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Antimicrobial activity of seaweeds extracts against multiresistant pathogens

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Abstract - Fourteen seaweeds collected from the intertidal zone of Southwest coast of India were tested against ten human pathogen bacteria and one human pathogen fungus using the well diffusion test in the casitone agar medium. The species used in the present study include five Chlorophyta (*Bryopsis plumosa, Ulva fasciata, Acrosiphonia orientalis, Chaetomorpha antennina, Grateloupia filicina*), five Rhodophyta (*Hypnea pannosa, Gracilaria corticata, Centroceras clavulatum, Portieria hornemannii, Cheilosporum spectabile*) and four Phaeophyta (*Padina tetrastromatica, Sargassum wightii, Stocheospermum marginatum, Chnoospora bicanaliculata*). Of these, seven species were determined to be highly bioactive and screened on the multiresistant pathogens. We found that drying process has eliminated the active principles in the seaweeds. In the present study, methanol:toluene (3:1) was found to be the best solvent for extracting the antimicrobial principles from fresh algae. However, the ethanolic extract showed no antibacterial activity. *Acrosiphonia orientalis* showed activity against 70% of the tested organisms. *Stocheospermum marginatum* was the only seaweed that showed activity against *Klebsiella pneumoniae*. The extract from *Gracilaria corticata* was highly active against *Proteus mirabilis,* a Gram negative pathogenic bacterium. The present findings revealed that the tested seaweeds were highly active against Gram negative bacteria than Gram positive bacteria. The antimicrobial principle from seaweed was found to be a lipophilic compound. The compound was stable over a wide range of temperature (30-60 °C). The active principles of highly active seaweeds *Acrosiphonia orientalis* and *Stocheospermum marginatum* were bactericidal.

Key words: green algae, brown algae, red algae, multiresistant pathogens, antimicrobial activity.

INTRODUCTION

As a consequence of an increasing demand in screening for new therapeutic drugs from natural products, there is a greater interest towards marine organisms. Several marine organisms produce bioactive metabolites in response to ecological pressures such as competition for space, maintenance of unfouled surfaces, deterrence of predation and the ability to successfully reproduce (Konig *et al.,* 1994). Seaweeds provide a rich source of structurally diverse secondary metabolites. These secondary metabolites offers defence against herbivores, fouling organisms and pathogens; they also play a role in reproduction, protection from UV radiation and as allelopathic agents (Hay, 1996; Watson and Cruz-Rivera, 2003). Harder (1917) was the pioneer to observe the antimicrobial potentials of seaweeds. Many algal species have been shown to have bactericidal or bacteriostatic substances (Glombitza, 1979; Michanek, 1979; Fenical and Paul, 1984; Paul and Puglisi, 2004). The bactericidal agents found in algae include aminoacids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids (Watson and Cruz-Rivera, 2003). In the present study, we report the efficacy of brown, red and green seaweeds collected from the peninsular coast of India against multiresistant human pathogens.

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MATERIALS AND METHODS

Collection of seaweeds. Five species of green algae, four species of red algae, four species of brown algae (Table 1) were collected in different seasons (April 2007 to March 2008) during the lowest tide of chart datum from the seaweed infested locations along the southwest coast of India, Kollam (08º 54′ N & 76º 38′ E) and Varkala (08º 28′ N & 76º 55′ E) area. The study area, southwest coast comprising of numerous sandy beaches and irregularly distributed rocky substratum interspersed with sandy intertidal pools inhabited with a wide variety of marine algae. The algae which infested exclusively on the intertidal rocky and other substratum were selected for the collection as to avoid other algal contamination. The algae were collected using a metal scraper. Epiphytic and extraneous matter were removed by washing first in seawater and then in fresh water. The collected algae were transported to the laboratory in polythene bags under ice and voucher specimens were frozen at -20 °C for identification and future reference.

Identification of the seaweeds. The morphological and anatomical characteristics of all species were observed under a zoom stereo microscope (Motic). The sections were taken with

TABLE 1 - Various species of algae collected from southwest coast of India and their biomass

Order	Species	Wet weight (g)	Dry weight (g)
Chlorophyta	*Bryopsis plumosa*	-	-
	Ulva fasciata	3400	558
	Acrosiphonia orientalis	5600	1248
	Chaetomorpha antennina	3080	719
	Grateloupia filicina	-	-
Phaeophyta	*Padina tetrastromatica*	6000	780
	Sargassum wightii	-	-
	Stocheospermum marginatum	-	-
	Chnoospora bicanaliculata	4400	558
Rhodophyta	*Hypnea pannosa*	5800	528
	Gracilaria corticata	7500	1186
	Centroceras clavulatum	4500	751
	Portieria hornemannii	-	-
	Cheilosporum spectabile	3000	1670

razor blade and stained in safranin for light microscopic analysis (Optica). Based on the microscopic analysis, morphometric features and ecological distribution pattern, the collected specimens were identified taxonomically with the help of seaweed taxonomist Mr. M.V.N. Panikkar, Department of Botany, Sree Narayana College, Kollam. The peculiar characteristics taken into account include length and diameter of the rhizome, its internal structure, distribution of rhizome length, width of the assimilator, colour, diameter of the peltate head, 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, reproductive characters like spermatia, tetra sporangia and cystocarp.

