent extracts of the seaweeds *S. wightii*, *Chaetomorpha linum (C. linum)* and *Padina gymnospora (P. gymnospora)* against some clinically important gram-positive and gram-negative human pathogenic bacteria species is reported.

2. Materials and methods

2.1. Plant material

Three species of marine algae [*S. wightii* Greville brown algae (Phaeophyceae), *C. linum* green algae (Cladophoraceae) and *P. gymnospora* – light brown algae (Dictyotaceae)] were collected during low tide by hand picking from the coast of Tuticorin, Tamil Nadu, India. The collected marine algae were identified and used for antibacterial studies.

2.2. Preparation of solvent extracts

The collected marine algae or seaweed samples were cleaned to remove the epiphytes and extraneous matter. The necrotic parts of the plants were also removed. The samples were washed carefully for about 3 to 4 times with sea water and then in fresh water. The algal samples were then transported to the laboratory in sterile plastic bags under ice. Voucher specimens of the collected samples were deposited in the department herbarium and some of them were also frozen at −20 ℃ for future reference. The samples were once again rinsed with sterile distilled water and shade dried. The dried samples were cut into small pieces and ground into fine powder in a clean mixer grinder. The powdered samples were soaked with hexane (100g/300mL) for 48 hours at room temperature. The extract was then filtered through a Buchner funnel with Whatmann No. 1 filter paper. The filtrate was evaporated to dryness under pressure using rotary vacuum evaporator at 50 ℃. The remains of the plant material were extracted using ethyl acetate, acetone and methanol sequentially in a similar manner using cold percolation method. These crude extracts were then tested for their antibacterial activity against selected human pathogens.

2.3. Test microorganisms and media

The gram-negative bacterial strains used for this experiment were *P. aeruginosa* (ATCC27853), *S. typhi*-B, *Erwinia amylovora* (MTCC2760) (*E. amylovora*), *Enterobacter aerogenes* (MTCC111) (*E. aerogenes*), *Proteus vulgaris* (MTCC1771) (*P. vulgaris*), *Klebsiella pneumonia* (ATCC15380) (*K. pneumonia*) and *E. coli* (ATCC25922). The gram-positive bacterial strains were Methicillin resistant *S. aureus*, *B. subtilis* (MTCC441) and *Enterococcus faecalis* (ATCC29212) (*E. faecalis*). These human pathogenic microorganisms were obtained from the Laboratory of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Mueller-Hinton Broth (MHB) was obtained from Hi-Media while solvents used were of HPLC grade.

2.4. Preparation of inoculums

Bacterial inoculums were prepared by transferring a huge number of bacterial strains from fresh culture plates to tubes containing 10 mL of Mueller Hinton Broth (Hi-media) and incubated for 24 hours at 37 ℃. The tubes were shaken occasionally to aerate and promote growth. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 10^8 CFU/mL.

2.5. Antibacterial activity

Antibacterial activity was carried out using the disc-diffusion method[58]. The petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations (5 mg/disc, 2.5 mg/disc and 1.25 mg/disc) of the crude extracts were prepared and loaded on the sterile discs (Hi-media) which were placed on the surface of the

solidified agar medium. Negative control was prepared using the respective solvent while streptomycin (0.01 mg/disc) was used as a positive control. The plates were incubated for 24 hours at 37 ℃ for bacterial growth. Zones of inhibition were recorded in millimeters and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

2.6. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was carried out according to the method of National Committee for Clinical Laboratory Standards (NCCLS)[59]. The marine algae extracts were selected for the effective solvents (i.e., hexane, ethyl acetate and acetone) and were dissolved in water containing 4% dimethyl sulfoxide (DMSO). The initial test concentration of extract was 5 mg/mL. It was then serially diluted into two folds. Each tube containing 5 ml of bacterial broth was inoculated with 5 μL of bacterial suspension containing 108 CFU/mL of bacteria. Streptomycin was used as positive control. The plates were incubated for 24 h at 37 ℃. MIC was determined as the lowest concentration of extract showing no visible growth on the agar plate. All the data were statistically analyzed.

