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Antibacterial activity of the seaweeds *Chaetomorpha linum* and *Padina gymnospora* on human bacterial pathogens

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Seaweeds are important source of bioactive molecules with known beneficial effects on human health. The present study is designed to investigate the antibacterial efficacy of two chosen marine algae namely *Chaetomorpha linum* and *Padina gymnospora* against some human pathogenic bacteria. Our earlier study on antibacterial screening using various solvent extracts revealed that the crude acetone extracts of the seaweeds *C. linum* and *P. gymnospora* reacted positively against selected human bacterial pathogens. The crude acetone extracts of these seaweeds were then fractionated by silica gel column chromatography using sequential gradient of solvent extraction. It yielded 61 fractions and they were pooled into 9 sub-fractions based on the molecular weight calculated using thin layer chromatography. The 9 (I to IX) sub-fractions were utilized for their antibacterial activity against human bacterial pathogens employing disc diffusion method. The sub-fractions I to V of *P. gymnospora* were revealed significant antibacterial activity against the tested bacterial strains except, Methicillin resistant *Staphylococcus aureus*, *Salmonella paratyphii* and *Klebsiella pneumonia*. However, in the green alga *C. linum*, the isolated sub-fraction IV responded well to two Gram-positive bacteria (*Bacillus subtilis* and *Enterococcus faecalis*) and the fraction V revealed inhibitory activity against few Gram-negative bacteria except *S. paratyphii*, *E. coli* and *Klebsiella pneumonia*. The fraction VIII responded to Gram-negative bacteria except *Salmonella paratyphii*, *Proteus vulgaris* and *Klebsiella pneumonia*. Although there are variations on the antibacterial activity of different column sub-fractions of these two seaweeds, sub-fractions with significant activity reported would be useful in isolation of effective antibacterial molecules.

KEYWORDS
antibacterial activity; *Chaetomorpha linum*; *Padina gymnospora*; sequential extraction

1. INTRODUCTION

Seaweeds are non-vascular, photosynthetic plants that inhabit the coastal regions commonly within rocky intertidal or submerged reef-like habitats and have been one of the richest and most promising sources of bioactive primary and secondary metabolites with antimicrobial properties (Faulkner, 2002; Lima-Filho et al., 2002; Fayaz et al., 2005; Tuney et al., 2006; Bansemir et al., 2006; Chew et al., 2008; Cox et al., 2010; Jebasingh et al., 2011). Amongst approximately 30,000 species of marine algae, only a small percentage is screened for use as potential bioactive compounds (Bhakuni and Rawat, 2005). The chemical forms of these compounds include haloforms, halogenated alkenes, alkenes, alcohols, aldehydes, hydroquinones and ketones that are used in the treatment of many diseases as antibiotics (Lincoln et al., 1991). Microorganisms have developed new strategies to evade the action of antibiotics, results in the simultaneous development of resistance to many antibiotic classes making extremely dangerous multidrug resistant (MDR) strains of bacteria such as “superbugs” (Sande-Bruinsma et al., 2008; Thanigaivel et al., 2015). Synthesis of different metabolites from seaweeds is an indicator of the

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presence of antimicrobial active compounds (Chiheb et al., 2009). It is also valuable to test these marine antimicrobials for possible synergism with existing drugs (Cheung et al., 2014). The crude extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria (Tuney et al., 2006). The antimicrobial activity displayed by a particular alga was demonstrated to be due to one or a few chemical entities (Robles and Ballantine, 1999) or a complex phenomenon of different active metabolites or specific metabolites vary in response to changes in physical conditions (Robles et al., 1996). Biomedical research has produced many synthetic drugs to prevent or control the infectious diseases caused by microbes, however, microbial resistance against the drugs (Sarma and Khan, 1980; Vickers and Zollman, 1999; De Smet, 2002; Dawson, 2005) have caused a search for an alternative non-resistant drug of minimum cost and the suggested bioactive substances from algae are considered most feasible. Thus, research on bioactive compounds or secondary metabolites in seaweeds with antibacterial activity against human pathogens are of potential interest in pharmaceutical industry. Restricting the genetically diverse pathogenic infectious diseases with new novel biologically active sources of potential antimicrobial agents from seaweeds are considered necessary (Moorthi and Balasubramanian, 2015). In the present study, the crude acetone extracts from brown seaweed *Padina gymnospora* and green seaweed *Chaetomorpha linum* known in our previous study as effective extract were sub-fractionated further by column chromatography and analysed for antibacterial property.