Extraction of bioactives. The seaweeds belonging to the 3 taxa collected during different seasons were subjected for extraction of the bioactive compounds. Small pieces (1 g wet weight) of each fresh species were blotted to dry for 1 min, weighed and homogenised in a mortar and pestle with different solvents including ethanol, methanol:toluene (3:1), methanol, and phosphate buffered saline (PBS). The extraction with different solvents was carried out separately. The extracts were concentrated to solvent free by evaporation in a rotary vacuum evaporator (Yamato). The residues obtained were finally dried in a vacuum desiccator and dissolved in the respective solvents. For extraction of bioactives in shade-dried seaweeds, 500 g of finely powdered algal material was refluxed three times in a 5 litre capacity round bottom flask in a water bath at 65 °C for about 6 h using dichloromethane:methanol (1:1) as a binary azeotropic solvent (Selvin and Lipton, 2004) and the above used solvents. The extracts were filtered and concentrated to recover the excess solvents in another distillation system. The concentrated extract (about 100 ml) was again filtered through a Whatman no. 1 filter paper fitted with a Buchner funnel using suction pressure. Finally, it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (Yamoto) at 40 °C, collected in air-tight plastic vials and stored in the refrigerator for further activity studies. The aliquots were tested for their antimicrobial activity on eleven clinical isolates.

Assay microorganisms. All extracts were tested against a panel of clinical isolates including one yeast *Candida albicans* FC1 (secondary pathogen of HIV infection) and ten bacteria (non-haemolytic *Streptococcus* PC1*, Enterococcus faecalis* PC2*, Staphylococcus epidermidis* PC3*, Escherichia coli* PC4*, Micrococcus luteus* PC5, *Bacillus subtilis* PC6, *Pseudomonas aeruginosa* PC7, *Klebsiella pneumoniae* PC8, *Proteus mirabilis* PC9*, Staphylococcus aureus* PC10) obtained from clinical laboratories. These isolates were established as multiresistant pathogens and deposited in Biomedical Diagnostic Laboratory, Bharathidasan University. The resistance patterns of these isolates were confirmed using selective antibiotics. Preliminary experiments confirmed that the isolates were resistance against chloramphenicol, streptomycin, oxytetracycline, ampicillin and erythromycin. The fungus *Candida albicans* (ATCC 90027) was resistant against ceftriaxone, vancomycin and amphotericin. All the bacterial strains were maintained on Nutrient agar at 4 °C. The *C. albicans* strains used in the study were maintained on Sabouraud Dextrose agar slants at 4 °C

Antimicrobial assay. The antibacterial assay was carried out as per Selvin and Lipton (2004). Briefly, the base layer was prepared with 10 ml (1.5%, w/v) of Muller Hinton agar (Himedia). Five numbers of sterile porcelain beads were placed on the base layer at 60° (angle) apart. The overlaid seed layer was prepared by pouring 15 ml of media containing 0.2 ml of prepared inoculum (~ 0.2 OD at 630 nm). The porcelain beads were removed carefully with sterile forceps. The resultant wells in triplicate were filled with 120 µl of the appropriate algal extract. The well with solvent used for dissolution was taken as negative control. The remaining well was filled with reference drugs (nystatin 30 µl/ml and nalidixic acid, 50 µl/ml). After 24 h of incubation at 37 °C, the diameter of inhibition around the wells was determined as average of triplicates. The aromatogram of pathogen was analysed for skewness using SPSS software.

Anticandidal activity of seaweeds against pathogenic yeast *C. albicans* (ATCC 90027), were determined preliminarily by cross streaking method, then potential strains were identified and their anticandidal activity was determined using modified agar well method (Egorov, 1957). Sabouraud Dextrose agar was used for bioactivity screening and routine propagation of *C. albicans*. For plate assay, a yeast suspension of 10^6 CFU/ml in sterile phosphate buffered saline was prepared (Forbes *et al.,* 1990). Appropriate seaweed extract was diluted in 1% dimethyl sulfoxide:methanol (1:1) and filled up to the brim of each well. Parallel reference standard (clotimazole 1% topical solution) and negative control (solvent used for dissolution) were used to validate the inferences. The plates were incubated at 30 °C for 48 h. After incubation, the bioactivity was determined by measuring the diameter of inhibition zone.

