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Marine sponge and its potential bioactive spectrum against CSF affected bacterial organisms

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Abstract
Sponges are sessile filter feeders that have developed efficient defense mechanisms against foreign invaders such as viruses, bacteria or eukaryotic organisms. Antimicrobial activities are known as principal functional important immune defense system in marine invertebrates. The aim of the present work was to study the antimicrobial properties of the eight Indian sponges against the CSF affected pathogenic bacterial organisms viz., *E. coli S. aureus H. influenzae*, *N. meningitides Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Pseudomonas fluorescens*, *Enterococcus faecalis*. From the present result clealrly expressed the maximum peak activity was noted on two experimental sponges such as *Ircinia fusca* and *Sigmadocia carnosa* crude extract showed that the peak activity 21±3.64 and 20±3.15 bioactivity against the pathogens of *H. influenzae* and *N. meningitides* respectively. Consequently another maximum zone of inhibition (17±2.32) formed with the sponge-5 extract on *K. pneumoniae*. The present result denoted on both sponge extract antimicrobial activity at 5% level of statistically significant. Although, least antimicrobial response was observed in *Cacospongia salaries* on *K. pneumoniae* (3.41±0.21) also *D. nigra* extract against *S. agalactiae* (2.30±0.41). From the over all results depicted statistically insignifiant response also noted on ms-2,3 against *E.coli* 5±2.58 and *H. influenzae* 7±2.10. The present study depicted that the chromatogram stands for compounds elucidation by GCMS on the methanol extract experimental sponge of *Ircinia fusca*. Initially nine compounds were categorized. Among the nine compounds, two antimicrobial compounds are present such as 2-Methoxy-1, 4-Benzenediol recognized as a peak compound subsequently 3-dimethylocta-1, 5-dien-3, 7-diol-dimethyltryptamine also been elucidated. The response of this kind of secondary metabolic compounds was act as potential antimicrobial compound against the experimental CSF pathogen derived from the tested marine sponges. Hence, this research focused a new source of marine sponge as a poised potential antimicrobial agent for the control the CNS involved pathogenic microbiota.

Keywords: Marine sponge, CSF Pathogen, Bioactive compounds.

1. Introduction
The world's oceans cover more than 70% of the earth's surface and represent an enormous resource for the discovery of chemotherapeutic agents [1]. Given the diversity of marine organisms and habitats, marine natural products encompass a wide variety of chemical classes such as terpenes, polyketides, acetogenins, peptides and alkaloids of varying structures representing biosynthetic schemes [2, 3]. Over the past 30–40 years marine organisms have been the focus of a worldwide effort for the discovery of novel natural products [5, 6]. Marine invertebrates that are sessile organisms like sponges were provided the largest number of marine derived secondary metabolites including some of the most interesting pharmacological important drug candidates. However, there are many difficulties regarding the origin of these natural compounds when sponges are studied in symbiotic relationships. Marine sponges are a rich source of biologically active secondary metabolites with novel chemical structures [8, 9]. Eighty four anti-inflammatory compounds have been isolated from marine sponges. This is the first comprehensive review presenting the pharmacological activities of marine sponge metabolites. To date majority of these chemicals ha-e been identified from marine invertebrates of which sponges predominate.
Cerebrospinal fluid (CSF) has been the mainstay of treatment for hydrocephalus for over 50 years Kestle, (2003). While CSF shunts allow children to survive and avoid further brain injury, they can cause new and often chronic surgical and medical problems. CSF shunt infections are one such complication, resulting in up to 2,400 pediatric hospital admissions each year in the U.S [34]. CSF shunt infections, usually associated with bacterial pathogens but

occasionally fungi [13, 25, 33] are notoriously difficult to treat, and adequate therapy generally requires both medical and surgical management. Medical treatment commonly involves prolonged intravenous, and occasionally intrathecal, antibiotic administration [13]. In addition, while the presence of bacteria during any infection has traditionally been determined by growth in conventional culture [17]. Given the recalcitrant nature of CSF shunt infection, the emerging evidence of a role for biofilms, and the increased application of culture-independent molecular approaches to medical microbiology, we sought to better characterize the diversity and natural history of the microbial communities (the microbiota) in CSF shunt infections using molecular approaches [26]. A serious obstacle to the ultimate development of most marine natural products that are currently undergoing clinical trails or are in preclinical evaluation is the problem of supply. The concentrations of many highly active compounds in marine invertebrates are often minute, accounting for less than a millionth of the wet weight [10].