2. MATERIALS AND METHODS

2.1. Collection of algal samples

Live and healthy specimens of the green algae *Chaetomorpha linum* (O.F.Müller) Kützing and brown algae *Padina gymnospora* Kützing were collected on the rocky and sandy intertidal coast of Tuticorin, Tamil Nadu, India during the pre-monsoon period (April to July, 2009). The algal material collected was identified by scientists at the Marine Algal Research Station in consultation with the Marine Algal Herbarium of Central Salt & Marine Chemical Research Institute, Mandapam, South India and confirmed with the help of Botanical Records and Monographs-2, Algal Taxonomy in India (Sarma and Khan, 1980).

2.2. Preparation of algal samples

The collected marine algal (seaweed) samples were washed 3 to 4 times with seawater to remove macroscopic epiphytes, extraneous matter and necrotic parts of the algae and rinsed in distilled water to remove the excess salt on the surface. The shade dried specimens (10 days at room temperature) were cut into small pieces and grounded to fine powder in a clean mixer grinder and sieved through 0.8 mm^2 sieves. The powdered samples were stored in sterile sealed polythene bags at room temperature and used immediately.

2.3. Sequential solvent extraction and column fractionation

Sequential extraction of dried marine algae or seaweed powder samples were performed using solvents (HPLC grade) of increasing polarity (sequential gradient partition with solvents) i.e., hexane < ethyl acetate < acetone < methanol. The extract of the algal sample (100 g / 300 mL) in hexane (48 h, RT) was filtered through a Buchner funnel with Whatmann No. 1 filter paper and the filtrate was evaporated to dryness under pressure using rotary vacuum evaporator at 50 ^oC. The filtered residue was extracted using ethyl acetate, acetone and methanol sequentially in a similar manner using cold percolation method.

Our earlier study on antibacterial screening using these solvent extracts revealed that the crude acetone extracts of the seaweeds *C. linum* and *P. gymnospora* showed significantly higher antibacterial activity with selected human bacterial pathogens (Rosaline et al., 2012). Column chromatography was thus carried out using crude acetone extracts of both of these algal samples for sub-fractionation in order to identify effective sub-fraction(s) against chosen human pathogenic bacteria. The crude acetone extract (20 g) of both marine algal samples were adsorbed on silica gel (250 g of 100-200 mesh size; SISCO Research Laboratories-SRL, Mumbai, India) in a glass column (125 x 3.5 cm) and sequential gradients of solvents were run as reported in our earlier work (Rosaline et al., 2016). The column was finally washed with 100% methanol and 61 sub-fractions (57 combination of solvent ratios + 4 absolute solvents) of 100 mL / 45 min were collected, concentrated under reduced pressure using a rotary vacuum evaporator at 45 ^oC and were subjected to thin layer chromatography (TLC) (Marston et al., 1997). The fractions showing similar Rf values on TLC were visualized under UV/

iodine and were pooled to 9 sub-fractions and stored at 4 °C (TLC data not shown).

2.4. Test microorganisms

Some chosen human pathogenic Gram-positive and Gram-negative bacterial strains were used for this experiment. The Gram-positive bacterial strains used were Methicillin resistant *Staphylococcus aureus* [MRSA] (ATCC33591), *Bacillus subtilis* (MTCC441) and *Enterococcus faecalis* (ATCC29212). The Gram-negative bacterial strains used were *Pseudomonas aeruginosa* (ATCC27853), *Salmonella paratyphi*-B, *Enterobacter aerogenes* (MTCC111), *Proteus vulgaris* (MTCC1771), *Klebsiella pneumoniae* (ATCC15380) and *Escherichia coli* (ATCC25922). Pure cultures of these human pathogenic microorganisms were obtained from the Laboratory of Microbiology, Christian Medical College, Vellore, India.

2.5. Preparation of inoculums and in vitro antibacterial assay

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 h at 37 °C. 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 108 CFU/mL. Activities of various seaweed extracts in controlling the growth of the bacterium under *in vitro* conditions were carried out using the disc-diffusion method (Murray et al., 1995). Prior to assay, the sub-fractions of seaweed extracts were mixed with 4% dimethyl sulfoxide (DMSO) in order to facilitate uniform diffusion of substances in the disc.