3. Results

The antibacterial activity of various solvent extracts of *C. linum*, *P. gymnospora* and *S. wightii* on ten different human bacterial pathogens are presented in Tables 1-3. Of the three marine algae screened in the present study for their antibacterial activity *P. gymnospera* and *S. wightii* were observed to be more active than *C. linum* against human pathogens in the control of their growth. Among the four solvents tested, acetone and ethyl acetate extracts exhibited maximum inhibition on the growth of the tested bacterial species. As observed, the acetone extracts of all the three marine algae showed the highest inhibitory activity for the chosen bacterial strains followed by other solvent extracts. Maximum activities were recorded in the brown marine algae *S. wightii* acetone (17.33±0.58 mm) and ethyl acetate (14.33 ±0.58 mm) extracts when compared to other solvent extracts as well as various solvent extracts of the marine algae *P. gymnospera* and *C. linum* (Table 3). Less inhibitory effects for all the test organisms were recorded in the *C. linum* and *P. gymnospora*. Among the three groups of marine algae tested, maximum activities were recorded in brown marine algae *S. wightii* and minimum activity was recorded in green and light brown marine algae. Methanol extract of the *C. linum* and *P. gymnospora* were not effective against any of the tested pathogenic organisms. All the four solvent extracts of the marine algae, *C. linum*, *P. gymnospora* and *S. wightii* were not revealed any activity against two gram-positive bacterial strains, *Salmonella paratyphi* (*S. paratyphi*) and *K. pneumonia*.

There were also specific antibacterial activities with reference to either the known solvent extract effective to a number of bacterial strains or specific effect of marine algae to some bacterial pathogens. The acetone extract of *S. whitii* showed excellent antibacterial activity. Specifically hexane extracts of *C. linum* indicated inhibition of bacteria such as *P. aeruginosa*, Methicillin resistant *S. aureus*. In *P. gymnospora* species hexane extract shows prominent activity against several bacteria such as *B. subtilis*, *E. faecalis*, *E. amylovora*, *E. coli* and *P. vulgaris*. It was observed that hexane extracts of *S. wightii* produced broad spectrum antibacterial activity against methicillin resistant *S. aureus*, *B. subtilis*, *E. amylovora*, *E. coli*, *E. aerogenes* and *P. vulgaris*. Ethyl acetate extract of all the three marine algae exhibited activity against Methicillin resistant *S. aureus*, *B.*

Table 1
Antibacterial activity of various crude solvent extracts of *C. linum*.

Solvents	Concentration (mg/disc)	Zone of inhibition (mm)									
		1	2	3	4	5	6	7	8	9	10
Hexane	1.25	–	–	–	–	–	–	–	7.33±0.58	–	–
	2.5	7.3±0.58	–	–	–	–	–	–	8.33±0.58	–	–
	5	9.67±0.58	–	–	–	–	–	–	10.00±0.00	–	–
Ethyl acetate	1.25	–	–	10.33±0.58	10.67±0.58	–	–	10.00±0.00	10.33±0.58	9.33±0.58	–
	2.5	–	–	11.33±0.58	11.67±0.58	–		12.67±0.58	11.67±0.58	10.00±0.00	–
	5	–	–	12.00±1.00	13.67±0.58			13.67±0.58	12.67±0.58	11.00±0.00	–
Acetone	1.25	–	–	12.67±0.58	12.67±0.58	10.00±0.00	–	12.67±0.58	12.67±0.58	10.67±0.58	10.00±0.00
	2.5	–	–	14.67±0.58	15.67±0.58	10.00±0.00	–	13.67±0.58	13.67±0.58	12.67±0.58	11.67±0.58
	5	–	–	16.67±0.58	16.67±0.58	11.00±0.00	–	15.67±0.58	14.67±0.58	15.67±0.58	13.67±0.58
Methanol	1.25	–	–	–	–	–	–	–	–	–	–
	2.5	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
Streptomycin (positive control)	0.01	28	16	24	22	27	10	28	22	35	37

Each value representing mean ± SD of 3 replicates, Gram negative bacteria: 1. *Pseudomonas aeruginosa*, 2. *Salmonella paratyphi* – B, 3. *Erwinia amylovora*, 4. *Enterobacter aerogenes*, 5. *Proteus vulgaris*, 6. *Klebsiella pneumonia*, 7. *Eschercia coli* Gram positive bacteria: 8. Methicillin resistant *Staphylococcus aureus*, 9. *Bacillus subtilis*, 10. *Enterococcus faecalis*, "–" indicating no activity

Table 2
Antibacterial activity of various crude solvent extracts of *P. gymnospora*.

