

Screening marine organisms for antimicrobial activity against clinical pathogens

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Bacterial resistance to existing antibiotics has led to the search for new therapeutic agents. With this aim, we have evaluated antimicrobial activity of extracts prepared from forty species of marine organisms belonging to different phyla and collected from Mandapam, south-east India, (9°16' N; 79°12'E) and Kanyakumari coasts, south India, 8°4' N; 77°34'E. The extracts were screened against clinical isolates of bacteria including multi-drug resistant (MDR) strains and fungi. The sponge, *Ectyobatzella enigmatica*-extract was quite promising as bactericidal agent especially against MDR *Streptococcus pyogenes* and except for its ineffectiveness against *Pseudomonas aeruginosa* and *Klebsiella* sp. it was as effective as standard streptomycin against each of the bacteria tested. Besides *E. enigmatica*, *Spongia officinalis* and the echinoderm *Pentaceraster affinis* extracts also exhibited good activity against MDR strains of *S. pyogenes* and *Acinetobacter* sp. An unidentified ascidian extract effectively inhibited the growth of *Shigella flexneri* and the soft corals, *Sinularia* sp.(iii) and *Sinularia kavarattiensis* were weakly active against some of the bacteria tested. Both the sea grasses (*Halodule* sp. and *Halophila ovalis*) were antifungal but not anti-bactericidal. Follow up studies showed that except *Ectyobatzella enigmatica* where the activity was distributed in all the fractions, the activity of the other extracts were concentrated mainly in the low polar hexane and chloroform fractions. Among the 7 fungal pathogens used in the study, *Fusarium* sp. was sensitive at varying degrees to 12 marine extracts while 17 extracts were weakly fungicidal against *Nocardia* sp. The extracts were practically inactive towards the remaining fungal strains. The standard antibiotic (ketoconazole) used was moderately effective only against *Cryptococcus neoformans* and *Aspergillus niger*.

[**Keywords:** Sponges, Soft corals, Sea grass, Ascidian, Starfish, Antibacterial activity, Antifungal activity].

Introduction

The marine environment is an exceptional reservoir of a wide variety of organisms which are prolific producers of novel and bioactive secondary metabolites with diverse chemical structures. This diversity of chemical compounds is believed to be a consequence of competition between organisms for space and resource in most marine habitats. Due to antimicrobial actions some of the biologically active natural products have become excellent sources of new and effective drugs such as antifungal, anti-inflammatory and anti-viral agents¹. Because of increasing resistance to antibiotics by several pathogens, marine extracts are of new interest as antimicrobial agents to combat infectious diseases.

Marine flora and fauna collected from the Indian coast have particularly proved to be very productive as far as secondary metabolites are concerned. This is further confirmed by the continuous flow of papers on

screening marine extracts with particular reference to samples from Indian coasts²⁻⁸. Thus, bromotyrosine alkaloids from Indian marine sponge *Psammaphysilla purpurea* showed antimicrobial activity⁶; jorumycin, a dimeric isoquinoline alkaloid isolated from the Indo-Pacific nudibranch, *Joruna funebris* collected from Mandapam coast of India, inhibited the growth of various Gram-positive bacteria and is anti-tumor against various human cell lines⁹, cyclic depsipeptides, kahalalides, from *Elysia grandifolia* and its algal diet *Bryopsis plumose* from Mandapam are known to be antimalarial, antituberculosis, antiviral and, primarily antitumor agents¹⁰ and several others.

In this study, methanolic extracts of forty marine organisms belonging to different phyla and fractions of active extracts were screened for their antimicrobial effects on human pathogens. A broad panel of microbial pathogens associated with various skin infections, urinary tract, gastrointestinal infections and waterborne diseases, has been included. The aim of the present study was to identify potential marine sources based on antimicrobial assay.

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Materials and methods

Sample collection and identification

Marine organisms were collected by SCUBA diving at depths ranging from 5-15m, at two different locations, Mandapam coast, south-east India (9°16'N; 79°12'E) and Kanyakumari coast, south India (8°4' N; 77°32' E) during the April-May months of 2004 (Table 1). Seagrasses and seaweeds were handpicked during low tide. Collected samples were frozen and transported to the laboratory for extraction. All the samples are deposited at the NIO Taxonomic Reference Center, Goa, India.