2.6. Antibacterial activity of various fractions of seaweeds

Overnight grown cultures of the human pathogenic bacteria with an OD of 0.5 at 580 nm were used for the study (Sonnenwirth, 1980). The culture media were swabbed on the top of the solidified agar containing 20 mL of sterile Mueller Hinton agar in petri plates and allowed to dry for 10 min. Three different concentrations (25 µL each containing 250 µg/disc, 500 µg/disc and 1000 µg/disc) of respective sub-fractions of marine algal extracts in 4% DMSO were loaded onto 6.0 mm diameter sterile paper discs (Hi-Media) and dried in sterile chamber. Then the discs were impregnated on the surface of the solidified agar medium. Negative control was prepared using 4% DMSO while streptomycin (10 µg/disc) was used as a positive control. The plates were incubated for 24 h at 37 °C for bacterial growth. Zones of inhibition were recorded in millimeters and the experiment was repeated thrice for concordant results. The standard deviation of mean and Student's t-test were calculated using SPSS (10.1) statistical package.

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity of column sub-fractions of P. gymnospora

Antibacterial activity of the column sub-fractions of *P. gymnospora* against human pathogenic Gram-positive bacteria is presented in Table 1. MRSA was inhibited by sub-fraction VII at 1000 µg concentration with the zone of inhibition (ZI) measuring 7.66 ± 0.57 mm ($p < 0.05$). The other tested Gram-positive bacteria *B. subtilis* and *E. faecalis* were inhibited by sub-fractions I to V. However, the ZI values for *B. subtilis* against sub-fraction I ranged from 8.33 ± 0.57 mm (250 µg) to 10.33 ± 0.57 mm at 1000 µg ($p < 0.05$). Also, sub-fraction II showed increase in ZI value and was measured as 11.33 ± 0.57 mm at 1000 µg concentration ($p < 0.05$). The sub-fractions IV and V responded at 1000 µg concentration with ZI values below 10.00 mm that were observed as resistant according to Kirby-Bauer SAIC table. Sub-fraction I of *P. gymnospora* responded against *E. faecalis* with the ZI 10.33 ± 0.57 mm at 250 µg to 12.00 ± 0.00 mm at 1000 µg concentration ($p < 0.05$).

Antibacterial activity of the sub-fractions of *P. gymnospora* against human pathogenic Gram-negative bacteria is shown in Table 2. Sub-fractions I to V of *P. gymnospora* inhibited *P. aeruginosa*, *E. aerogenes*, *P. vulgaris* and *E. coli*. The ZI as seen for *P. aeruginosa* ranged from 10.33 mm at 250 µg and 12.33 mm for 1000 µg concentration ($p < 0.05$) for sub-fraction I and 8.66 ± 0.57 mm at 250 µg to 12.33 ± 0.57 mm at 1000 µg concentration ($p < 0.05$) for sub-fraction II. The observed antibacterial response as deduced by Kirby-Bauer SAIC table seemed to lack antibacterial activity. Sub-fractions III, IV, V and VII showed antibacterial activity with ZI values less than 11 mm that clearly revealing the resistant nature of the microbes against the human pathogens. Sub-fractions I to V and VIII, showed antibacterial activity against *E. aerogenes* but the ZI measured was less

Table 1. Antibacterial activity of various column sub-fractions of *Padina gymnospora* against human pathogenic Gram-positive bacteria.

Fractions	Concentration (µg/disc)	Zone of inhibition (mm)		
		Methicillin resistant *Staphylococcus aureus*	*Bacillus subtilis*	*Enterococcus faecalis*
I	250	-	8.33 ± 0.57	10.33 ± 0.57
	500	-	8.66 ± 0.57^{NS}	11.33 ± 0.57^{NS}
	1000	-	10.33 ± 0.57*	12.00 ± 0.00*
II	250	-	8.33 ± 0.57	-
	500	-	9.66 ± 0.57^{NS}	9.33 ± 0.57*
	1000	-	11.33 ± 0.57*	10.33 ± 0.57*
III	250	-	-	-
	500	-	9.33 ± 0.57*	8.66 ± 0.57*
	1000	-	10.33 ± 0.57*	10.33 ± 0.57*
IV	250	-	-	-
	500	-	-	-
	1000	-	6.66 ± 0.57*	9.00 ± 0.00*
V	250	-	-	-
	500	-	-	-
	1000	-	9.33 ± 0.57*	9.33 ± 0.57*
VI	250	-	-	-
	500	-	-	-
	1000	-	-	-
VII	250	-	-	-
	500	-	-	-
	1000	7.66 ± 0.57*	-	-
VIII	250	-	-	-
	500	-	-	-
	1000	-	-	-
IX	250	-	-	-
	500	-	-	-
	1000	-	-	-
4% DMSO	Negative Control	-	-	-
Streptomycin (10 µg)	Positive Control	22.33 ± 0.58		

