Antibacterial Properties Of The Green Alga *Pithophora Oedogonia* (Mont.) Wittrock

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ABSTRACT: Methanol and n-hexane extracts of the green alga, *Pithophora oedogonia* was tested for antibacterial activity against clinical isolates of common human pathogenic bacteria namely, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae, Shigella flexnerii, Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus faecalis*. Methanolic extract residue dissolved in diethylether exhibited good activity against *Streptococcus pyogenes, Streptococcus faecalis* and *Escherichia coli*. Activity of silica gel column fractions is significant and comparable to that of standard antibiotics. Chromatatron fractions recorded very low MIC values for *Streptococcus pyogenes, Streptococcus faecalis* and *Escherichia coli* as compared to that of standard antibiotics. The findings presented in this paper suggest that the 'nuisance alga'. *Pithophora oedogonia*, could serve as a potential source of biologically active natural products for pharmaceutical application.
[Pamela Sukumaran and Thevanathan, R. **Antibacterial Properties Of The Green Alga *Pithophora Oedogonia* (Mont.) Wittrock**. Report and Opinion 2011;3(1):53-60]. (ISSN: 1553-9873). http://www.sciencepub.net.

Keywords: Antibacterial; Green Alga; *Pithophora Oedogonia* (Mont.); Wittrock

INTRODUCTION

In the past four decades, algae have been shown as a potential source of compounds with antibiotic properties. Crude extracts of marine macroalgae have been extensively studied for their antimicrobial properties (Allen and Dawson, 1960: Burkholder *et al*.,1960; Duff *et al*.,1966; Hornsey and Hide, 1974; Glombitza, 1979; Henriquez *et al*.,1979; Pesando *et al*.,1979; Pesando and Caram, 1984; Caccamese *et al*.,1979; 1985; Calvo *et al*.,1986; Alam, 1994; Avelin Mary *et al*.,1995; Lara *et al*.,1996; Horikawa *et al*.,1966; Robles Centeno *et al*.,1996; Rovirosa, 1997; Khaliq Un and Zamam, 1998; Robles Centeno and Ballantine, 1999; Walter and Mahesh, 2000; Dhamotharan, 2002;Noemi *et al*.,2007). However, reports on fresh water algae are few (Pratt *et al*.,1944; Harder and Opperman, 1954; Davidson, 1961; Gupta and Shrivastava, 1965; Stangenberg, 1968; Pandey *et al*.,1977; Debro and Ward, 1979; Mason *et al*.,1982; Cannell *et al*.,1988;Prashantkumar *et al*., 2006).

Pithophora oedogonia (Mont.) Wittrock, the 'horse hair' or 'cotton ball alga' is a fresh water green alga of the order Cladophorales. The alga produces free floating mats of vegetation in static or slow moving bodies of water. Its luxuriant growth in shallow lakes and ponds as thick clumps or mats with profusely branched filaments having rigid, coarse cell walls, biomass production in huge quantities and high degree of resistance to many algicides have placed the alga in a prominent position as a filter clogging or nuisance alga of water systems. Though the alga has been studied as a nuisance alga, its nutritive and bioactive properties are not explored. The present investigation explores the possibilities of exploiting this alga in pharmaceutical industries as a source of antimicrobial compounds.