Solvents	Concentration (mg/disc)	Zone of inhibition (mm)									
		1	2	3	4	5	6	7	8	9	10
Hexane	1.25	–	–	–	–	7.33±0.58	–	8.00±0.00	–	–	–
	2.5	–	–	6.33±0.58	–	8.67±0.58	–	10.33±0.58	–	8.33±0.58	7.33±0.58
	5	–	–	7.33±0.58	–	10.00±0.00	–	11.33±0.58	–	8.67±0.58	8.00±0.00
Ethyl acetate	1.25	7.33±0.58	–	–	6.33±0.58	–	–	7.00±0.00	8.33±0.58	–	–
	2.5	9.00±0.00	–	7.33±0.58	7.33±0.58	–	–	8.33±0.58	9.67±0.58	7.33±0.58	6.33±0.58
	5	10.33±0.58	–	8.33±0.58	9.33±0.58	–	–	9.33±0.58	10.67±0.58	8.33±0.58	7.33±0.58
Acetone	1.25	10.67±0.58	–	9.00±0.00	10.33±0.58	8.33±0.58	–	8.00±0.00	–	8.33±0.58	9.00±0.00
	2.5	12.67±0.58	–	10.67±0.58	11.67±0.58	10.33±0.58	–	10.33±0.58	–	10.00±0.00	10.67±0.58
	5	15.67±0.58	–	15.33±0.58	13.67±0.58	12.00±0.00	–	12.67±0.58	–	11.67±0.58	14.33±0.58
Methanol	1.25	–	–	–	–	–	–	–	–	–	–
	2.5	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
Streptomycin (positive control)	0.01	28	16	24	22	27	10	28	22	35	37

Table 3
Antibacterial activity of various crude solvent extracts of *S. wightii*.

Solvents	Concentration (mg/disc)	Zone of inhibition (mm)									
		1	2	3	4	5	6	7	8	9	10
Hexane	1.25	–	–	8.33±0.58	–	6.67±0.58	–	–	–	8.33±0.58	–
	2.5	–	–	11.00±0.00	10.67±0.58	8.67±0.58	–	10.33±0.58	12.00±0.00	10.33±0.58	–
	5	–	–	12.33±0.58	12.00±0.00	10.33±0.58	–	11.33±0.58	12.67±0.58	10.67±0.58	–
Ethyl acetate	1.25	–	–	–	10.33±0.58	–	–	11.33±0.58	11.67±0.58	8.67±0.58	8.67±0.58
	2.5	–	–	8.67±0.58	12.33±0.58	–	–	12.00±0.00	13.33±0.58	10.33±0.58	10.00±0.00
	5	–	–	10.33±0.58	13.33±0.58	–	–	13.33±0.58	14.33±0.58	11.33±0.58	11.33±0.58
Acetone	1.25	9.67±0.58	–	11.33±0.58	11.67±0.58	8.33±0.58	–	14.33±0.58	14.33±0.58	9.33±0.58	9.33±0.58
	2.5	10.67±0.58	–	12.33±0.58	14.33±0.58	10.33±0.58	–	15.33±0.58	16.33±0.58	10.00±0.00	10.67±0.58
	5	11.67±0.58	–	13.33±0.58	15.33±0.58	11.67±0.58	–	16.33±0.58	17.33±0.58	11.33±0.58	11.33±0.58
Methanol	1.25	–	–	–	–	–	–	–	–	–	–
	2.5	–	–	–	–	–	–	–	–	–	–
	5	–	–	10.00±0.00	11.67±0.58	–	–	10.67±0.58	–	–	–
Streptomycin (positive control)	0.01	28	16	24	22	27	10	28	22	35	37

subtilis, *E. amylovora*, *E. coli* and *E. aerogenes*. The marine algae, *P. gymnospora* ethyl acetate extract showed activity against *P. aeruginosa* whereas, ethyl acetate extract of inhibited *E. faecalis*. Acetone extract of *C. linum* produced a wide range of activity against *P. aeruginosa*, Methicillin resistant *S. aureus*, *B. subtilis*, *E. faecalis*, *E. amylovora*, *E. coli*, *E. aerogenes* and *P. vulgaris*. In *P. gymnospora*, the activity was seen in *P. aeruginosa*, *B. subtilis*, *E. faecalis*, *E. amylovora*, *E. coli*, *E. aerogenes* and *P. vulgaris*. There were no antibacterial activities of extracts of all the three seaweeds to *S. paratyphi*-B and *K. pneumonia*. Methanolic extracts of *C. linum* and *P. gymnospora* were not revealed any activity against all the bacteria (Tables 1 & 2) except *S. wightii* in which it inhibited the bacterial strains *E. amylovora*, *E. coli* and *E. aerogenes* (Table 3).

Minimum inhibitory concentrations for the various marine algae were carried out using the better performing solvent extracts in the disc diffusion assays like extracts of various plants using hexane, ethyl acetate and acetone. As observed in the disc diffusion assays, all the selected crude solvent extracts of the three different marine algal plants revealed no minimum inhibitory concentration (i.e., the initial test concentration of 5mg/ml used) for *S. paratyphi*-B and *K. pneumonia*. However, there were values of MIC to certain bacterial strains which were not responded to disc diffusion assays. Such values of MIC were observed as 2.5 for *P. aeruginosa* to crude ethyl acetate extracts of *S. wightii* and 5 for *E. faecalis* to crude hexane extracts of *S. wightii* (Table 4). A minimum value of MIC as 0.625 was observed for *S. aureus* to the crude acetone extracts of *S. wightii*. Among various crude solvent extracts tested, acetone extracts of all the three marine algae performed better than the other solvent extracts.