Identification of the active principles. Highly active seaweed (methanol:toluene) extracts were fractionated using thin layer chromatography (TLC). The chromatogram was developed using different solvent systems including 96% ethanol:water, 7:3 (for aminoacids), chloroform:aceticacid:water, 60:70:10 (for carbohy-

drates) and hexane:diethylether:acetic acid, 80:20:1 (for lipids). The fractions obtained were subjected for antimicrobial assay. To study the chemical behaviour of the bioactive principles, the TLC fractions were incubated at different temperatures ranging from 30-80 °C for 30 min. The residual antimicrobial activity of the fractions were determined against one Gram positive (non-haemolytic *Streptococcus*) and one Gram negative microorganism (*Pseudomonas aeruginosa*) as determined earlier.

Determination of mechanisms of antibiosis (bacteriostatic or bactericidal). The minimal inhibitory concentration (MIC) was determined by the broth dilution method. The multidrug resistant *Streptococcus* PC1 and *Pseudomonas aeruginosa* PC7 were used for the determination of MIC. The 96-well microtiter plates were filled with 0.1 ml TLC fractions prepared in Mueller-Hinton broth (MHB). The microtiter plates were incubated in a 10% CO_2 atmosphere at 37 °C for 48 h. In every microtiter plate, one row was set for control (without TLC fractions) and standard (nystatin and nalidixic acid). After incubation, the OD was read at 610 nm (Spectronic 20 UV-Vis spectrophotometer). MICs were recorded as the lowest concentrations inhibiting visible growth.

To measure the minimal bactericidal concentrations (MBC), the MIC cultures were plated on Mueller-Hinton agar with 5% lysed horse blood and incubated them for 24 h at 37 °C in a 10% CO_2 atmosphere. A reduction of at least 90% of the colonies, compared with the culture of the initial inoculum of the strain, was regarded as evidence of bactericidal activity. When the ratio of MBC/MIC is ≤ 2, the active fractions were considered as bactericidal otherwise as bacteriostatic. If the ratio is ≥ 16 the fractions were considered as ineffective.

RESULTS AND DISCUSSION

The main objective of the work was to evaluate and compare the ability of different macroalgal species from southwest coast of India to produce bioactive compounds of potential therapeutic interests. The production of antimicrobial activities was considered to be an effective indicator of the capability of the seaweeds to synthesize bioactive secondary metabolites.

Identification and seasonal variability of seaweeds

Based on the anatomical and morphological characteristics, the seaweeds collected were identified and the results are presented in Table 1. The species of division chlorophyta are collected during post monsoon season (April 2007 to March 2008) and are distributed in the rocky outcrops of intertidal zone. Among the five members of chlorophyta, *Ulva fasciata*, *Acrosiphonia orientalis*, and *Chaetomorpha antennina* were dominated in southwest coast. While the samples of phaeophyta were distributed widely in subtidal zone and their abundance was observed only in November. The red algae were observed in different habitats of upper intertidal, intertidal and subtidal zones during summer (April and May). Extensive bed of *Gracilaria corticata* was found throughout the year. A sporadic distribution of *Centroceras clavulatum* was also observed for all the seasons. Data related to the wet and dry weight of seaweeds are presented in Table 1.

Activity of seaweeds extracts

Dried seaweeds extracts have less or no effects on bacteria in comparison to the fresh seaweeds extracts (data not shown). In the present study, we found that the drying process has eliminated the active principles, since enhanced activity was observed with the extracts from fresh seaweeds compared to the shade dried ones. As observed in the present study, the organic extract of fresh seaweeds from the Turkey coast showed highest antibacterial activity compared to the dried extracts (Tuney *et al.,* 2006). This result can be related to the presence of volatile antimicrobial components in the samples, such as hydrogen peroxide, terpenoids, volatile fatty acids and bromoether compounds (Rosell and Srivastava, 1987; Masuda *et al.,* 1997).

Among the solvent system used in the present study, methanol:toluene (3:1) was the best solvent for extracting the antimicrobial principles from fresh algae. The ethanolic extracts had no antibacterial activity. These findings evidenced that the extraction method had definite effect on the isolation of bioactive principles. For instance, the methanol:toluene extracts of the seaweeds were ineffective against *C. albicans*. Whereas the PBS extract of the algae showed bioactivity against *C. albicans*. This implies that the anticandidal bioactive metabolite in the algae might be readily soluble in PBS rather sparingly soluble in the organic solvents. Earlier reports on the effectiveness of extraction methods evidenced that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate (Rosell and Srivatsava, 1987; Sastry and Rao, 1994; Paul and Puglisi, 2004). Whereas others reported that chloroform is a better solvent than methanol and benzene (Fables *et al.,* 1995). It is clear that using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based methods (Masuda *et al.,* 1997; Lima-Filho *et al.,* 2002).

