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STUDIES ON ANTIBACTERIAL ACTIVITY OF SEAWEEDS, *ENTEROMORPHA INTESTINALIS* (LINNAEUS) AND *GELIDIELLA ACEROSA* (FORSSKAL) FROM PUDUCHERRY AND RAMESWARAM (SOUTHEAST COAST OF INDIA)

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ABSTRACT

The main objective of the work was to evaluate and compare the *invitro* antibacterial activity of seaweed using different solvent system. Seaweeds, *Enteromorpha intestinalis* and *Gelidiella acerosa*, collected from Puducherry and Rameshwaram (South East Coast of India). From the dried seaweeds extracts of crude bioactive compounds were obtained using the solvents of acetone, diethyl ether, methanol:chloroform (1:1). The seaweed extracts were tested against 12 human bacterial pathogens by disc diffusion method. The methanol:chloroform extracts showed maximum zone of inhibition against *Bacillus subtilis, Streptococcus pneumonia, Vibrio cholera* and *Proteus mirabilis*. The HPTLC analysis of E.*intestinalis* methanol:chloroform extracts showed 14 different phytochemical compounds. From the present study, it can be concluded that the green alga *Enteromorpha intestinalis* is a potential source of bioactive compounds.

KEY WORDS: ***Enteromorpha intestinalis*, *Gelidiella acerosa*, antibacterial activity, HPTLC.**

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1. INTRODUCTION

Seaweeds are a group of larger marine plants attached to the bottom in relatively shallow coastal waters. Seaweeds are not as complex as that of flowering plants. Seaweeds lack roots, flowers, seeds, and true leaves. Approximately 841 species of marine algae are found in both inter-tidal and deep water regions of the Indian coast[1]. Several seaweeds have been used in the treatment of lung, kidney, stomach, and intestinal disorders as vermifuges, purgatives and also in the treatment of oncology patients. Many substances obtained from marine algae such as alginate, carrageenan and agar as phycocolliods have been used for decades in medicine and pharmacy[2]. Seaweeds have energy interest in the biomedical area, mainly due to their contents of bioactive substrate which show great potential as anti-inflammatory, antimicrobial, antiviral and anti-tumoral drugs[3][4]. Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest for the pharmacological industry[5]. More recent reports indicate that in many parts of the world marine algae is still used in folk medicine for the treatment of a variety of disease. The present study was aimed to determine the antibacterial activity of two marine algae belonging two families, Chlorophyceae (*Enteromorpha intestinalis*) and Rhodophyceae (*Gelidiella acerosa*). The efficiency of solvent like methanol:chloroform, diethyl ether and acetone for extracting antibiotics from seaweeds and to obtain species with highly active antibacterial compounds.

2. MATERIALS AND METHODS

2.1. Collection and identification of seaweeds

The samples of seaweed, *Enteromorpha intestinalis* (Chlorophyta) were collected from Puducherry coast and the *Gelidiella acerosa* samples were collected from the Rameshwaram, along the south coast of India. The samples taken in the polythene bags were labeled and preserved for identification in the laboratory. The seaweeds species were identification based on their morphological and anatomical observation. For identification, the key provided by various taxonomic literatures were used[6][7]. The peculiar characteristics taken into account include length and diameter of the rhizome, its internal structure, colour, length of the plant, number and length of the lateral branches, size and shape of primary, secondary, and tertiary leaves, size and shape of air bladders, branching and length of receptacles, shape and size of hold fast.

2.2. Preparation of seaweed extracts

The isolated seaweed samples were washed with tap water to remove epiphytes and other marine organisms and then washed with distilled water to remove all the salt on the surface. For extraction of bio actives, seaweeds were shade-dried at room temperature and ground into fine powder, from that 25g of finely powdered algal material was extracted using three different types of solvent systems are viz: chloroform:methonal(1:1v/v), acetone and diethyl ether. The extraction with different solvents was carried out in Scott Durant bottles containing 250ml of respective solvents. They were placed at 35℃ on a shaker at 120 rpm for one week to allow full extraction of the active compounds[8].The concentrated extract was filtered through a What man no.1 filter paper fitted with a Buchner funnel. The resultant extract was evaporated under vacuum. Finally, it was reduced to thick oily natured crude extracts which were collected in air-tight plastic vials and stored in the refrigerator for further activity studies. The aliquots were tested for their antimicrobial activity on twelve clinical isolates.