Kirby-Bauer standard interpretative chart for positive control (Quality Control-NCCLS, 1995):
1. Carbenicillin (10 µg) for *P. aeruginosa* - Resistant(R) <13 mm; Intermediate (I) 14-16 mm; Sensitive/ Susceptibility >17 mm
2. Streptomycin (10 µg) for other bacteria - Resistant(R) <11 mm; Intermediate (I) 12-14 mm; Sensitive/ Susceptibility >15 mm
* – Significant at 95% level, NS –Non-significant, ZI – Zone of inhibition, "-" No activity; Each value representing mean ± SD of 3 replicates

than 11.5 mm and it was deduced that the antibacterial response was resistant when evaluated against the Kirby-Bauer SAIC. Also the antibacterial activity against *P. vulgaris*, measured as significant ZI values for sub-fractions I to V. The ZI values were less than 11 mm and the antibacterial response was considered resistant on the same criteria. The ZI measured against *E. coli* was also less than 11 mm with sub-fractions I to V and the antibacterial response was suggested as resistant. The above observations clearly showed that the antibacterial response of sub-fractions I and II was specific and against *P. aeruginosa* only.

Table 2. Antibacterial activity of various column sub-fractions of *Padina gymnospora* against human pathogenic Gram-negative bacteria

Fractions	Concentration (µg/disc)	Zone of inhibition (mm)					
		Pseudomonas aeruginosa	*Salmonella paratyphi – B*	*Enterobacter aerogenes*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Escherchia coli*
I	250	10.33 ± 0.57	-	-	-	-	-
	500	11.33 ± 0.57^{NS}	-	7.33 ± 0.57*	-	-	6.66 ± 0.57*
	1000	12.33 ± 0.57*	-	8.33 ± 0.57*	7.33 ± 0.57*	-	7.66 ± 0.57*
II	250	8.66 ± 0.57	-	-	-	-	-
	500	11.33 ± 0.57*	-	-	-	-	-
	1000	12.33 ± 0.57*	-	8.66 ± 0.57*	7.66 ± 0.57*	-	9.33 ± 0.57*
III	250	-	-	-	-	-	-
	500	10.33 ± 0.57*	-	10.33 ± 0.57*	9.33 ± 0.57*	-	-
	1000	11.00 ± 0.00*	-	11.33 ± 0.57*	10.66 ± 0.57*	-	8.66 ± 0.57*
IV	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	7.33 ± 0.57*	-	9.33 ± 0.57*	9.33 ± 0.57*	-	9.33 ± 0.57*
V	250	-	-	-	-	-	-
	500	9.33 ± 0.57*	-	9.33 ± 0.57*	-	-	10.33 ± 0.57*
	1000	9.66 ± 0.57*	-	10.33 ± 0.57*	9.33 ± 0.57*		10.66 ± 0.57*
VI	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
VII	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	9.33 ± 0.57*	-	-	-	-	-
VIII	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	8.33 ± 0.57*	-	-	-
IX	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
4% DMSO	Negative control	-	-	-		-	
Streptomy-cin (10 µg)	Positive Control	22.33 ± 0.58					

Kirby-Bauer standard interpretative chart for positive control (Quality Control-NCCLS, 1995):
1. Carbenicillin (10µg) for *P. aeruginosa* - Resistant(R) <13mm; Intermediate (I) 14-16 mm; Sensitive/ Susceptibility >17 mm; 2. Streptomycin (10 µg) for other bacteria - Resistant(R) <11 mm; Intermediate (I) 12-14 mm; Sensitive/ Susceptibility >15 mm; * – Significant at 95% level, NS –Non-significant, ZI – Zone of inhibition, "-" No activity; Each value representing mean ± SD of 3 replicates

3.2. Antibacterial activity of column sub-fractions of C. linum

Antibacterial activity of the column sub-fractions of *C. linum* against human pathogenic Gram-positive bacteria is presented in Table 3. The Gram-positive MRSA was inhibited by sub-fraction V but the ZI recorded was 9.33 ± 0.57 mm at 1000 µg concentration ($p < 0.05$). The sub-fraction V, VI, VIII and IX inhibited *B. subtilis* but the ZI did not exceed 11.50 mm. *E. faecalis* was inhibited by sub-fraction V and VIII but the ZI remained within 11.50 mm. Thus it was inferred that the antibacterial response was not significant and evaluated as resistant in Kirby-Bauer SAIC.