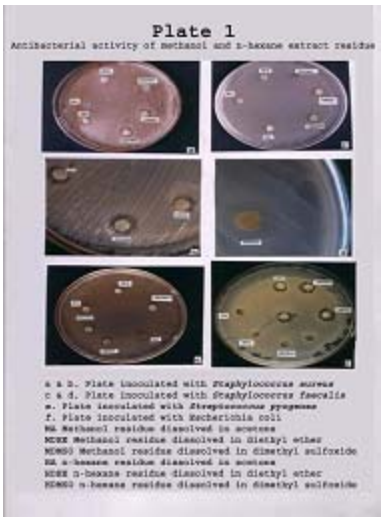
MATERIALS AND METHODS

Filaments of *Pithophora oedogonia* were collected from cultures maintained in cement tanks containing Bold basal medium at 27 ± 2°C and a light intensity of 50 μ Einsteins m^{-2} s^{-1}. Harvested filaments (250 g) were shade dried and extracted with n-hexane for 48 hrs and the marc was re-extracted with methanol. Both extracts were dried separately in flash evaporator and the residues were redissolved in known quantities of respective solvents and used as crude extracts of the solvents. The residues of these solvent extracts were dissolved in acetone, DMSO (dimethyl sulfoxide), and DEE (diethyl ether) separately to get a concentration of 100 and 200 μg extract residue/mL of solvent. They were then tested for their antibacterial activity against clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae, Shigella flexnerii, Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus faecalis* by disc diffusion method (Bauer – Kirby, 1965). All the test organisms were maintained on nutrient agar except *Streptococcus pyogenes*, which was maintained on blood agar. Discs loaded with the extracts at desired concentrations were aseptically placed on seeded medium and gently pressed down to ensure contact. The plates were then incubated at 37°C and observed after 12 hrs for zones of inhibition. Simultaneously, the antibiotics ampicillin, erythromycin, streptomycin

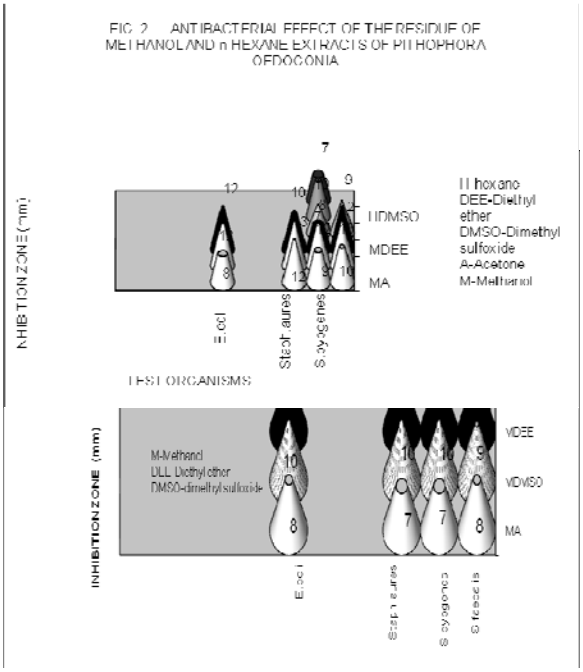
and cifroflaxin were also tested at similar conditions. Column fractionations ofl extract residues was carried out with Silica gel G (60 – 120 mesh, 150 g, 45 x 14 cm) using hexane, 5%, 20%, 30%, 40%, 50%,60%, 80% ethyl acetate in hexane, ethyl acetate, chloroform, 1 – 50% methanol in chloroform and methanol. Based on similarities on TLC, the eluates were pooled and designated as MCC-1, MCC-2, etc. These pooled fractions were dried and redissolved in DMSO (dimethyl sulfoxide) and serially diluted to get desired concentrations of extract residue/mL of solvent and tested again for activity against the test organisms. Active fractions were further fractionated by chromatatron or flash chromatography as and when necessary (Pamela, 2001).

RESULTS

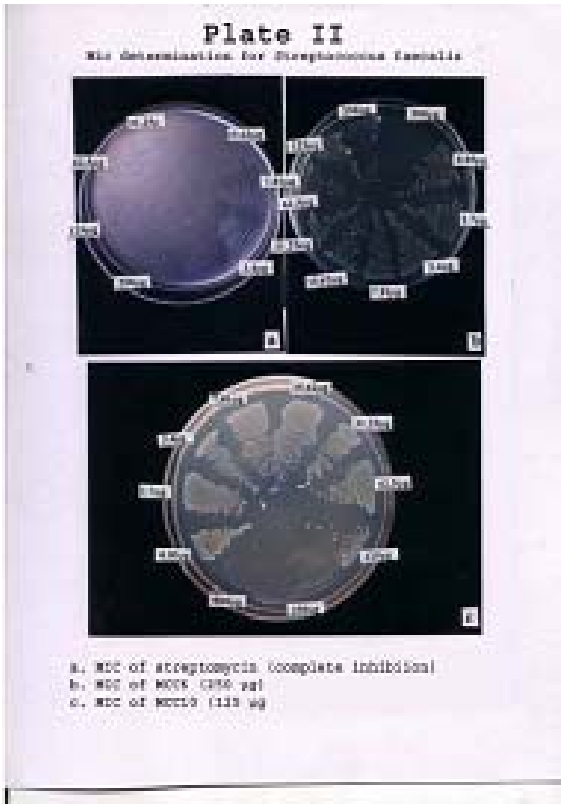
Methanol and n-hexane extracts of *Pithophora oedogonia* were dried and redissolved in acetone, dimethyl sulphoxide (DMSO) and diethyl ether (DEE) and tested for antibacterial activity against ten common pathogenic bacteria namely, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae, Shigella flexnerii* and *Staphylococcus faecalis* employing Bauer – Kirby method.