2.3. Bacterial Strains Used For Assay microorganisms

The seaweed extracts were tested against a panel of clinical isolates viz: *E.coli, Bacillus cereus, Pseudomonas* sp*., Klebsiella pneumoniae, Proteus mirabilis, Vibrio cholera*, *streptococcus pneumonia*, *salmonella typhi, salmonella paratyphi*, *Shigella* sp.,

Staphylococcus epidermidis and methicillin resistant *Staphylococcus aureus* [MRSA]. The resistance patterns of these isolates were confirmed using selective antibiotics. All the bacterial strains were maintained on Nutrient agar at 4 °C. Microbial strains were obtained from Department of Microbiology, PGP College of arts and science, Namakkal.

2.4. Antibacterial activity test

Test was performed by the disc diffusion method[9], Sterile discs 6mm were prepared by pipetting 100µl of extract to each disc then the discs were allowed to dry for 15min, to reduce the toxic effect of solvent on test strains and placed on the agar Mueller-Hinton (pH 7.2± 0.2) and incubated at 37°C during 24 h overnight[10]. Inhibition results have been expressed as width of the clear halo surrounding each disc on cultivated agar plats. Control discs were prepared by just loading the sterile discs with the respective solvent alone. All experiments were performed in triplicate. Diameter of the zones of inhibition was measured in millimeters.

2.5. High Performance Thin Layer Chromatography (HPTLC)

HPTLC for chloroform:methanol extracted samples were processed on the automated HPTLC system (CAMAG, Switzerland) according to the instructions of the manufacturer. Ready-to-use silica coated plates were activated by blowing hot air for 5min and placed in the automatic sample applicator. The HPTLC was programmed to automatically spray 2.5, 5, 10µl of each sample in band from using specialized Hamilton syringe on one-side of the TLC plate in individual tracks. Totally six tracks were prepared from two seaweed samples. The TLC plate was developed in chloroform:methonal (ratio 95:5 v/v) solvent system and the plate was developed in the automated developing chamber (CAMAG) until the solvent front reached the maximum distance (80 mm distance in a typical 20 x 10 cm plate). The extract was subjected to colum chromatography on silica gel (Merk) eluted with mixtures of chloroform and methanol. After colour development of the separated constituents, compounds were quantified using TLC scanner[11][12].The developed plate was dried with a plate drier and subjected to UV analysis in the dedicated UV detector. All tracks in the plate were scanned at user-defined wavelength (254 and 366 nm) and individual R_f values of peaks were calculated.

3. RESULTS

Presently, the crude extracts derived from the seaweeds were screened for various *invitro* antibacterial activities. The results of antimicrobial activity are seaweeds summarized in Table 1. For the present investigation, extractions were obtained from two different species of seaweeds viz: *Enteromorpha intestinalis* and *Gelidiella acerosa* using three different solvent systems viz: diethyl ether, acetone and chloroform: methanol. Totally 9 extracts were obtained and tested against 12 human bacterial pathogens.

3.1. Chloroform:methanol

The effect of choloroform:methanol (1:1) extract against the bacterial pathogens is shown in Table 1 (figure: 1). The *E.intestinal* fractions showed 8mm inhibition zone against *S.pneumonia*, *B.cereus*, 7mm inhibition zone against *V.cholerae*, 6mm in *P.mirabilis*, 3mm in *E.coli*, 2mm in *Pseudomonas* sp. and 1mm in *K.pneumoniae* and *S.epidermidis*. These extract did not shown any antibacterial activity against *S.typhi, S.paratyphi, Shigella* sp., *MRSA* etc. The *Gelidiella acerosa* fractions were effective by inhibiting 7mm in *S.pneumoniae*, 6mm in *V.cholerae*, 4mm zone occur *P.mirabilis*, *E.coli* and no activity was found all other organisms. The respective solvent impregnated negative control discs showed no zone of inhibition against all the test bacterial pathogens.