Extract preparation and fractionation

Marine organisms were extracted as described earlier¹¹. Briefly, marine organisms were homogenized in a blender and extracted in methanol (3×500 mL) at room temperature and then filtered through GF/C filter paper (Whatman 1). The solvent from combined filtrates was evaporated at reduced pressure and temperatures and the residue obtained i.e. the crude methanolic extract of different organisms was used for primary screening. Crude extracts showing significant activity were further fractionated for secondary screening. For the purpose, methanol extract was dissolved in 5% aqueous methanol and then partitioned successively with hexane, chloroform, ethyl acetate and n-butanol. Organic layer was separated and concentrated under *vacuum* to obtain respective fractions.

Microorganisms used for screening

Details of the pathogenic strains used for screening marine extracts for antibiotic activity are listed in Table 2. All the test strains were clinical isolates collected from sick patients and were generous contribution from the Department of Medical Microbiology of Goa Medical College (GMC) and Hospital.

Antimicrobial assay

Antibacterial activity of different extracts was assessed using standard disc-diffusion assay on marine agar as described earlier⁵. Extracts and fractions, each, were dissolved in the respective solvents and loaded onto sterile paper discs (Whatman GF/C), to yield a final concentration of 300 µg/disc. Each test organism of known concentration (~1.2×10⁸ CFU/ml) was inoculated on nutrient agar plates (HiMedia) and spread to form a lawn of individual bacterium. Crude Extract and fraction loaded discs as

well as positive and negative control discs for comparison were also placed on the plates. Positive control discs comprised of streptomycin (10 µg/disc) while negative control discs were discs with the respective solvents. Plates were incubated at 37°C for 17-24 h until bacteria developed a confluent film. Observed zones of growth inhibition around each disc were measured in millimeters.

Antifungal assay

This activity was tested against fungal pathogens using the disc diffusion assay method as described for antibacterial assay. The sterile paper discs impregnated with 500 µg/disc of extract was used for antifungal assay. The inoculum concentration was 0.5×10³ – 2.8×10³ CFU/mL. Ketoconazole was used as positive control (100 µg/disc). The plates were incubated for 48 h and the zone of growth inhibition around each disc was measured in millimeters.

Both the antibacterial and antifungal assays were repeated twice and each assay was performed in duplicate.

Results and Discussion

Marine samples collected from, both, Mandapam and Kanyakumari are listed in Table 1. These areas are particularly known to harvest wide range of organisms capable of showing biological activity. Initially, methanolic extracts of these organisms were subjected to primary screening against human pathogens. The pathogens which include bacteria, fungi and multidrug resistant strains (MDR) are listed in Table 2.

The result of primary screening for antibiotic activity as well as comparison with the respective standards (streptomycin/ketoconazole) which measures diameter of growth inhibition is summarized in Table 3. As evident from Table 3, three of the samples studied appeared to be quite promising (two sponges; *Ectyobatzella enigmatica* and *Spongia officinalis*, and an echinoderm *Pentacaster affinis*) for their ability to inhibit the growth of Multi Drug Resistant (MDR) strains as well as their broad spectrum activity against other selected pathogenic bacteria. The activity of *Ectyobatzella enigmatica* (Fig. 1a) was comparable with that of standard antibiotic streptomycin. The MDR *Streptococcus pyogenes* was insensitive to streptomycin but strongly sensitive to the extract of *E. enigmatica* with an inhibition zone of 17 mm against both *Shigella flexineri* and multi-drug resistant

Streptococcus pyogenes. It also showed good activity against *E. coli*, *Vibrio cholerae*, and moderate activity against *Staphylococcus aureus* and *Salmonella typhi* (i). *E. enigmatica*, however, was insensitive to all the

fungal strains tested in the present study. Hence it did not exhibit any antifungal characteristics.