Extracts of 14 species of seaweed were tested against multiresistant pathogen bacteria and *C. albicans* (ATCC 90027). Particularly the highly active fresh seaweed extracts were assayed against the panel of pathogenic microbes used in our study. The results of primary screening revealed that 7 species of algae including *Acrosiphonia orientalis, Ulva fasciata, Padina tetrastromatica, Sargassum wightii, Stocheospermum marginatum, Hypnea pannosa, Gracilaria corticata* possessed considerable antibacterial activity. Based on the preliminary findings, the methanol:toluene and PBS extracts of 7 selected algal species were used for comprehensive antimicrobial screening.

Antibacterial activity of the tested seaweeds

Of the 7 highly active algal species analysed, 100% showed antimicrobial activity against at least one tested organism. Particularly one phaeophyceae (*Stocheospermum marginatum)* showed activity against all the test organisms except *E. coli*. In the

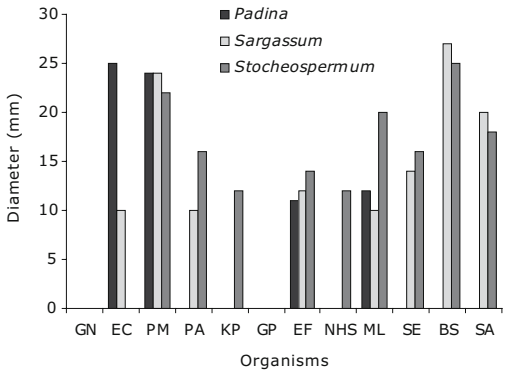

FIG. 1 - Antibacterial potential of Phaeophyta.
GN - Gram negative group: EC - *Escherichia coli,* PM - *Proteus mirabilis,* PA - *Pseudomonas aeruginosa,* KP - *Klebsiella pneumoniae.*
GP - Gram positive group: EF - *Enterococcus faecalis,* NHS – non-haemolytic *Streptococcus,* ML - *Micrococcus luteus,* SE - *Streptococcus epidermidis,* BS - *Bacillus subtilis,* SA - *Staphylococcus aureus.*

phaeophyceae group (Fig. 1), *Sargassum wightii* also recorded highest antimicrobial activity against 8 test microorganisms. Among the tested seaweed taxa, 86% showed activity against *M. luteus* followed by *E. faecalis* (71%)*, S. epidermidis* (71%), *E. coli* (71%), *P. aeruginosa* (71%), *P. mirabilis* (71%), *Streptococcus* sp. (57%)*, S. aureus* (43%) *B. subtilis* (29%) and *K. pneumoniae* (14%). Higher frequency of activity against Gram positive bacteria have been observed in most of the surveys of antimicrobial activities from seaweeds reported in the literature (Ballatine *et al.,* 1987; Gerwick *et al.,* 1985). However in the present study the antibiogram showed a positive skewness towards Gram negative bacteria. It is known fact that Gram negative bacteria are the most prominent pathogens compared to the Gram positive bacteria. Therefore the present finding brings out a new insight towards the development of antimicrobials against Gram negative bacteria from seaweed based natural products.

The brown algae (phaeophyceae) comprised 42% of species surveyed in this study. Phaeophyceae had the highest percentage of active species. Invariably, all the brown algae tested were active against *P. mirabilis, M. luteus, E. coli ,* and *E. faecalis* (Fig. 1). However, only 67% of tested brown algae showed activity against *S. aureus, P. aeruginosa, S. epidermidis* and *B. subtilis.* Antimicrobial activities were highly prevalent among the extracts from species belonging to the families of Sargassaceae and Dictyotaceae. The extract of *Stocheospermum marginatum* showed significant activity against multiresistant *K. pneumoniae.* In addition, the extract from *S. wightii* and *Stocheospermum marginatum* showed antibacterial activity against Gram negative bacteria. In a previous report (Gonzalez del val *et al.,* 2001), methanol extracts of *Padina pavonica* showed antibacterial activity against only *Bacillus.* The narrow spectrum antimicrobial activity of *P. pavonica* was confirmed in the later studies as the diethyl ether extracts of *P. pavonica* showed weak activity against *Candida, P. aeruginosa, E. coli,* and *E. faecalis* (Tuney *et al.,* 2006). The results of the present study showed that methanol:toulene extract of *Padina* was effective against *E. coli, P. mirabilis, M. luteus* and *E. faecalis.* Previous studies have demonstrated that brown algae produce a wide variety of isoprenoid metabolites as defences against herbivory a well as to prevent antifouling (Paul and Puglisi, 2004). From the evolutionary perspective, it would be important to determine if the same molecules that daunt herbivory and/or prevent biofouling also function as antimicrobial chemical defences.