Antibacterial activity of the column sub-fractions of *C. linum* against Gram- negative bacteria

Table 3. Antibacterial activity of various column sub-fractions of *Chaetomorpha linum* against human pathogenic Gram-positive bacteria

Fractions	Concentration (µg/ disc)	Zone of inhibition (mm)		
		Methicillin resistant *Staphylococcus aureus*	*Bacillus subtilis*	*Enterococcus faecalis*
I	250	-	-	-
	500	-	-	-
	1000	-	-	-
II	250	-	-	-
	500	-	-	-
	1000	-	-	-
III	250	-	-	-
	500	-	-	-
	1000	-	-	-
IV	250	-	-	-
	500	-	-	-
	1000	-	-	-
V	250	-	7.33 ± 0.57	-
	500	-	8.33 ± 0.57^{NS}	10.33 ± 0.57*
	1000	9.33 ± 0.57*	10.66 ± 0.57*	11.33 ± 0.57*
VI	250	-	-	-
	500	-	9.66 ± 0.57*	-
	1000	-	11.33 ± 0.57*	-
VII	250	-	-	-
	500	-	-	-
	1000	-	-	-
VIII	250	-	8.66 ± 0.57	-
	500	-	10.33 ± 0.57^{NS}	9.33 ± 0.57*
	1000	-	10.66 ± 0.57*	10.33 ± 0.57*
IX	250	-	-	-
	500	-	-	-
	1000	-	8.66 ± 0.57*	-
4% DMSO	Negative Control	-	-	-
Streptomycin (10 µg)	Positive Control	22.33 ± 0.58		

Kirby-Bauer standard interpretative chart for positive control (Quality Control-NCCLS, 1995):
1. Carbenicillin (10 µg) for *P. aeruginosa* - Resistant(R) <13 mm; Intermediate (I) 14-16 mm; Sensitive/ Susceptibility >17 mm;
2. Streptomycin (10 µg) for other bacteria - Resistant(R) <11 mm; Intermediate (I) 12-14 mm; Sensitive/ Susceptibility >15 mm
* – Significant at 95% level, NS –Non-significant, ZI – Zone of inhibition, "-" No activity; Each value representing mean ± SD of 3 replicates

Table 4. Antibacterial activity of various column sub-fractions of *Chaetomorpha linum* against human pathogenic Gram-negative bacteria

Fractions	Concentration (µg/disc)	Zone of inhibition (mm)					
		Pseudomonas aeruginosa	*Salmonella paratyphi – B*	*Enterobacter aerogenes*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Escherchia coli*
I	250	9.66 ± 0.57	-	-	-	-	-
	500	10.66 ± 0.57^{NS}	-	-	-	-	-
	1000	12.33 ± 0.57*	-	-	-	-	-
II	250	-	-	-	-	-	-
	500	9.66 ± 0.57*	-	-	-	-	-
	1000	11.33 ± 0.57*	-	-	-	-	-
III	250	-	-	-	-	-	-
	500	7.66 ± 0.57*	-	-	-	-	-
	1000	9.66 ± 0.57*	-	-	-	-	-
IV	250	-	-	-	-	-	-
	500	9.33 ± 0.57*	-	-	-	-	-
	1000	10.33 ± 0.57*	-	-	-	-	-
V	250	9.33 ± 0.57	-	-	-	-	-
	500	11.00 ± 0.00*	-	9.66 ± 0.57*	8.33 ± 0.57*	-	-
	1000	11.66 ± 0.57*	-	13.33 ± 0.57*	10.33 ± 0.57*		-
VI	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
VII	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
VIII	250	-	-	-	-	-	-
	500	10.33 ± 0.57*	-	8.66 ± 0.57*	-	-	6.66 ± 0.57*
	1000	10.66 ± 0.57*	-	9.66 ± 0.57*	-	-	8.33 ± 0.57*
IX	250	-	-	-	-	-	-
	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
4% DMSO	Negative control	-	-	-		-	
Streptomycin (10 µg)	Positive control	22.33 ± 0.58					

Kirby-Bauer standard interpretative chart for positive control (Quality Control-NCCLS, 1995):
1. Carbenicillin (10 µg) for *P. aeruginosa* - Resistant(R) <13 mm; Intermediate (I) 14-16 mm; Sensitive/ Susceptibility >17 mm; 2. Streptomycin (10 µg) for other bacteria - Resistant(R) <11 mm; Intermediate (I) 12-14 mm; Sensitive/ Susceptibility >15 mm; * – Significant at 95% level, NS –Non-significant, ZI – Zone of inhibition, "-" No activity; Each value representing mean ± SD of 3 replicates