Samples showing significant activity in the primary screening are shown in Fig. 1 (a-h). Results of

Table 1—List of samples used for antimicrobial activity and their location

Sr. No.	NIO No.	Species	Location
		Porifera	
1	NIO 741	<i>Ircinia</i> sp. (i) (SP)	80°4' N; 77°32' E
2	NIO 743	<i>Ectyobatzella enigmatica</i> Burton & Rao (SP)	-do-
3	NIO 745	<i>Sigmatocia carnosus</i> (Dendy) (SP)	-do-
4	NIO 746	<i>Hyattella cribriformis</i> (Hyatt) (SP)	-do-
5	NIO 747	<i>Myxilla arenaria</i> (Dendy) (SP)	-do-
6	NIO 748	<i>Spongia</i> sp. (i) (SP)	-do-
7	NIO 749	<i>Ircinia fusca</i> (Carter) (SP)	-do-
8	NIO 753	<i>Axinella donnani</i> (Bowerbank) (SP)	-do-
9	NIO 755	<i>Sigmatocia fibulata</i> (Schmidt) (SP)	9°16' N; 79°12'E
10	NIO 756	<i>Fasciospongia cavernosa</i> (Schmidt) (SP)	-do-
11	NIO 759	<i>Psammaphysilla purpurea</i> (Carter) (SP)	-do-
12	NIO 760	<i>Aurora globostellata</i> (Carter) (SP)	-do-
13	NIO 763	<i>Cliona varians</i> (Duch. & Mich.) (SP)	-do-
14	NIO 765	<i>Haliclona</i> sp. (SP)	-do-
15	NIO 766	<i>Cinachyra cavernosa</i> (Lamarck) (SP)	-do-
16	NIO 767	<i>Hyattella cribriformis</i> (Hyatt) (SP)	-do-
17	NIO 769	<i>Spirastrella inconstans</i> var. <i>digitata</i> Dendy (SP)	-do-
18	NIO 771	<i>Spongia officinalis</i> var. <i>ceylonensis</i> Dendy (SP)	-do-
19	NIO 773	<i>Clathria reinwardti</i> vosmaer (SP)	-do-
20	NIO 775	<i>Hyattella cribriformis</i> (Hyatt) (SP)	-do-
21	NIO 776	<i>Cliona varians</i> (Duch. & Mich.) (SP)	-do-
22	NIO 777	<i>Haliclona pigmentifera</i> (Dendy) (SP)	-do-
23	NIO 778	<i>Endectyon fruticosa</i> (Dendy) (SP)	-do-
24	KK-8	<i>Callyspongia reticulata</i> Var. <i>solomonensis</i> (Dendy) (SP)	80°4' N; 77°32' E
25	KK-3	<i>Ircinia</i> sp. (ii) (SP)	-do-
		Coelenterata	
26	NIO 754	<i>Sinularia kavarattiensis</i> (Alderslade & Shirwaiker) (SC)	9°16' N; 79°12'E
27	NIO 761	<i>Sarcophyton</i> sp. (SC)	-do-
28	NIO 764	<i>Sinularia</i> sp. (i) (SC)	-do-
29	NIO 768	<i>Lobophytum</i> sp. (SC)	-do-
30	NIO 770	<i>Sinularia</i> sp. (ii) (SC)	-do-
31	NIO 772	<i>Sinularia inelegans</i> Tixier-Durivault (SC)	-do-
32	NIO 774	<i>Sinularia</i> sp. (iii) (SC)	-do-
		Echinodermata	
33	NIO 740	Red sea Urchin (SU)	80°4' N; 77°32' E
34	NIO 751	<i>Pentaceraster affinis</i> (Miller & Troschel) (SF)	-do-
35	NIO 752	Cushion star (SF)	-do-
		Eurochordata	
36	NIO 742	Ascidian (AS) (unidentified)	80°4' N; 77°32' E
		Marine Plants	
37	NIO 750	<i>Halodule</i> sp. (Zannichelleaceae) (SG)	80°4' N; 77°32' E
38	NIO 757	<i>Halophila ovalis</i> (Hydrocharitaceae) (SG)	9°16' N; 79°12'E
39	NIO 758	<i>Enteromorpha</i> SP. (SW)	-do-
40	NIO 762	<i>Eucheuma kappaphycus</i> (SW)	-do-

SP-Sponge, SC-Soft coral, SF-Star fish, SU-Sea urchin, AS-Ascidian, SW- Sea weed, SG- Sea grass.

secondary screening are presented in Table 4. It is evident from Table 4, that all the fractions of *Ectyobatzella enigmatica* were active against all the bacterial and drug resistant pathogens used in the study but to varying degrees. This suggests that different class of compounds with different polarity is responsible for the observed activity. This is the first report on antibacterial activity of the sponge *Ectyobatzella enigmatica*. However, due to small quantity of the sample obtained, further study could not be carried out.