(Plate I). The solvent fractions of both hexane and methanol extract residues of the alga exhibited considerable activity against *E. coli, Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus faecalis* (Figs 1 & 2). However, activity could not be observed against *Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae* and *Shigella flexnerii* regardless of the solvent fraction of the residues used (Fig.1&2).

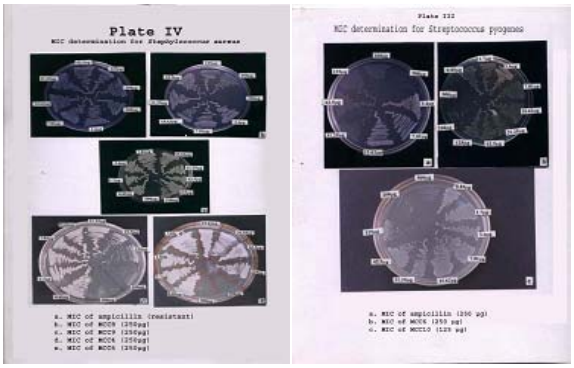


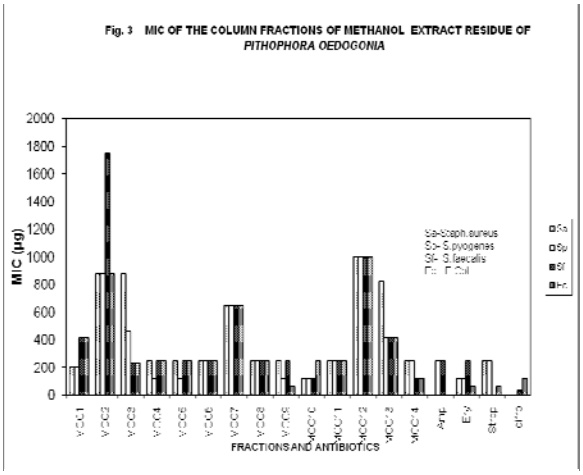
The methanolic residue dissolved in DMSO, DEE and acetone at a concentration of 100 µg showed considerable activity against *E. coli, Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus faecalis* (Fig. 1). The DMSO fraction of the methanol extract residue recorded maximum activity for all these organisms. Solvent fractions of n-hexane residue of the alga (100 µg) did not show any activity against any of the ten test organisms. However, at a concentration of 250 µg, some activity could be observed against a few test organisms (Fig. 2). Acetone fraction of the residue exhibited activity against *Streptococcus pyogenes* and *Streptococcus faecalis* and the DMSO and DEE fractions showed activity against *Streptococcus pyogenes* only (Fig. 2. The methanolic extract residue was taken up for column fractionation using silica gel G and the MIC and MBC for these fractions were determined and compared with those of standard antibiotics such as ampicillin, erythromycin, cifroflaxin and streptomycin (Plates II - IV).