is represented in Table 4. The sub-fractions I, II, III, IV, V and VIII showed antibacterial activity against *P. aeruginosa*. The ZI observed with the fraction I ranged from 9.66 ± 0.57 mm at 250 µg concentration to 12.33 ± 0.57 mm at concentration of 1000 µg ($p < 0.05$). The antibacterial response at 1000 µg was inferred as an intermediary response when compared to Kirby-Bauer SAIC. The sub-fraction V showed a significant increase in the ZI ranging from 9.33 ± 0.57 mm at 250 µg, 11.00 ± 0.00 mm at 500 µg concentration and 11.66 ± 0.57 mm at 1000 µg concentration ($p < 0.05$). The response against *P. aeruginosa* was intermediate

when compared to Kirby-Bauer SAIC. However, the ZI measured for sub-fraction II, III, IV, and VIII inhibited *P. aeruginosa* with ZI measurements within 11.00 mm. Hence, the antibacterial response was considered as resistant and insensitive when compared in Kirby-Bauer SAIC. The sub-fractions V and VIII inhibited *E. aerogenes*. The ZI of the sub-fraction V ranged from 9.66 ± 0.57 mm at 500 µg to 13.33 ± 0.57 mm at 1000 µg concentration ($p < 0.05$). The antibacterial response was considered as intermediate. However, the sub-fraction VIII showed ZI values below 10.00 mm and the bacterial response was deduced as resistant. An antibacterial activity against the Gram-negative bacteria *P. vulgaris* was reported for the sub-fraction V, also inhibition of *E. coli* was observed by sub-fraction VIII. The bacterial response against *P. vulgaris* and *E. coli* was not sufficient as it was less than the effective range of 11.00 mm (ZI). Thus it was resistant when compared with the Kirby-Bauer SAIC.

Recently, the increase in antibacterial resistance has risen to a global problem demanding an alternate source of antibiotics against fatal diseases (Westh et al., 2004) and marine macro-algae are considered as the potential producers of bioactive compounds (Shimizu, 1996; Blunt et al., 2005) for the development of new antipathogenic products (Blunt et al., 2005; Abedin and Taha, 2008; El Gamal, 2010). In the present study, brown alga *P. gymnospora* and green alga *C. linum* collected from the coastal region of Tuticorin, South India were explored for the antibacterial responses against chosen Gram-positive and Gram-negative human pathogenic bacteria. Reports from various marine algae have revealed that the antibacterial response of an alga differs and changes based on numerous factors, of which, season, location, age and method of extraction are a few (Rao, 1998). The antibacterial response of various solvent sub-fractions of selected two seaweeds in the present analysis revealed that, sub-fraction-I of *P. gymnospora* showed potent antibacterial activity against Gram-positive bacteria, *B. subtilis* and *E. faecalis* and Gram-negative bacteria, *P. aeruginosa* at minimum concentration of 250 µg. Sub-fraction-II was active against *B. subtilis* and *P. aeruginosa* at 250 µg. Likewise, *C. linum* showed antibacterial potency with sub-fractions V and VIII at a minimum concentration (250 µg) against *B. subtilis* and sub-fraction V against *P. aeruginosa*. The antibacterial response of column sub-fractions of algal extract of *C. linum* clearly revealed no significant inhibitory activity against various Gram-positive bacteria screened whereas, Gram-negative bacteria *P. aeurginosa* responded with strong inhibition. Similarly, the sub-fraction V responded to *E. aerogenes* and *P. vulgaris* and sub-fraction VIII showed inhibitory response to 3 out of the 6 tested Gram-negative bacteria. A comparative report presented by Patra et al. (2009) on algal extract preparation by different organic solvent system showed that chloroform extract of *C. linum* was effective against most of the pathogens whereas, the *P. subtilissima* extract was effective against only *S. flexneri* and *B. subtilis*. In similar such studies, Senthilkumar and Sudha (2012) observed inhibition on the growth of *B. cereus* and *P. mirabilis* with the methanolic extract of *C. linum*. In another study conducted by Sivakumar and Safhi (2013) revealed petroleum ether extract of *C. antennina* showed effective response against *E. coli*, *S. aureus* and *P. aeurginosa*, and no response against *B. subtilis* and *K. pneumoniae*. These results suggested that the extract of the marine macroalgae have the ability to inhibit the growth of bacteria irrespective of its nature. But variations in its activity are accounted to the specific antimicrobial response determined by the metabolites extracted using a suitable solvent system (Tuney et al., 2006; Patra et al., 2009; Ibtissam et al., 2009; Rajasulochana et al., 2009; Rangaiah et al., 2010; Jebasingh et al., 2011; Rosaline et al., 2012). In conclusion, the column sub-fractions I, II, V and VIII of the marine alga, *P. gymnospora* and sub-fractions V and VIII of marine alga, *C. linum* revealed significant inhibitory activity to few human pathogenic bacteria used in this study. Further biochemical analyses of these sub-fractions could reveal potential antibacterial substances.