Spongia officinalis (Fig. 1b) exhibited good activity against *Shigella flexineri*, *V. cholera* and multi-drug resistant *Streptococcus pyogenes* and *Acinetobacter* sp. with an inhibition zone of 9-15 mm. It showed, weak bactericidal activity against *E. coli*, *S. aureus* and *S. typhi* (i) its extract was moderately antifungal against *Fusarium* sp. and weakly fungicidal against *Nocardia* sp. Secondary screening, showed the antimicrobial activity to reside in the chloroform fraction. Interestingly, *Spongia officinalis* extract was as effective as streptomycin against some of the bacterial pathogens. Earlier report showed that methanol-toluene extract of *Spongia officinalis* inhibited growth of bacterial strains of *Staphylococcus epidermides* (5-12 mm), *Bacillus subtilis* (2-5 mm) and *Streptococcus lactis* (5-12mm) while its methylene chloride extract was effective against *B. subtilis* (2-5 mm) and *S. lactis* (2-5 mm)¹². Similarly, Becerro *et al.*¹³ reported antimicrobial activity of *Spongia officinalis* against marine bacteria. They also showed that, both, Gram+ve and Gram-ve bacteria were equally affected by the sponge extracts. Some of the antimicrobial compounds identified from *Spongia officinalis* whose crude extract was active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus spherical*, include isoagatholactone and its analogs¹⁴. Later on Gonzalez *et al.*¹⁵ also identified antifungal diterpenes from the same source.

The starfish, *Pentaceraster affinis* (Fig. 1c), though less active than the standard streptomycin showed considerable antibiotic activity against *Shigella flexineri*, MDR *Acinetobacter* sp., and moderate activity against *Streptococcus pyogenes*. It also exhibited weak activity against *Salmonella typhi*. All the three MDR strains of bacteria were insensitive to streptomycin. Secondary screening was suggestive of the antibacterial activity being concentrated in the low polar hexane fraction. The starfish was mildly

Table 2—List of pathogens (bacteria and fungi) used in the study

Sr. No.	Microorganisms	Code	Strain no. characteristics	Disease they cause
Bacterial pathogens				
1.	<i>Escherichia coli</i>	B ₁	Gram Negative	Gastrointestinal infection
2.	<i>Pseudomonas aeruginosa</i>	B ₂	Gram Negative	Urinary tract infection
3.	<i>Staphylococcus aureus</i>	B ₃	Gram Positive	Skin infection
4.	<i>Salmonella typhi</i> (i)	B ₄	Gram Negative	Typhoid
5.	<i>Shigella flexineri</i>	B ₅	Gram Negative	Gastrointestinal infection
6.	<i>Klebsiella</i> sp.	B ₆	Gram Negative	Urinary tract infection
7.	<i>Vibrio cholerae</i>	B ₇	Gram Negative	Cholera
Multi-drug resistant Bacteria				
1.	<i>Streptococcus pyogenes</i>	D ₁	Gram Positive	Skin infection
2.	<i>Salmonella typhi</i> (ii)	D ₂	Gram Negative	Typhoid
3.	<i>Acinetobacter</i> sp.	D ₃	Gram Negative	Urinary tract infection
Fungal pathogens				
1.	<i>Aspergillus fumigatus</i>	F ₁		Skin infection
2.	<i>Fusarium</i> sp.	F ₂		Skin infection
3.	<i>Cryptococcus neoformans</i>	F ₃		Skin infection
4.	<i>Aspergillus niger</i>	F ₄		Skin infection
5.	<i>Rhodotorula</i> sp.	F ₅		Skin infection
6.	<i>Nocardia</i> sp.	F ₆		Skin infection
7.	<i>Candida albicans</i>	F ₇		Candidiasis

B- Bacterial pathogen, D- Multi-Drug resistant bacteria, F-Fungal pathogen.

fungicidal with an inhibition zone between 1-4 mm against *Cryptococcus neoformans*, *Nocardia* sp. and *Candida albicans*. Antimicrobial effects against fouling bacteria by body wall extract of starfish *Pentaceraster affinis* have been described earlier⁴. The activity has been attributed primarily to saponins and saponin like steroid derivatives¹⁶.