2. Materials and Methods

Collection of sponges and preparation of crude extracts Sponges were collected from Muttom coastal region in Cape Comorin coasts of Indian Ocean, at depths varying from 10 - 15 feet by snorkeling and SCUBA-diving process. Sponges were gently removed from the substratum and cut into small pieces and then soaked in methanol for preparing crude extracts. The experimental samples namely *Sigmadocia fibulata*, *Spirastrella inconstans*, *Ircinia campana*, *Callyspongia fibrosa*, *Dendrilla nigra*, *Cacospongia salaries*, *Sigmadocia carnosa and Ircinia fusca*

The intact sample specimens were sent to the Central Marine Fisheries Research Institute (CMFRI), Trivandram, Kerala, India for identification. The initial methanol extract was concentrated in the laboratory under reduced pressure and lyophilized. The lyophilized powder was extracted with 1:1 mixture of methanol solvent. At the same time the methanol soaked cut pieces (100 g) were further diced and extracted with the same mixture of solvents. The extracts were pooled and the organic portions evaporated for obtaining solvent free crude extract. Then the tested solutions with desired concentrations were prepared by mixing the known amount of crude extract in a carrier, methanol (w/v).

2.1. Laboratory – based report

As a part of NNIS-ICARE hospitals also reported susceptibility data to selected organisms from clinical specimens (CSF) obtained from the patients in the same ICUs whether associated with hospital acquired or community acquired infection or colonization. Then these organisms allowed for determining the cumulative susceptibility report or antibiogram of all organisms processed from the clinical specimens submitted to the clinical microbiology laboratory.

2.2. Primary screening

A modified cross- streak method was used for antimicrobial activity .Single streak of actinomycetes was made on surface of the modified nutrient agar and incubated at 28 °C. After observing a good stretch like growth of the actinomycetes on the plates, the overnight pathogenic bacterial strains, such as *E. coli S. aureus H. influenzae*, *N. meningitides Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Pseudomonas fluorescens*, *Enterococcus faecalis* which (isolated from the admitted patients from Karakkonam Hospital) were streaked at right angles to the original streak of actinomycetes and incubated at 28 °C and the incubation distance was measured after 24-48 hrs. A control plate was also maintained without inoculating the microbes to assess the normal growth of bacteria.

2.3. Antibacterial assay

Antibacterial activity was determined against *E. coli*, *S. aureus*, *H. influenzae*, *N. meningitides*, *K. pneumoniae*, *S. agalactiae, E. faecalis*, using the paper disk assay method (12). Whatman No. 1 filter paper disk of 6-mm diameter was sterilized by autoclaving for 15 min at 121 ^{0}C. The sterile disks were impregnated with different extracts (500 Ag/ ml). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately $1.2X10^8$ CFU/ml. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37 ^{0}C for 24 h. Disk of Streptomycin (400 Ag/ml) was used as a positive control. The diameter (mm) of the growth inhibition halos caused by the methanolic extracts of marine organisms was examined. All the assays were carried out in duplicate.

2.4. GC-MS Analysis

The crude plant extracts were subjected to centrifugation at about 10,000 rpm for about 30 minutes to remove the particulates. The clear supernatant was aspirated using a pipette and transferred into a clean vial and labeled. Then the supernatants were subjected to gas chromatography analysis using a Varian Cp 3,800 model gas chromatography equipped with two flame ionization detectors and connected with two flame ionization detectors and connected with two flame ionization detectors and connected with Cp-ware (Polyethylene glycol) (60 m x 0.25 nm) and Cp-5 (100% dimethyl polysiloxane) capillary column. The peak area calculations were done by star work station and peak identification by comparison with authentic, wherever available calculations of Kovats Retention index was done. The Kovats index system has been widely used in the analysis of food flavors, pesticides and essential oil analysis. Kovats retention index, (1) is defined and calculated by following equation (Douglas, 2000).

$$1= 100\ N + 100\ \log'R\ (N+n) – \log'R(N).$$

Whereas,

t'R (N) = adjusted retention time of n paraffin hydrocarbon of Carbon number eluting before solute-A.

T'R (N+n) = adjusted retention time of n paraffin hydrocarbon of Carbon number (N+n) eluting after solute A.
T'R (A) = adjusted retention time of solute-A.

Mass spectrometry analysis was performed on a Shimadzu GC 17 A QP 5,000 MS coupled with a mass detector, fitted non-polar DB-5 (Diphenyl siloxane) capillary column of length 25 m × 0.25 mm id GC MS operation conditions at initial temperature 60–300 ^{0}C. The injection volume was 0.1 µl with helium gas as carrier at the flow rate of 0.6 ml per minute. Relative retention times (RRts) of constituents were determined using c5-c30 straight chain alkanes as standards.