4. Discussion

The main objective of this work was to evaluate and compare the ability of different macroalgae or seaweed species from southern coastal region of Tamil Nadu to produce bioactive compounds of potential therapeutic interest. The production of antibacterial activities was

Table 4
Minimum inhibitory concentration (MIC) of the effective crude solvent extracts of selected sea weeds.

Name of the sea weed	Solvent	MIC	1	2	3	4	5	6	7	8	9	10
Chaetomorpha linum	He	mg/mL	5	–	–	–	–	–	–	5	–	–
	Ea		–	–	2.5	1.25	–	–	2.5	5	5	–
	Ac		–	–	1.25	2.5	5	–	1.25	2.5	1.25	1.25
Padina gymnospora	He		–	–	5	–	5	–	5	–	5	5
	Ea		2.5	–	5	2.5	–	–	2.5	2.5	5	5
	Ac		1.25	–	2.5	1.25	2.5	–	1.25	–	2.5	1.25
Sargassum wightii	He		–	–	5	2.5	5	–	2.5	2.5	5	5*
	Ea		2.5*	–	5	1.25	–	–	2.5	1.25	2.5	2.5
	Ac		2.5	–	2.5	1.25	2.5	–	1.25	0.625	2.5	2.5
Streptomycin	–	(10 μg/mL)	1.25	5	1.25	1.25	1.25	5	1.25	1.25	0.625	0.312
4 % DMSO			–	–	–	–	–	–	–	–	–	–

Three replicates were maintained for each concentration gram negative bacteria, He = Hexane, Ea = Ethyl acetate, Ac = Acetone; Values followed by "*" mark indicating MIC for additional bacterial strains which was not reported in disc diffusion assays.

considered to be an indicator for the capability of the seaweeds to synthesize bioactive compounds. Because, marine natural products contain a wide range of novel bioactive compounds or antibiotics with distinctive complex structures because they developed unique metabolic and physiological capability. 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[60]. There have been a number of reports that demonstrating the antimicrobial activity of marine plants[61], marine algae or seaweeds[36,46-51,62], mangrove flora[63] and seagrass[64,65]. Still, in India only limited information is available on marine algae. Hence it was intended to evaluate and compare the ability of some abundantly available marine algae in the coastal regions of Tamil Nadu, India in order to identify the bioactive potential of these sea weeds against selected human bacterial pathogens. It was earlier reported that hexane and ethyl acetate extracts of Trichodesmium erythraeum (microalgae) showed antibacterial activity[66]. Seaweeds belonging to red, brown and green algae exhibit inhibitory action against both gram-positive and gram-negative bacteria[67-69]. Vallinayagam et al has reported that the red algae showed higher activity than the brown and green algae when tested against seven human pathogenic bacteria. The organic solvent chloroform and methanol extracts of some red and brown algae showed maximum activity against certain human pathogenic bacteria[69]. In our study it was reported that the brown algae (*S. wightii*) showed antibacterial activity against several Gram-negative and Gram-positive bacteria. Maximum activities were recorded in the brown algae *S. wightii* against Methicillin resistant *S. aureus* in acetone and ethyl acetate extracts when compared to other solvent extracts of the marine algae *P. gymnospera* and *C. linum*. Among the various organic solvents such as methanol, acetone, diethyl ether and ethanol extracts of eleven macroalgae screened for antimicrobial activity against human pathogens, the extracts of diethyl ether was found to possess bioactive compounds[70]. In another study, acetone was found best among several solvents used for extracting antibacterial substances[71]. Some other studies performed in the extraction of seaweeds using chloroform and ethyl acetate also exhibited good antibacterial activity[72,73]. It was reported that methanol extracts of seven different seaweeds tested showed broad spectrum antibacterial activity against human pathogenic bacteria[74]. This kind of less or more activity could also be attributed to the sequential extraction of marine algae using solvents from low polar to high polar. In the present study, methanol extracts were not exhibited any activity except *S. wightii* where it produced very little activity against only three bacterial pathogens. In general, the acetone extracts of all the three marine algae showed antibacterial activity against both gram positive and gram negative bacteria with very well known higher levels of antibacterial activity of *S. wightii*. It is thus concluded from this study that the acetone extract of marine alga, *S. wightii* could be used for further investigation to identify actual components against human bacterial pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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