In the case of ascidian (Fig. 1d), methanolic extract showed good antibacterial activity against *Shigella flexineri* (9-15 mm) but weakly antifungal against *Fusarium* sp., *Nocardia* sp. and *Rhodotorula* sp. with inhibition zone less than 4 mm. Secondary screening showed hexane fraction to be responsible for the antimicrobial activity in both starfish *Pentaceraster affinis*, as well as in the unidentified ascidian.

Table 3—Primary screening of methanolic extracts of marine organisms against pathogens: bacteria, including multidrug resistant (MDR) and fungi.

Sp No	Bacterial pathogens							MDR bacteria			Fungal pathogens							TSS
	B ₁	B ₂	B ₃	B ₄	B ₅	B ₆	B ₇	D ₁	D ₂	D ₃	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	
NIO 741	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	4
NIO 743	+++	-	++	++	++++	-	+++	++++	-	-	-	-	-	-	-	-	-	6
NIO 745	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 746	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 747	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 748	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 749	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	+	-	2
NIO 753	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 755	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 756	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
NIO 759	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 760	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	2
NIO 763	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 765	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 766	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 767	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
NIO 769	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 771	+	-	+	+	+++	-	+++	+++	-	+++	-	++	-	-	-	+	-	9
NIO 773	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 775	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 776	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 777	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
NIO 778	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KK-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
KK-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
NIO 754	-	-	-	+	-	-	+	+	-	-	-	++	-	-	-	+	-	5
NIO 761	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 764	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 768	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	2
NIO 770	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	2
NIO 772	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	1
NIO 774	+	-	+	+	+	-	+	+	-	-	-	+	-	-	-	+	-	8
NIO 740	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	3
NIO 751	+	-	+	+	+++	-	+	++	+	+++	-	-	+	-	-	+	+	11
NIO 752	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
NIO 742	-	-	-	-	+++	-	-	-	-	-	-	+	-	-	+	+	-	4
NIO 750	-	-	-	-	-	-	-	-	-	-	+++	++	-	+	+	+	-	5
NIO 757	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	+	-	2
NIO 758	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIO 762	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Std	+++	+++	++	++	++++	+	+++	-	-	-	-	-	++	++	-	-	-	9

- = No activity, + = weak activity (inhibition zone 1-4 mm), ++ = moderate activity (inhibition zone 5-8 mm), +++ = good activity (inhibition zone 9-15 mm), ++++ = highly active (inhibition zone 16-25 mm). TSS- Total no. of sensitive strains. Std = Streptomycin for bacteria/ ketoconazole for fungi

The Present study show that, invertebrates belonging to phylum Coelenterata, were either practically ineffective or exhibited mild activity against all the pathogens tested except for moderate fungicidal activity against *Fusarium* sp. and *Nocardia*

sp. exhibited by the extract of *Sinularia kavarrattensis* (Fig. 1f) and weakly antifungal activity shown by *Sinularia inelegans*, *Sinularia* sp. (ii) and *Lobophytum* sp. The activity observed in the soft corals of genus *Sinularia* and *Lobophytum* sp. could

be due to soft coral-derived terpenes. Recently, two new antimicrobial norcembrane diterpenoids, from *Sinularia inelegans*, showed a moderate-to-high activity against *Vibrio cholerae*, *Aspergillus fumigatus*, *Escherichia coli* and *Staphylococcus aureus* have been reported from our laboratory¹⁷. The compounds identified earlier from genus *Sinularia* and *Lobophytum* and possessing antimicrobial properties are flexibilide, sinulariolide, epi-

sinulariolide, etc especially active against the growth of *Bacillus* sp. and *S. aureus*¹⁸⁻²⁰. Vanisree and Subbaraju², identified antibacterial ceramides in *Lobophytum crassum*. A rare sesquiterpene spathulenol possessing significant antibacterial activity was reported from *Sinularia kavarrattensis*²¹.