Individual constituents of the extract were identified by WILEY and NIST database matching by comparison of mass spectra with published data and by comparison of Mass spectra with published data and by their Comparison of their RRts.

2.5. Statistical Analysis

One-way ANOVA were used to determine the significance of antibacterial broad spectrum activity of 12 actinomycetes with nine bacterial pathogens. Data's were analyzed with a one way of ANOVA Test using pp Version-4 Window. Result with $P<0.05$ were considered as statistically significant.

3. Results

Table 1: Measurement of Zone of Inhibition (Antibacterial activity) of ethanolic extract of the marine against CSF disease causative agent of pathogenic bacteria

Name of the sponge	Name of the tested Organisms (Inhibition Zone Diameter in mm)						
	E.coli	***S. a***	*H. i*	*N. m*	***K. pneu***	***St. ag***	***E. f***
Sigmadocia fibulata Ms 1	-	12±2.64*	9±3.54	-	11±0.50	6±2.36*	12.±2.3
Spirastrella inconstans (Ms- 2)	5±2.58^{is}	12±1.57	-	10±2.87	-	12±1.64**	9±4.11
Ircinia campana (Ms-3)	-	5±0.02**	7±2.10^{is}	-	10±1.91**	9±1.43	-
Callyspongia fibrosa (Ms-4)		8±0.66	9±1.05	12.54.±2.2	-	10±0.34**	11±3.6
Dendrilla nigra (Ms-5)	3.65±0.6	11±1.49	9±1.08	5±1.08	17±2.32**	2.30±0.41	6.3±2.6
Cacospongia salaries (Ms-6)	-	6±2.33	-	-	3.41±0.21	10±0.95	-
Sigmadocia carnosa (Ms-7)	9±0.36	12±0.23*	12±0.09	20±0.57**	-	14±2.01*	-
Ircinia fusa (Ms-8)	-	4±1.77*	-	-	21±3.64	10±1.06*	12±0.5

E. coli; S. a - S. aureus; H. i - H. influenzae; N. m - N. meningitides; K. pneu- K. pneumoniae; St. ag- Streptococcus agalactiae; E. f - E. faecalis;

Values are means of three replications.

NA = not applicable. (-) = no inhibition of growth at the concentrations tested.

Values are Means ± SEM of triplicates

Asterisks indicate values which are significantly different.

- $P < 0.05$- Significant
- *** $P < 0.001$- Highly significant at 0.05% level

*Zone in mm indicates the distance from the border of the disc to the edge of the clear zone

From the present result clearly expressed the maximum peak activity was noted on two experimental sponges such as MS-8 and MS-7 crude extract showed that the peak activity 21±3.64 and 20±3.15 bioactivity against the pathogens of *H. influenzae* and *N. meningitides* respectively.

Subsequently another maximum zone of inhibition (17±2.32) formed with the sponge-5 extract on *K. pneumoniae*. The present result denoted on both sponge extract antimicrobial activity at 5% level of statistically significant. Furthermore, similar antibacterial activity was noticed on MS-7 against *S. aureus* and *H. influenzae* followed by Ms-4 against 12.±2.20 particularly its range between 11±1.49 to 12.54.±2.2 Ms - 4 *C. fibrosa* against the similar bacteria of *N. meningitides*. Additionally, MS-1 and ms-2 sponge extracts were well pronouncedly activate against the *S. aureus* (12±2.64*), *E. faecalis* (12.±2.30) and *St. ag* (12±1.64). The above said result was p=0.05% level of significant when compared with other bacterial microorganisms (Figure-1).

The present result expressed the optimum effect noticed on 6±2.33 to 9±0.36, *E. coli, S. aureus* and *H. influenzae* and its sponge named as the Ms-1, 4, 5 and 6. Though, the least activity also been observed ms-6 on *K. pneum* (3.41±0.21) MS-5 *St. agaricus* (2.30±0.41). From the over all results depicted statistically insignifiant response also noted on ms-2,3 against *E.coli* 5±2.58 and *H. influenzae* 7±2.10. Interestingly, the over all result indicated all the sponges had a remarkable activity against *Staphylococcus aureus* and *S. agalactiae* along with *H. influenzae* and *K. pneumoniae* bacterial strains.