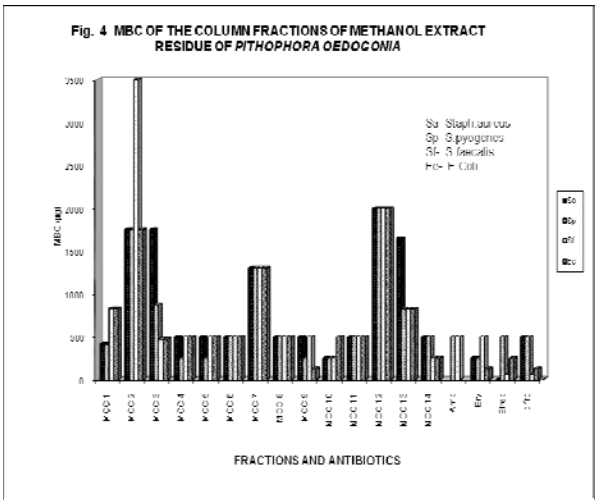
The MIC of column fractions for *Streptococcus pyogeness* and *Streptococcus faecalis* were similar to that observed for ampicillin (Table 1; Fig. 3).

With respect to *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus faecalis* MCC-10 appeared to be more effective than ampicillin and erythromycin with its low MIC for these organisms. In the case of *Streptococcus pyogenes*, the fraction recorded a low MIC against that of cifroflaxin. Among the various column fractions, MCC-9 proved to be highly effective against *E. coli*.





Based on these findings, MCC-10 was further fractionated by chromatatron and the fractions were tested against *Streptococcus pyogenes* and *Streptococcus faecalis* for MIC and MBC determinations. The chromatatron fractions CT-1, CT-2 and CT-3 were found to be highly effective against *Streptococcus pyogenes* and *Streptococcus faecalis* as compared to the four antibiotic compounds (Table II & Fig. 5). With respect to *Streptococcus pyogenes*, the fractions CT-7, CT-8, CT-10 and CT-12 prove to be very efficient than the standard antibiotics tested and in fact, CT-7 completely inhibited the growth of the organism at a low concentration of 3.4 µg/mL. Determinations of MBC reflected the trend observed for MIC determinations (Figs 4 & 6). Of the various fractions of n-hexane extract residue of the alga, HFL-5 alone showed some activity against *Staphylococcus aureus*, *Streptococcus faecalis* and *Streptococcus pyogenes* at 370 µg, 750 µg and 180 µg concentrations (Table 3&Fig.7). Other fractions of n-hexane residue required high concentrations to exhibit activity against the test organisms and hence, may not be of significance. Further studies to characterize the bioactive ingredients in the active fractions are in progress.



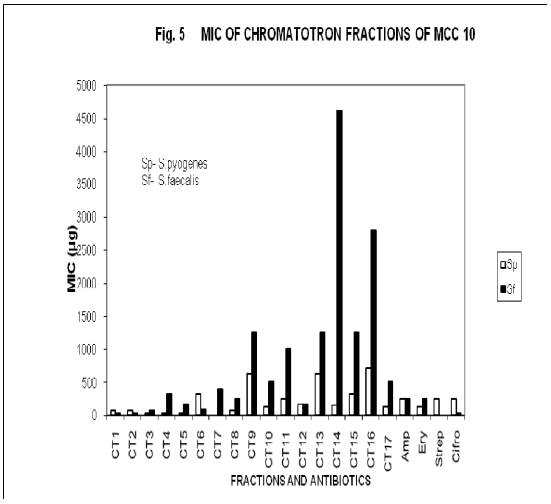


Table 1 Minimum inhibitory and minimum bactericidal concentrations for the column fractions of the meathanolic extract residue of *P. oedogonia*