3.2. Diethyl ether extract

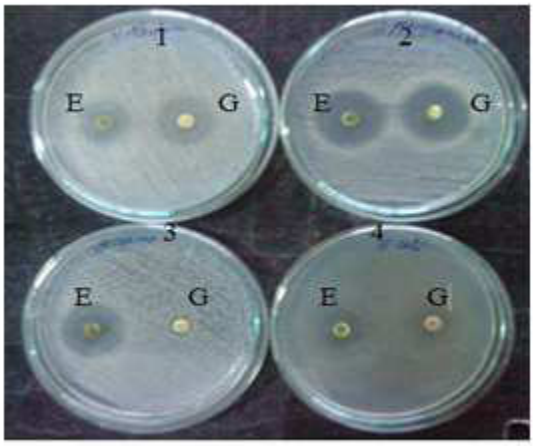
The effect of Diethyl ether extracts of *E.intestinal* fractions showed 5mm inhibition zone against *S.pneumoniae* and 2mm inhibition in *V.cholerae, B.cereus, S.epidermidis*, *K.pneumonia*, 1mm on *P.mirabilis* and *E.coli*. These extracts did not show any antibacterial activity against *S.typhi, S.paratyphi,*

pseudomonas sp. and *MRSA*. The *Gelidiella acerosa* fractions were effective by inhibiting 4mm in *Pseudomonas* sp., *MRSA*, 3mm in E.coli, 2mm in *P.mirabilis*, 1mm in *V.cholerae*, *S.epidermidis, S.pneumoniae* and no activity was found in all other organisms (Figure 2).

3.3. Acetone Extract

The effect of acetone extract of *E.intestinal* fractions showed 7mm inhibition zone against *B.cereus*, 3mm in *V.cholerae*, 2mm in *Pseudomonas sp*., *S.pneumonia,* 1mm in *P.mirabilis*. These extracts did not show any antibacterial activity against all other organisms. The *Gelidiella acerosa* fractions were effective by inhibiting 1mm inhibition zone against *P.mirabilis* and *V.cholerae,* and no activity was found in all other organisms (Figure 3).

Figure 1
Antibacterial activity of seaweed extracts (chloroform:methanol) against bacterial pathogen.



1-Vibrio cholera, 2-Streptococcus pneumonia, 3-Bacillus cereus, 4-Escherichia coli, E-Enteromorpha intestinalis, G-Gelidiella acerosa

Figure 2
Antibacterial activity of seaweed extracts (diethyl ether) against bacterial pathogen.

Staphylococcus epidermidis

Pseudomonas sp.

Figure 3
Antibacterial activity of seaweed extracts (acetone) against bacterial pathogen.

Bacillus cereus *Pseudomonas* sp.

Table 1
Antimicrobial activity of seaweed extracts against bacterial pathogens.

S.N	Bacterial pathogen	Methanol: chloroform		Diethyl ether		Acetone	
		Ei	Ga	Ei	Ga	Ei	Ga
1.	*Escherichia coli*	3	4	1	3	-	-
2.	*Bacillus cereus*	8	-	2	-	7	1
3.	*Proteus mirabilis*	6	4	1	2	1	-
4.	*Pseudomonas* sp.	2	-	-	4	2	-
5.	*Salmonella typhi*	-	-	-	-	-	-
6.	*Salmonella paratyphi*	-	-	-	-	-	1
7.	*Vibrio cholerae*	7	6	2	1	3	-
8.	*Staphylococcus epidermidis*	1	-	2	1	-	-
9.	*Streptococcus pneumoniae*	8	7	5	1	2	-
10.	*Shigella* sp.	-	-	-	-	-	-
11.	*Klebsiella pneumoniae*	1	-	2	-	-	-
12.	*MRSA*	-	-	-	4	-	-