Green algae comprised 29% of total species assessed in this study. Among the green algae, antimicrobial activity was prevalent in the extract from *Acrosiphonia orientalis* (Fig. 2). The significant result obtained in the present study is that the methanol:toluene extract of *A. orientalis* was effective against 70% of the test organisms (both Gram positive and Gram negative bacteria). The extract from *A. orientalis* was found to be active against 67% of the Gram positive bacteria. The extract showed no inhibition against *B. subtilis*. The methanol:toluene extract of *A. orientalis* was also found to be inhibitory for all the tested Gram negative organisms except *K. pneumoniae.* Unto our knowledge, this is the first report that demonstrated the antimicrobial activity of *A. orientalis*. In the Chlorophyceae, *U. fasciata* showed a narrow spectrum of activity against Gram positive bacteria. However in the previous report the extract of *U. fasciata* had no antimicrobial activity (Perez *et al.,* 1990). In contrast, in the present study, the methanol:toulene extract of *U. fasciata* inhibited 40% of the tested bacteria. Awad (2000) isolated 3-O-β-D glucopyranosyl stigmasta-5, 25-dien, a steroid from *U. fasciata* which had both anti-inflammatory and antimicrobial activity.

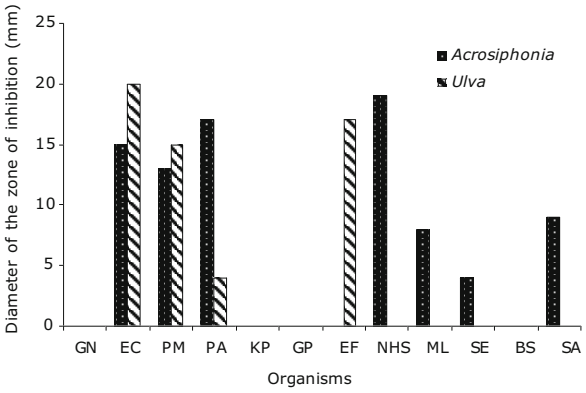

FIG. 2 - Antibacterial potential of Chlorophyta.

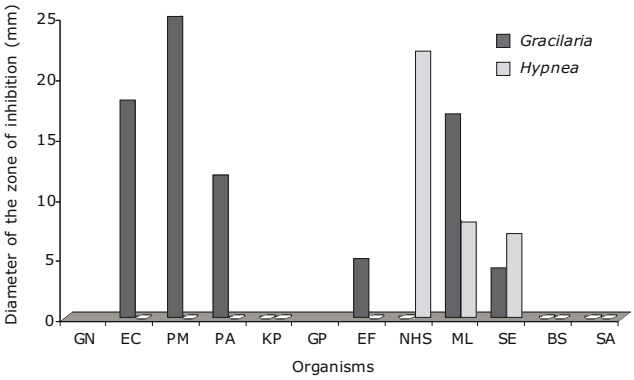

FIG. 3 - Antibacterial potential of Rhodophyta.

Red algae comprised 36% of all the species surveyed in this study. The extracts from *G. corticata* were found to be more effective against all Gram negative bacteria used in the present study except *K. pneumoniae*. It was also effective against two Gram positive bacteria (*M. luteus,* and *S. epidermidis* and *E. faecalis*). In contrast, the extract of *Hypnea* was active against Gram positive bacteria than Gram negative bacteria (Fig. 3). Lima Filho *et al.* (2002) found that the hexane extract of *G. corticata* species inhibited only *Bacillus subtilis.* In contrast, our results showed that methanol:toulene extract of *G. corticata* contained broad spectrum of antibacterial agents against *P. mirabilis, E. coli, P. aeruginosa, M. luteus, S. epidermidis,* and *E. faecalis*.

Anticandidal activity of the tested seaweeds

The PBS extract from all species of Chlorophyta, Rhodophyta and Phaeophyta taxa were active against the yeast *Candida albicans*

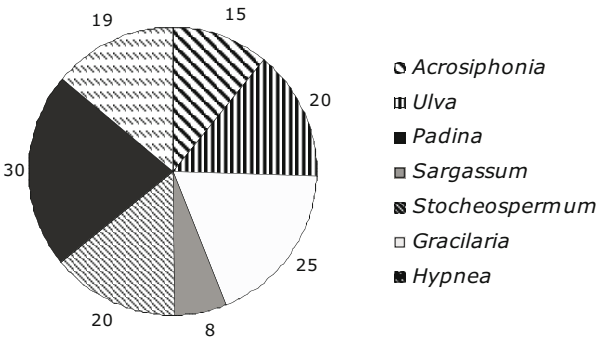

FIG. 4 - Anticandidal activity of seaweeds. Values are expressed in terms of zone of inhibition (mm).