Fraction No.	*Staphylococcus Aureus*		*Streptococcus pyogenes*		*Streptococcus faecalis*		*Escherichia coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MCC 1	207.5±1.4	415±2.69	207.5±1.4	415±2.89	415±2.89	830±5.78	415±2.89	830±5.78
MCC 2	875±2.89	1750±5.7	875±2.89	1750±5.7	1750±5.7	3500±11.5	875±2.89	3500±5.78
MCC 3	875 ± 2.5	1750±2.8	468.7±0.2	875±0.7	234.3±0.1	468.7±0.2	243.3±0.1	468.7±0.2
MCC 4	250±0.57	500±1.15	125±0.25	250±0.57	250±0.57	500±1.15	250±0.57	500±1.15
MCC 5	250±0.57	500±1.15	125±0.25	250±1.15	250±0.57	500±1.15	250±0.57	500±1.15
MCC 6	250±0.57	500±1.15	250±1.5	500±1.6	250±0.57	500±1.15	250±0.57	500±1.15
MCC 7	650±1.45	1300±2.8	650±1.44	1300±2.8	650±1.44	1300±2.89	650±1.44	1300±2.89
MCC 8	250±1.45	500±2.3	250±1.14	500±1.7	250±1.15	500±1.5	250±1.15	500±2.3
MCC 9	250±1.15	500±2.3	125±0.5	250±0.57	250±0.57	500±0.57	62.5±0.25	125±0.28
MCC 10	125±0.28	250±0.57	125±0.5	250±1.15	125±0.28	250±1.15	250±0.57	500±1.15
MCC 11	250±0.72	500±1.15	250±0.72	500±1.15	250±0.72	500±1.15	250±0.72	500±1.15
MCC 12	1000±2.8	2000±5.7	1000±2.8	2000±5.7	1000±2.8	2000±5.78	1000±2.8	2000±5.78
MCC 13	825±2.8	1650±5.7	412.5±1.4	825±2.8	412.5±1.4	825±2.8	412.5±1.4	825±2.8
MCC 14	250±0.72	500±1.15	250±0.72	500±1.15	125±0.43	250±1.15	125±0.43	250±0.57
Ampicillin	Resistant	Resistant	250	500	250	500	Resistant	Resistant
Erythromycin	125	250	125	250	250	500	62.5	125
Streptomycin	250	500	250	500	inhibition	Inhibition	62.5	125
Cifroflaxin	1.9	3.9	250	500	31.25	62.5	250	500

Mean ± standard error

Table 2 Minimum inhibitory and minimum bactericidal concentration for the chromatotron fractions of column fraction MCC 10

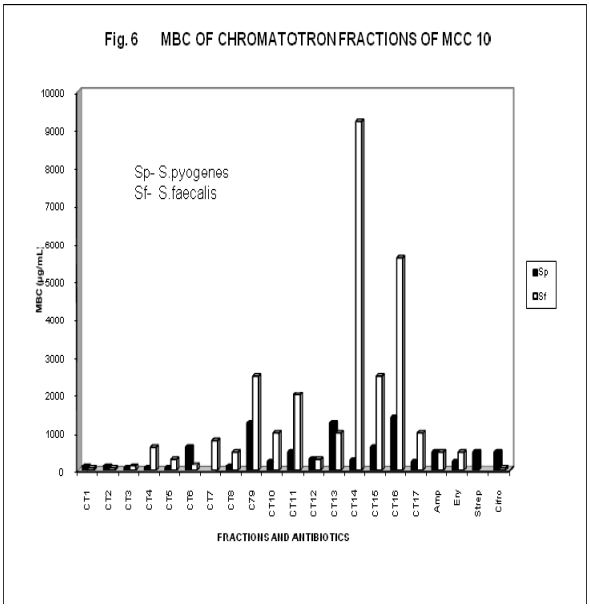
Fraction No.	**Steptococcus pyogenes**		***Steptococcus faecalis***	
	MICμg/mL	**MBCμg/mL**	MICμg/mL	MBCμg/mL
CT 1	62.5 ± 0.14	125 ± 0.28	31.25 ± 0.072	62.5 ± 0.14
CT 2	62.5 ± 0.14	125 ± 0.28	31.25 ± 0.072	62.5 ± 0.14
CT 3	31.2 ± 0.07	62.5± 0.14	62.5 ± 0.14	125 ± 1.4
CT 4	31.2 ± 0.07	62.5± 0.14	312.5 ± 0.72	625 ± 1.4
CT 5	312.5± 0.72	625 ± 1.4	156.25± 0.36	312.5±0.72
CT 6	312.5± 0.72	625 ± 1.4	78.125± 0.25	156.5±0.36
CT 7	*	*	400 ± 0.6	800 ± 1.4
CT 8	62.5 ± 0.14	125 ± 0.28	250 ± 0.57	500 ± 0.6
CT 9	625.5± 1.4	1250± 2.8	1250 ± 2.8	2500 ± 5.7
CT 10	125 ± 1.4	250 ± 0.57	500 ± 0.6	1000 ± 2.3
CT 11	250 ± 0.57	500 ± 0.6	1000 ± 2.3	2000 ± 4.6
CT 12	156.2± 0.36	312.5± 0.72	156.25 ± 0.36	312.5± 0.72
CT 13	312.5± 0.72	625 ± 1.4	500 ± 0.6	1000 ± 2.3
CT 14	143.7± 0.02	287 ±0.04	4600 ± 1.6	9200 ± 3.3
CT 15	312.5± 0.72	625 ± 1.4	1250 ± 2.8	2500 ± 5.7
CT 16	700 ± 0.72	1400 ± 7.5	2800 ± 2.8	5600 ± 3.3
CT 17	125 ± 1.4	250 ± 0.5	500 ± 0.6	1000 ± 2.3
Ampicillin	250	500	250	500
Erythromycin	125	250	250	500
Streptomycin	250	500	1nhibition	Inhibition
Cifroflaxin	250	500	31.25	62.5