Sponges belonging to genus *Ircinia*, *Ircinia* sp. (i), *Ircinia* sp. (ii) and *Ircinia fusca*, collected from the same location but different marine environment



Fig. 1(a-h)—Marine samples from Mandapam and Kanyakumari coast whose crude extract showed significant antimicrobial activity [1a-*Ectyobatzella enigmatica*; 1b-*Spongia officinalis*; 1c-*Pentaceraster affinis*; 1d-Unidentified Ascidian; 1e-*Sinularia* sp; 1f-*Sinularia kavarrattensis*; 1g-*Ircinia fusca*; 1h-*Halodule* sp; 1i- *Halophila ovalis*]

exhibited different activities. *Ircinia* sp. (i) was bactericidal against *E.coli*, *Shigella flexneri*, *Vibrio cholerae* and MDR *Streptococcus pyogenes* while the other two were totally inactive against bacteria. All the three sponge extracts were moderate to weakly antifungal against *Fusarium* and *Nocardia* sp. Since *Ircinia* from different locations exhibit different activity, being either antibacterial or antifungal, it is likely that the observed activity may be due to the associated microorganisms present on the surface of the invertebrate animal. Studies on *Ircinia fusca* by Thakur et al.²² have shown that epibacterial population of the sponge is influenced by temporal changes in the environment and that sponges seem to have selective control on the bacterial epibiosis on its surface. Ortiz and Rodriguez²³ reported Palominin, a geometrical isomer of ircinic acid possessing antibiotic properties²⁴, from a Caribbean sponge *Ircinia* species. Pangan et al.²⁵ report antimicrobial activity exhibited by the ethanol extracts of *Haliclona* sp. and *Ircinia* sp. from Phillipines against *E coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The former (*Haliclona* sp.) being more potent with inhibition zone of 24 mm, 16.5 mm and 21 mm against *B. subtilis*, *E. coli*, and *P. aeruginosa* respectively than the latter (*Ircinia* sp.) with growth inhibition zone of 23 mm, 11.5 mm and 15.5 mm respectively against the same pathogens. Similarly, methanol-toluene extract of *Ircinia* sp. to show moderate activity (2-5 mm) against *S. lactis* and *B. subtilis*¹².

Sea grasses *Halodule* sp. and *Halophila ovalis* (Fig. 1g & 1h) were antifungal with the former being considerably active against *Aspergillus fumigatus* (inhibition zone 9-15 mm), followed by *Fusarium* sp. (5-8 mm), and weakly active against *Aspergillus niger*, *Rhodotorula* sp., and *Nocardia* sp. (1-4 mm). On the other hand, *Halophila ovalis* showed moderate activity against *Fusarium* sp. and weak activity against *Nocardia* sp. Secondary screening showed hexane fraction of *Halophila ovalis* and ethyl acetate fraction of sea grass *Halodule* sp. to be responsible for the antifungal activity. Sea grasses are a rich source of secondary metabolites particularly phenolic sulfate, flavone sulfate, nonsulfated phenol, fatty acids and sterols²⁶. It has long been suspected that phenolic compounds produced by marine plants function in chemical defense and possess antimicrobial properties²⁷. It was later confirmed that phenolic compounds from sea grass have significant,

Table 4—Secondary screening of selected samples to determine the fraction [hexane (H), chloroform (C), ethyl acetate (E) and butanol (B)] responsible for activity.