4. CONCLUSIONS

The crude acetone extracts of seaweeds of *Padina gymnospora* and *Chaetomorpha linum* were fractionated using sequential gradient of solvent extraction with silica gel column chromatography. Nine sub-fractions were used for their antibacterial activity against human bacterial pathogens. The sub-fractions I to V of *P. gymnospora* revealed significant antibacterial activity against majority of tested bacterial strains. However, the isolated sub-fraction IV of *C. linum* responded well to *Bacillus subtilis* and *Enterococcus faecalis* and its sub-fraction V revealed inhibitory activity against few Gram-negative bacteria. The sub-fraction VIII showed antibacterial activity to few Gram-negative bacteria. Though there are differences, identified effective sub-fractions is a beneficial outcome of this study for future isolation of antibacterial molecules.

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REFERENCES

Abedin, R.M.A. and Taha, H.M. (2008) Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*. Global Journal of Biotechnology and Biochemistry, 3, 22-31.

Bansemir, A., Blume, M., Schröder, S. and Lindequist, U. (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252, 79-84.

Bhakuni, D.S. and Rawat, D.S. (2005) Bioactive marine natural products. New Delhi, India: Anamaya Publishers.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T. and Prinsep, M.R. (2005) Marine natural products. Natural Product Reports, 22, 15-61.

Cheung, R.C.F., Wong, J.H., Pan, W.L., Chan, Y.S., Yin, C.M., Dan, X.L., Wang, H.X., Fang, E.F., Lam, S.K., Ngai, P.H., Xia, L.X., Liu, F., Ye, X.Y., Zhand, G.Q., Liu, Q.H., Sha, O., Lin, P., Ki, C., Bekhit, A.A., Bekhit, Ael-D., Wan, D.C., Ye, X.J., Xia, J. and Nq, T.B. (2014) Antifungal and antiviral products of marine organisms. Applied Microbiology and Biotechnology, 98, 3475-3494.

Chew, Y.L., Lim, Y.Y., Omar, M. and Khoo, K.S. (2008) Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Science and Technology, 4, 1067-1072.

Chiheb, I., Hassane, R., Martinez, L., Jose, D., Francisco, G., Antonio, B., Hassan, B. and Mohamed, K. (2009) Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. African Journal of Biotechnology, 8, 1258-1262.

Cox, S., Ghannam, A.N. and Gupta, S. (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal, 17, 205-220.

Dawson, W. (2005) Herbal medicine and the EU directive. Journal of the Royal College of Physicians of Edinburgh, 35, 25-27.

De Smet, P.A. (2002) Herbal remedies. The New England Journal of Medicine, 347, 2046-2056.

El Gamal, A.A. (2010) Biological importance of marine algae. Saudi Pharmaceutical Journal, 18, 1-25.

Faulkner, D.J. (2002) Marine natural products. Natural Product Reports, 19, 1-48.

Fayaz, M., Namitha, K.K., Murthy, K.N.C., Swamy, M.M., Sarada, R., Khanam, S., Subbarao, P.V. and Ravishankar, G.A. (2005) Chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezzi* (Doty). Journal of Agricultural and Food Chemistry, 53, 792-797.

Ibtissam, C., Hassane, R., José, L.R., Francisco, D.S.J., Antonio, G.V.J., Hassan, B. and Mohamed, K. (2009) Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. African Journal of Biotechnology, 8, 1258-1262.

Jebasingh, E.J.S., Rosenmary, R., Elaiyaraja, S., Sivaraman, K., Lakshmikandan, K., Murugan, A. and Raja. P. (2011) Potential antibacterial activity of selected green and red seaweeds. Journal of Pharmaceutical and Biomedical Sciences, 5, 1-7.

Lima-Filho, J.V.M., Carvalho, A.F.F.U. and Freitas, S.M. (2002) Antibacterial activity of extracts of six macroalgae from the Northeastern Brazillian coast. Brazilian Journal of Microbiology, 33, 311-313.

Lincoln, R.A., Strupinski, K. and Walker, J.M. (1991) Bioactive compounds from algae. Life Chemistry Reports, 8, 97-183.