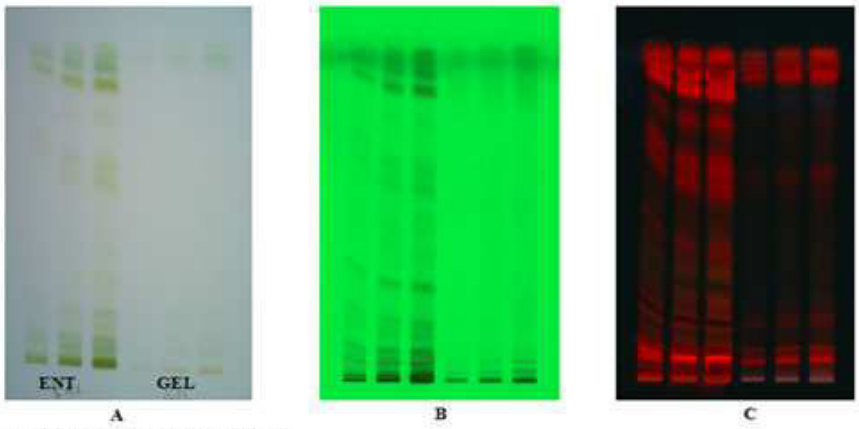
Zone of inhibition in mm, Ei- Enteromorpha intestinalis, Ga- Gelidiella acerosa

3.4. High Performance Thin Layer Chromatography (HPTLC)

HPTLC was found to be a powerful technique for the detection and potential quantization of drugs and compounds in clinical samples[13]. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks (Fig.4). The desired aim was achieved using chloroform:methanol (95:5 v/v) as the mobile phase. The wave length of 360 nm was found to be optimal for the highest sensitivity. Chloroform: methanol aqueous extract of *E.intestinalis* revealed 14 peaks at R_f -0.00, 0.02, 0.05, 0.07, 0.09, 0.16, 0.21, 0.33, 0.51, 0.69, 0.72, 0.86, 0.92, 0.96 and the respective areas (%) covered by the individual peaks are:2.79, 0.78, 7.41, 3.34, 9.47, 7.74, 12.90, 17.30, 11.72, 10.70, 7.18, 1.44, 4.53, 2.69 (Fig. 5). Chloroform: methanol aqueous extract of *G.acerosa* revealed 9 peaks at R_f 0.03, 0.07, 0.10, 0.15, 0.30, 0.46, 0.67, 0.86, 0.94 and the respective areas (%) covered by the individual peaks are: 19.02, 4.53, 0.94, 6.49, 2.30, 3.42, 10.55, 21.45, 31.30 (Fig.6).

Figure 4

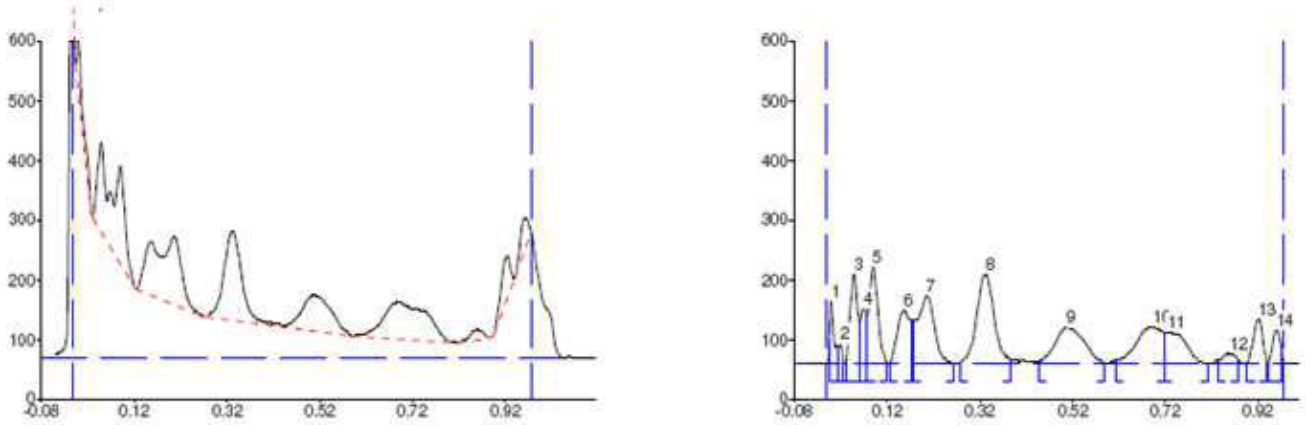
HPTLC Profiles of seaweed (Enteromorpha intestinalis and Gelidiella acerosa)