S. No.	Sample Name	Pathogen	H	C	E	B
NIO 743	<i>Ectyobatzella enigmatica</i> (Sponge)	<i>Escherichia coli</i>	X	X	√	X
		<i>Staphylococcus aureus</i>	√	√	√	√
		<i>Salmonella typhi</i> (i)	√	√	√	√
		<i>Shigella flexneri</i>	√	√	√	√
		<i>Vibrio cholerae</i>	√	√	√	√
		<i>Streptococcus pyogenes</i>	√	√	√	√
NIO 771	<i>Spongia officinalis</i> (Sponge)	<i>Escherichia coli</i>	X	√	X	X
		<i>Staphylococcus aureus</i>	X	√	X	X
		<i>Salmonella typhi</i> (i)	X	√	X	X
		<i>Shigella flexneri</i>	X	√	X	X
		<i>Vibrio cholerae</i>	X	√	X	X
		<i>Streptococcus pyogenes</i>	X	√	X	X
		<i>Acinetobacter</i> sp.	X	√	X	X
		<i>Fusarium</i> sp.	X	√	X	X
		<i>Nocardia</i> sp.	X	√	X	X
NIO 751	<i>Pentaceraster affinis</i> (Starfish)	<i>Escherichia coli</i>	√	X	X	X
		<i>Staphylococcus aureus</i>	√	X	X	X
		<i>Salmonella typhi</i> (i)	√	X	X	X
		<i>Shigella flexneri</i>	√	X	X	X
		<i>Vibrio cholerae</i>	√	X	X	X
		<i>Streptococcus pyogenes</i>	√	X	X	X
		<i>Salmonella typhi</i> (ii)	√	X	X	X
		<i>Acinetobacter</i> sp.	√	X	X	X
		<i>Cryptococcus neoformans</i>	√	X	X	X
		<i>Nocardia</i> sp.	√	X	X	X
NIO 742	Unidentified Ascidian	<i>Shigella flexneri</i>	√	X	X	X
		<i>Fusarium</i> sp.	√	X	X	X
		<i>Rhodotorula</i> sp.	√	X	X	X
		<i>Nocardia</i> sp.	√	X	X	X
		<i>Escherichia coli</i>	√	√	X	X
NIO 774	<i>Sinularia</i> sp. (iii) (Soft coral)	<i>Staphylococcus aureus</i>	√	√	X	X
		<i>Salmonella typhi</i> (i)	√	√	X	X
		<i>Shigella flexneri</i>	√	√	X	X
		<i>Vibrio cholerae</i>	√	√	X	X
		<i>Streptococcus pyogenes</i>	√	√	X	X
		<i>Fusarium</i> sp.	√	√	X	X
		<i>Nocardia</i> sp.	√	√	X	X
		<i>Escherichia coli</i>	√	√	X	X
		<i>Salmonella typhi</i> (i)	√	√	X	X
NIO 754	<i>Sinularia kavarrattensis</i> (Soft coral)	<i>Vibrio cholerae</i>	√	√	X	X
		<i>Streptococcus pyogenes</i>	√	√	X	X
		<i>Fusarium</i> sp.	√	√	X	X
		<i>Nocardia</i> sp.	√	√	X	X
NIO 749	<i>Ircinia fusca</i> (Sponge)	<i>Fusarium</i> sp.	X	√	X	X
		<i>Nocardia</i> sp.	X	√	X	X
NIO 750	<i>Halodule</i> sp. (Seagrass)	<i>Aspergillus fumigatus</i>	X	X	√	X
		<i>Fusarium</i> sp.	X	X	√	X
		<i>Aspergillus niger</i>	X	X	√	X
		<i>Rhodotorula</i> sp.	X	X	√	X
NIO 757	<i>Halophila ovalis</i> (Seagrass)	<i>Nocardia</i> sp.	X	X	√	X
		<i>Fusarium</i> sp.	√	X	X	X
		<i>Nocardia</i> sp.	√	X	X	X

ecologically relevant microbiological activity and further report that this activity can be accounted for the presence of a sulfated flavone glycoside, simple phenolic acids similar to those implicated as the primary agents of chemical defense in land plants²⁸. Both the sea grasses in the present study, *Halodule* sp. and *Halophila ovalis* showed selective activity only against fungal pathogens.

Conclusion

Present study indicated that marine organisms remain an interesting source for new antimicrobial metabolites and also suggest that *Ectyobatzella enigmatica* could be a source of the secondary metabolite with a strong antibacterial activity. Hitherto this sponge has not been investigated either for its chemistry or for its activity. Further, *E. enigmatica* was followed in activity by *Spongia officinalis*, *Pentaceraster affinis* (star fish), an unidentified ascidian and soft coral, *Sinularia* sp (iii), and *S. kavarrattiensis*. The sea grasses were particularly antifungal in nature. The problem of drug resistance by many microbes may now be dealt with by considering marine organisms for antibiotic activity. The small quantities of the extract did not permit further studies using the active samples.

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