Marston, A., Maillard, M. and Hostettmann, K. (1997) The role of TLC in the investigation of medicinal plants of Africa, South America and other tropical regions. GIT Lab J, 1, 36-39.

Moorthi, P.V. and Balasubramanian, C. (2015) Antimicrobial properties of marine seaweed, *Sargassum muticum* against human pathogens. Journal of Coastal Life Medicine, 3, 122-125.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (1995) Manual of clinical microbiology. Washington DC:ASM. 6, 441-484.

National Committee for Clinical Laboratory Standards. (1995) Performance standards for antimicrobial susceptibility testing: Sixth informational supplement M100-S6. NCCLS, Wayne, PA.

Rao, P.P.S. (1998) Biological investigations of Indian Phaeophyceae: 17. Seasonal variation of antibacterial activity of total sterols obtained from frozen samples of *Sargassum johnstonii* Setchell et Gardner. Seaweed Research and Utilization, 20, 91-95.

Patra, J.K., Patra, A.P., Mahapatra, N.K., Thatoi, H.N., Das, S., Sahu, R.K. and Swain, G.C. (2009) Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. Malaysian Journal of Microbiology, 5, 128-131.

Rajasulochana, P., Dhamotharan, R., Krishnamoorthy, P. and Murugesan, S. (2009) Antibacterial activity of the extracts of marine red and brown algae. Journal of American Science, 5, 20-25.

Rangaiah, G.S., Lakshmi, P. and Manjula, E. (2010) Antimicrobial activity of seaweeds *Gracillaria*, *Padina* and *Sargassum* sps. on clinical and phytopathogens. International Journal of Chemical Analytical Science, 1, 114-117.

Robles, C.P.O. and Ballantine, D.L. (1999) Effects of culture conditions on production of antibiotically active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae: Rhodophyta). I. Light. Journal of Applied Phycology, 11, 217-224.

Robles, C.P.O., Ballantine, D.L. and Gerwick, W.H. (1996) Dynamics of antibacterial activity in three species of Caribbean marine algae as a function of habitat and life history. Hydrobiologia, 326/327, 457-462.

Rosaline, X.D., Sakthivelkumar, S., Rajendran, K. and Janarthanan, S. (2012) Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity. Asian Pacific Journal of Tropical Biomedicine, S140-S146.

Rosaline, X.D., Sakthivelkumar, S., Rajendran, K. and Janarthanan, S. (2016) Detection of antibacterial activity and its characterization from the marine macro-algae *Sargassum wightii* (Greville). Indian Journal of Geo-Marine Science, 45, 1365-1371.

Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H. and MatusFerech, M. (2008) Antimicrobial drug use and resistance in Europe. Emerging Infectious Diseases, 14, 1722-1730.

Sarma, Y.S.R.K. and Khan, M. (1980) Algal taxonomy in India. New Delhi. Today's and Tomorrrow's printers and publishers (Botanical records and monographs 2), pp. 153.

Senthilkumar, P. and Sudha, S. (2012) Antioxidant and antibacterial properties of methanolic extract of green seaweed *Chaetomorpha linum* from Gulf of Mannar: Southeast coast of India. Jundishapur Journal Microbiology, 5, 411-415.

Shimizu, Y. (1996) Microalgal metabolites: a new perspective. Annual Review Microbiology, 50, 431-465.

Sivakumar, S.M. and Safhi, M.M. (2013) Isolation and screening of bioactive principle from *Chaetomorpha antennina* against certain bacterial strains. Saudi Pharmaceutical Journal, 21, 119-121.

Sonnenwirth, A.C. (1980) Bacteriology. In Sonnenwirth, A.C. and Jarrett, L (Eds.), Gradwohl's clinical laboratory methods and diagnosis 8th edition. Missouri, US: The C. V. Mosby Company, pp.1391-1405.

Thanigaivel, S., Vidhya, S.H., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N. and Thomas, J. (2015) Differential solvent extraction of two seaweeds and their efficacy in controlling *Aeromonas salmonicida* infection in *Oreochromis mossambicus* a novel therapeutic approach. Aquaculture, 433, 56–64.

Tuney, I., Cadirci, B.H., Unal, D. and Sukatar, A. (2006) Antimicrobial activities of the extracts of marine algae from the coast of Urla (İzmir, Turkey). Turkish Journal of Biology, 30, 1-5.

Vickers, A. and Zollman, C. (1999) ABC of complementary medicine: Herbal medicine. British Medical Journal, 319, 1050-1053.

Westh, H., Zinn, C.S. and Rosdahl, V.T. (2004) An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microbial Drug Resistance (MDR), 10, 169-176.