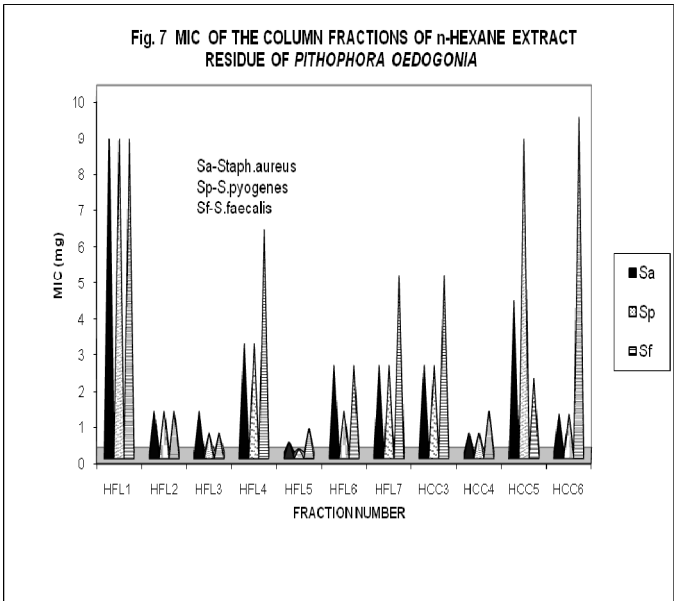
* Complete inhibition at 3.4μg/ mL Mean ± standard error

Table 3 Minimum inhibitory and minimum bactericidal concentration for the -hexane residue of column fractions

Fraction No.	Staphylococcus aureus		Streptococcus pyogenes		Streptococcus faecalis	
	MIC mg/mL	MBC mg/mL	MIC mg/mL MBCmg/mL		MIC mg/mL	MBC mg/mL
HFL 1	8.75 ± 0.14	17.5 ± 0.28	8.75 ± 0.14	17.5 ± 0.28	8.75 ± 0.14	17.5 ± 0.28
HFL 2	1.25 ± 0.28	2.5 ± 0.57	1.25 ± 0.28	2.5 ± 0.57	1.25 ± 0.28	2.5 ± 0.57
HFL 3	1.25 ± 0.28	2.5 ± 0.57	0.62 ± 0.14	1.25 ± 0.28	0.62 ± 0.14	1.25 ± 0.28
HFL 4	3.12 ± 0.72	6.25 ± 0.14	6.25 ± 1.41	12.5 ± 2.89	3.12 ± 0.72	6.25 ± 1.4
HFL 5	0.37 ± 0.01	0.75 ± 0.02	0.75 ± 0.08	1.5 ± 0.16	0.18 ± 0.28	0.37 ± 0.56
HFL 6	2.5 ± 0.57	5.0 ± 1.15	2.5 ± 0.57	5.0 ± 1.15	1.25 ± 0.28	2.5 ± 0.56
HFL 7	2.5 ± 0.57	5.0 ± 1.15	5.0 ± 1.44	10.0 ± 2.89	2.5 ± 0.57	5.0 ± 1.47
HCC 3	2.5 ± 0.57	5.0 ± 1.15	5.0 ± 1.44	10.0 ± 2.89	2.5 ± 0.57	5.0 ± 1.47
HCC 4	0.62 ± 0.14	1.25 ± 0.28	1.25 ± 0.28	2.5 ± 0.57	0.62 ± 0.14	1.25 ± 0.28
HCC 5	4.3 ± 0.72	8.75 ± 1.41	2.15 ± 0.72	4.3 ± 1.44	8.75 ± 0.14	17.5 ± 0.2
HCC 6	1.17 ± 0.36	2.3 ± 0.72	9.37 ± 2.30	18.7 ± 4.60	1.17 ± 0.36	2.13 ± 0.72
Ampicillin	Resistant	Resistant	250μg/mL	500μg/mL	250μg/mL	500μg/mL
Erythromycin	125μg/mL	250μg/mL	125μg/mL	250μg/mL	250μg/mL	500μg/mL
Streptomycin	250μg/mL	500μg/mL	50μg/mL	500μg/mL	inhibition	inhibition
cifroflaxin	1.9μg/mL	3.9μg/mL	250μg/mL	500μg/mL	31.25μg/mL	62.5μg/mL