A = After derivation of under day light
B = Under UV 254nm
C = Under 366nm

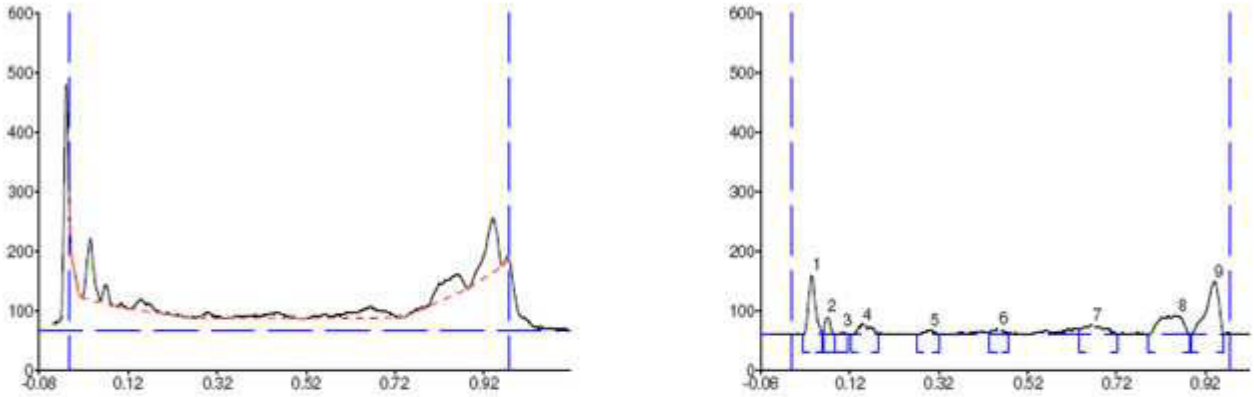
Figure 5

HPTLC Separation chromatogram of Enteromorpha intestinalis extracts.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	41.7	-0.00	104.9	8.61	0.01	28.5	706.3	2.79	unknown *
2	0.01	27.6	0.02	32.9	2.70	0.03	0.3	198.9	0.78	unknown *
3	0.03	3.8	0.05	150.2	12.31	0.06	68.8	1878.8	7.41	unknown *
4	0.06	71.2	0.07	93.2	7.64	0.07	79.9	847.5	3.34	unknown *
5	0.08	80.6	0.09	162.7	13.34	0.12	1.2	2400.2	9.47	unknown *
6	0.12	0.7	0.16	89.5	7.34	0.17	71.4	1961.4	7.74	unknown *
7	0.17	71.4	0.21	113.9	9.34	0.26	0.4	3271.6	12.90	unknown *
8	0.28	0.3	0.33	149.5	12.26	0.39	6.9	4387.6	17.30	unknown *
9	0.45	3.5	0.51	60.3	4.95	0.59	1.0	2972.8	11.72	unknown *
10	0.61	4.5	0.69	61.8	5.07	0.72	51.1	2712.8	10.70	unknown *
11	0.72	51.2	0.72	52.2	4.28	0.81	0.2	1821.7	7.18	unknown *
12	0.83	4.5	0.86	18.0	1.47	0.88	6.7	364.2	1.44	unknown *
13	0.90	1.4	0.92	74.4	6.10	0.94	1.3	1148.7	4.53	unknown *
14	0.94	0.7	0.96	56.0	4.59	0.97	29.8	683.2	2.69	unknown *