TABLE 2 - Biomass availability versus activity of *Acrosiphonia orientalis*

Species	Bimonths	Wet wt(g)	Dry wt(g)	Activity
Acrosiphonia orientalis	Apr-May	5000	200 ± 18	+
	Jun-Jul	5000	420 ± 10	+++
	Aug-Sep	5000	466 ± 12	+++
	Oct-Nov	5000	402 ± 16	++
	Dec-Jan	5000	380 ± 13	+
	Feb-Mar	5000	366 ± 10	+
Stocheospermum marginatum	Oct-Nov	6000	630 ± 15	++
	Dec-Jan	6000	550 ± 13	+

(ATCC 90027) (Fig. 4). The PBS extract of one Phaeophyceae (*Padina*) and one Rhodophyceae (*Gracilaria*) showed significant inhibition zone of greater than 20 mm diameter.

Seasonal variation on the most active seaweeds *Acrosiphonia orientalis* and *Stocheospermum marginatum*

Two seaweeds, one belonging to chlorophyta (*A. orientalis)* and other belonging to phaeophyta (*S. marginatum)* were found to be more active against the panel of organisms used and therefore selected for further studies. The relation between the wet, dry weight and antimicrobial activity of *A. orientalis* and *S. marginatum* are shown in Table 2.

Acrosiphonia orientalis was found to be abundant throughout the year. The antimicrobial activity was found to be maximum during June-September when this alga flourished abundantly along the coast. The antimicrobial activity of *A. orientalis* and *S. marginatum* showed a positive correlation with biomass abundance (dry wt). With increasing biomass, the antimicrobial activity increased and reached a steady state when the biomass was constant.

Compound identification

The TLC fractionation based on amino acids did not yield any fractions. The fractionation based carbohydrates from *A. orientalis* and *S. marginatum* yielded three and four fractions respectively (Fig. 5). But none of the fractions contained antimicrobial activity. The results suggested that the bioactive principle is a compound other than protein and carbohydrates.

The fractionation based on lipids yielded 3 fractions from *A. orientalis* (Fig. 6). TLC fraction 1 of methanol:toluene extract of *A. orientalis* inhibited the growth of 70% of the organisms tested. In the case of *S. marginatum*, totally 4 fractions were obtained (Fig. 6). The TLC fraction 3 and 4 showed clear zones around all the tested microorganisms after 24 h of incubation. Our preliminary results suggested that the antimicrobial activity observed in the methanol:toluene extracts of the seaweeds could be due to a lipophilic compounds. This hypothesis needs to be investigated further by GC-MS, NMR and chemical elucidation.

The results obtained in the present study suggested that lipid soluble extracts from marine macroalgae as a source of substances with pharmacological properties. Olessen *et al.* (1964) related antibacterial activity in the chloroform and acetone extracts of *Falkenbergia hillebrandii* against *S. aureus*. Sastry *et al.* (1994) reported antibacterial activity against Gram positive and Gram negative pathogenic strains after successive extraction with benzene, chloroform and methanol. The chemical nature of active principles in lipophilic compounds was scarcely reported. Rossel and Srivasta (1987) demonstrated the antibiotic activity from 10 Xantophyta was due to the presence of unsaturated fatty acids, organic acids and phenol compounds.

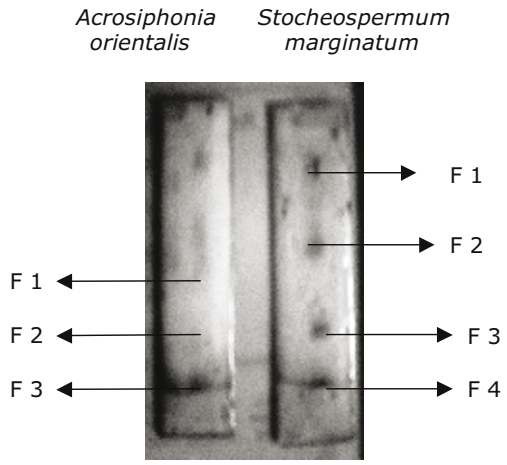

FIG. 5 - TLC for carbohydrates.

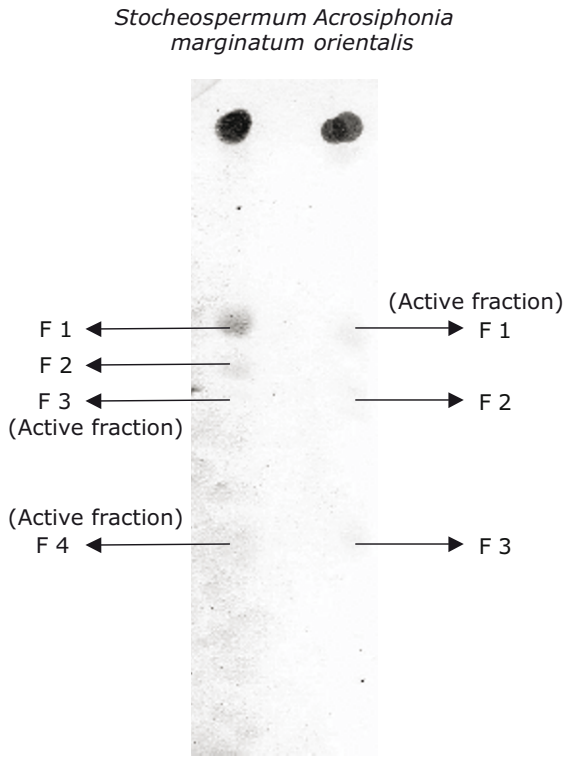

FIG. 6 - TLC for lipids.