Mean ± standard error





DISCUSSION

Crude extracts of many species of algae are reported to contain substances with antibiotic properties against bacteria, fungi and viruses. As outlined earlier, fresh-water algae have been largely precluded in the search for bioactive natural products. Several solvent systems have been used to extract the antibacterial substances from marine algae. Methanol, acetone, toluene, chloroform, hexane, diethyl ether, ethanol, and ethyl acetate have been widely used either alone or in combination and the choice of the solvent for the extraction of antibacterial substance appeared to be selective for an alga (Allen and Dawson, 1960; Martinez Nadal *et al*.,1966; Bhakuni and Silva, 1974; Debro and Ward, 1979; Caccamese *et al*.,1980; 1985; Rao and Parekh, 1981; Pesando and Caram, 1984; Rao *et al*.,1986; Rao, 1991; 1995; Sastry and Rao, 1994; Robles Centeno *et al*.,1996; Centeno and Ballantine, 1999; Dhamotharan, 2002). Our investigation revealed the effectiveness of the methanolic extract residue of the fresh-water green alga, *Pithophora oedogonia* in inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus faecalis* at a concentration of 100 µg (Figs 1 & 2). At the same concentration, n-hexane residue did not show any activity against the test organisms. Nevertheless hexane extract residue too was able to register activity against species of *Streptococcus* at a concentration of 250 µg (Fig. 2). Of the three solvents used to dissolve the extract residues for the test against the bacterial pathogens, DEE proved to be the best with positive results that showed maximum inhibition of the test organisms as compared to that of acetone or DMSO dissolved fractions.

The MIC and MBC determinations of the column fractions of the methanolic extract residue of the alga revealed high antibacterial activity of some of the fractions. With their low MIC and MBC values, the column fractions MCC-9 and MCC-10 proved to be highly effective than the standard antibiotics ampicillin, erythromycin and cifroflaxin when tested against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus faecalis* . Fraction MCC-10 had a low MIC for *S. pyogenes* and *S. faecalis* while MCC-9 was active against *E. coli*. When MCC-10 was further fractionated by chromatatron and test against *S. pyogenes* and *S. faecalis*, the fractions CT-7 completely inhibited the growth of the bacteria at 3.4 µg. Fractions of hexane extract residue could not be considered significant since they were required at high concentrations to register antibacterial activity comparable to that of standard antibiotics.

Observed antibacterial activity of the methanolic extract of *Pithophora oedogonia* against *Streptococcus pyogenes*, *Streptococcus faecalis* and *Escherichia coli* is significant and comparable to that of standard antibiotics. In some instance, the alga proved to be more effective than a few branded antibiotics. Easy availability of the alga, simple growth requirements and fast growth under enriched conditions are added advantage for exploitation of the

alga commercially for antibiotic production. The findings presented in this paper would certainly form a basis to view *Pithophora oedogonia*, the 'nuisance alga'. as a potential source of biologically active natural products for pharmaceutical application.

Acknowledgment
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Submitted on 17th july2010