Figure 6
HPTLC seperation chromatogram of Gelidiella acerosa extracts.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.4	0.03	102.5	31.98	0.06	4.6	1223.5	19.02	unknown *
2	0.06	5.9	0.07	32.1	10.03	0.08	0.3	291.7	4.53	unknown *
3	0.09	0.2	0.10	7.3	2.29	0.11	1.6	60.2	0.94	unknown *
4	0.12	0.6	0.15	18.8	5.87	0.18	2.7	417.5	6.49	unknown *
5	0.27	0.2	0.30	8.3	2.60	0.32	1.0	148.1	2.30	unknown *
6	0.43	6.3	0.46	10.3	3.20	0.47	4.5	220.0	3.42	unknown *
7	0.63	9.3	0.67	16.7	5.20	0.72	0.3	678.5	10.55	unknown *
8	0.79	0.7	0.86	33.2	10.35	0.88	0.1	1379.6	21.45	unknown *
9	0.89	1.7	0.94	91.3	28.48	0.96	1.9	2013.0	31.30	unknown *

DISCUSSION

The present investigation showed that the solvent chloroform:methanol(1:1) was found to be very effective in extracting the antibacterial substance from the two seaweeds. Similar observation was made in earlier studies noted that chloroform:methanol is the best solution for extracting the effective antibacterial materials from the algal species[14][15]. It is clear that the use of organic solvents always provides a higher efficiency in extracting antimicrobial compounds. The effectiveness of extraction methods reported by many authors showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate [16][17]. There are few reports that chloroform is a better solvent than methanol and benzene [18]. The diethyl ether extract also showed good antimicrobial activity against few pathogens (figure: II). Thus several research workers used different solvent systems to extract antibiotic principle maximum and strongly feels that diethyl ether is the most suitable solvent to extract antibacterial substance from seaweeds [19]. According to our results, diethyl ether extracts of *E.intestinalis* showed high and low antimicrobial activities, respectively[20][21]. The other solvent acetone extract appeared to be ineffective in inhibiting various organisms. The chloroform:methanol extracts showed more activity compared to other solvent systems used. This could probably be because bio-active compounds could be soluble in chloroform and methanol [22]. As opined by many researchers, the diethylether solvent could also cause similar effect. But the acetone extracts showed very less antibacterial activity, probably suggesting that this solvent is not much suitable for extraction. From the present work, it could be concluded that the

chloroform:methanol is good solvent system to extract antibacterial substance from seaweeds. Presently, *S.typhi*, *S.paratyphi*, *Shigella sp*. And methicillin resistant S.aureus showed more resistant to all kinds of seaweed extracts and also 65-70% of gram negative bacteria susceptible to *E.intestinalis* extracts[23][24]. The brown, yellow, green color spots was detected in UV after derivatization derivetaization in the chromatogram, confirms the presence of poly phenols and various phytoconstituents. The seaweed *E.intestinalis* produce a large number of secondary metabolite, including terpens, aromatic compounds, acetogens, amino acid derivatives and polypehnolics, which might be responsible for the antibacterial activity of seaweeds[13]. Some antibacterial compounds identified from the seaweeds such as chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpens, sulphur containing heterocyclic compounds alginates, sterols, catehic tannin, phenolics compounds[25][26]. However, further characterization using mass spectra and NMR will be useful and warranted in the structural elucidation of the compounds in the extracts tested in the present study.

6. CONCLUSION

Finally we conclude from this study, the extracts of seaweed, *Enteromorpha intestinalis* showed maximum activity against *Proteus mirabilis*, *Streptococcus pneumonia, Vibrio cholera* and *Pseudomonas* sp. and the chloroform:methanol was found to be best solvent for the extraction of effective antibacterial materials. The HPTLC finger print of chloroform:methanol extracts of seaweeds showed many peaks, indicating different groups of phytochemicals, some of which might be responsible for antibacterial resistance. Further studies are necessary for the by isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases. From the present study, it can be concluded that the green alga *Enteromorpha intestinalis* is a potential source of bioactive compounds. These compounds can be utilized for the development of natural antibiotic against bacteria.

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