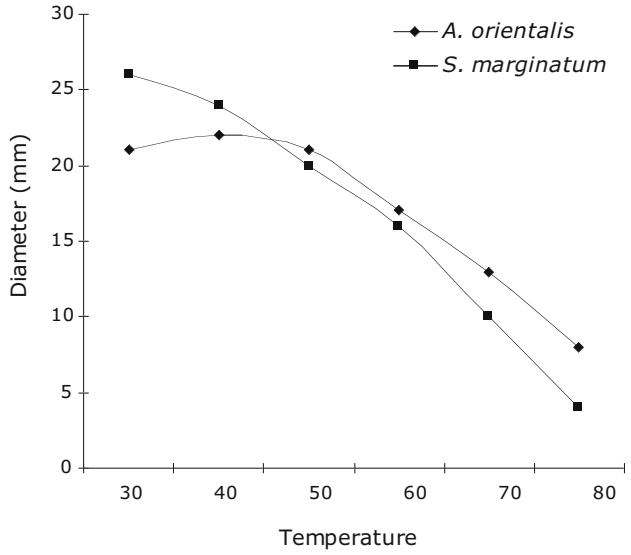

FIG. 7 - Effect of temperature on the antimicrobial activity of the active principles against *Pseudomonas aeruginosa*

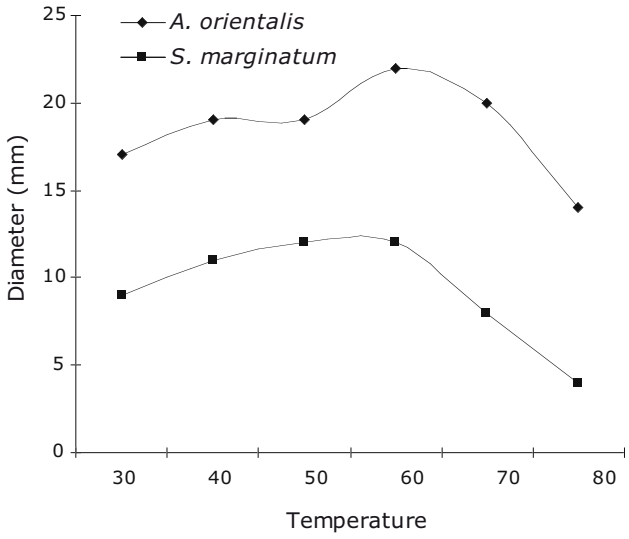

FIG. 8 - Effect of temperature on the antimicrobial activity of the active principles against non-haemolytic *Streptococcus.*

TABLE 3 - MIC and MBC values of the active principles against two organisms

Organisms	Seaweeds	MIC	MBC	MBC/MIC
Pseudomonas aeruginosa	*A. orientalis*	50	10	0.02
	S. marginatum	10	0.5	0.05
Non hemolytic *Streptococcus*	*A. orientalis*	5	5	1
	S. marginatum	100	10	0.01

After incubation of the TLC fractions of *A. orientalis* and *S. marginatum* at different temperatures, the antimicrobial assay was performed against *Pseudomonas aeruginosa* and non-haemolytic *Streptococcus*. The fractions obtained were found to be active over a broad range of temperature (30-60 °C). Beyond 60 °C, there was a steady decrease in the antimicrobial activity. This envisaged that the active principles were thermo-tolerant rather than thermo-stable. The effect of temperature on the stability of antimicrobial fractions against *Pseudomonas aeruginosa* and non-haemolytic *Streptococcus* are depicted in Fig. 7 and 8 respectively.

Mechanism of antibiosis

The MBC/MIC ratio was determined to identify whether the active principle was a bactericidal or a bacteriostatic compound. The results of MIC and MBC of the active principles from *A. orientalis*, *S. marginatum* are presented in Table 3. Since the MBC/MIC ratio is less than 1 the active principles can be considered to be a bactericidal agent.

CONCLUSION

The remarkable difference between our results and the results obtained in previous studies may be due to several factors. The main reason can be due to the difference in the seasonal variation of the seaweeds which has been well established from our results. Another important reason can be due to difference in the extraction procedure to recover the active metabolites and differences in assay methods that would result in different susceptibilities of the target strains. The prevalence of antimicrobial activities observed in the extracts from the southwest coast of India provides credible evidence that algae maintain effective antimicrobial chemical defences. From the present study, it can be concluded that the macroalgae are potential sources of bioactive compounds. Further studies are necessary to identify the chemical structure of the active lipophilic compound.

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REFERENCES

Awad N.E. (2000). Biologically active steroid from the green algae *Ulva lactica*. Phytother. Res., 14: 641-643.

Ballatine D.I., Gerwick W.H., Velez S.M., Alexander E., Guevara P. (1987). Antibiotic activity of lipid soluble extracts from Caribbean marine algae. Hydrobiologia, 151: 463-469.

Campos-Takaki G.M., Diu M.B.S., Koening M.l., Pereira E.C. (1988). Screening of marine algae from Brazilian northeastern coast for antimicrobial activity. Bot. Mar., 31: 375-377.

Egorov N.S. (1957). Determination of antibiotic activity of microorganisms by the agar block method. Antibioki, 2: 50-53.

Fables C.I., Arias A., Gill-Rodriguez M.C. (1995). *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta, Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuario del Estudios Canneries, 34: 181-192.

Fenical W., Paul V.S. (1984). Antimicrobial and cytotoxic terpenoids from tropical green algae of the family Udoteaceae. In: Bird C.J., Ragan M.A., Eds, 11th International Seaweed Symposium, Dr. W. Junk Publishers, Dordrccht, Boston, Lancaster, pp. 135-140.

Forbes B.A., Sahm D.F., Weissfeld A.S., Trevino E.A. (1990). Methods for testing antimicrobial effectiveness. In: Baron E.J. *et al.*, Eds, Bailey and Scott' Diagnostic Microbiology, Mosby Co, St Louis, Missouri, pp. 171-194.

Gerwick W.H., Fenical W., Norris J.N. (1985). Chemical variation in the tropical seaweed *Stypopodium zonale* (Dictyotaceae). Phytochemistry, 24: 1279-1283.

Glombitza K.W. (1979). Antibiotics from algae. In: Hoppe I.L.A., Ed., Marine Algae in Pharmaceutical Science, Waiter de Gruyter, Berlin, New York, 303f.

Gonzalez del Val A., Platas G., Basillio A. (2001). Screening of antimicrobial activities in red, green, and brown algae from Gran Canaria (Canary Islands, Spain). Int. Microbiol., 4: 35-40.

Harder R. (1917). Ernahrungphysiologische untersuchughen an Cyanophyceen, Hauptsachlich am endophytischen *Nostoc punctiformie*. Z. Bot., 9: 145.

Hay M.E. (1996). Marine chemical ecology. What's known and what's next? J. Exp. Mar. Biol. Ecol., 200: 103-134.

Konig G.M., Wright A.D., Stiche O., Angerhofer C.K., Pezzuto J.M. (1994). Biological activities of selected marine natural products. Planta Med., 60: 532-537.

Lima-Filho J.V.M., Carvalho A.F.F.U., Freitas S.M. (2002). Antibacterial activity of extracts of six macroalgae from the Northeastern Brazillian coast. Brazilian J. Microbiol., 33: 311-333.

Masuda M., Abe T., Sato S. (1997). Diversity of halogenated secondary metabolites in the red algae *Laurencia nipponica* (Rhodomelaceae Ceramiales). J. Phycol., 33: 196-208.

Michanek G. (1979). Seaweed resources for pharmaceutical uses. In: Hoppe I.L.A., Ed., Marine Algae in Pharmaceutical Science, Waiter de Gruyter, Berlin, New York, pp. 203-235.

Olessen P.E., Maretzki A., Almodovar L.A. (1963). An investigation of antimicrobial substances from marine algae. Bot. Mar., 6: 226-232.

Paul V.J., Puglisi M.P. (2004). Chemical mediation of interactions among marine organisms. Nat. Prod. Rep., 21: 189-209.

Perez R.M., Avila J.G., Perez G. (1990). Antimicrobial activity of some American algae. J. Ethnopharmacol., 29: 111-118.

Rosell K.G., Srivastava L.M. (1987). Fattyacids as antimicrobial substances in brown algae. Hydrobiologia, 151: 471-475.

Sastry V.M.V.S., Rao G.R.K. (1994). Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. Bot. Mar., 37: 357-360.

Selvin J. (2002) Shrimp disease management using secondary metabolites isolated from marine organisms. Ph.D dissertation, Manonmaniam Sundaranar University, Tirunelveli, India.

Selvin J., Lipton A.P. (2004). Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. J. Mar. Sci. Technol., 12: 1-6.

Tuney I, Cadirci BH, Unal D, Sukatar A. (2006). Antimicrobial activities of the extracts of marine algae from the coast of Urla (Imzir, Turkey). Turk. J. Biol., 30: 171-175.

Watson S.B., Cruz-Rivera E. (2003). Algal chemical ecology: an introduction to the special issue. Phycologia, 42: 319-323.