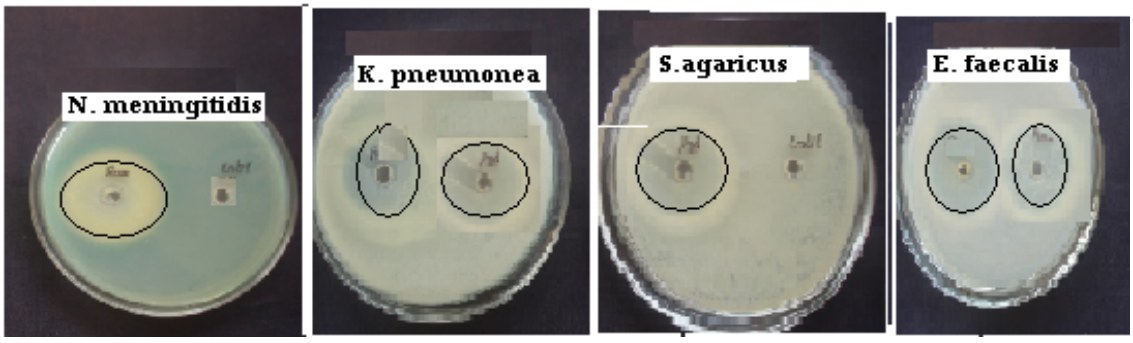

Fig 1: Secondary screening of antibacterial activity of eight Marine sponges against the various CSF influenced Bacterial Pathogens

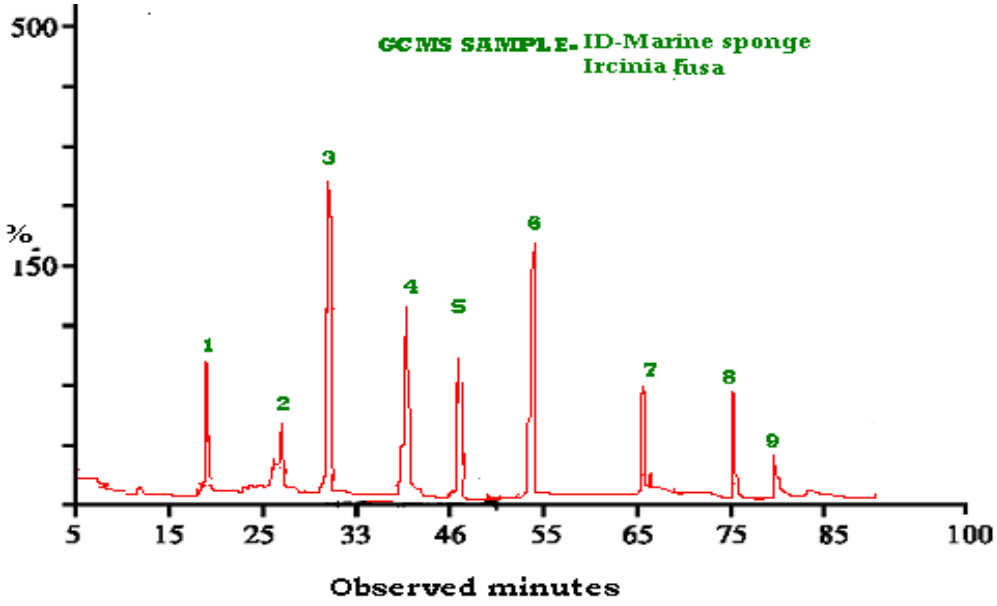

Fig 2: GCMS Chromatogram Analysis of Dominant antimicrobial activity showed from the marine sponge of *Ircinia fusca*

Table 2: Bioactive Compound elucidation from the marine sponge of *Ircinia fusca* by GCMS Analysis

S. no.	Retention time (rt)	Compounds separated (name of the analytes)	Abundance (%)
1	9.21	1,2-Benzenedicarboxylic Safranal	87.5
2.	27.30	3-methyldecanoic acid	89.2
3.	30.53	2-Methoxy-1, 4-Benzenediol	375.1
4.	41.7	4,4'-(1-methylethyl) – 2,4-tetradecadiene	145.2
5.	49.28.	Para-methoxy1, 2-Benzenedicarboxylic acid	179.8
6.	54.70	3-dimethylocta-1,5-dien-3,7-diol- dimethyltryptamine	394.7
7	61.15	Triphenylphosphine	65.36
8.	75	Pyranosyl apigenin	64.1
9.	80	1, 6-Dihydro-beta - Ethyl Palmitate	62.4

These chromatogram stands for compounds elucidation by GCMS on the methanol extract experimental sponge of *Ircinia fusca*. Initially nine compounds and its abundance were categorized such as 1,2-Benzenedicarboxylic Safranal (87.5), 3-methyldecanoic acid (89.2%), 2-Methoxy-1, 4-Benzenediol (375.1), 4,4'-(1-methylethyl) – tetradecadiene (145.2), Para-methoxy, 2-Benzenedicarboxylic acid (179.8), 3, dimethylocta-1, 5-dien- 3,7- diol- dimethyltryptamine (394.7), Triphenylphosphine (65.36), Pyranosyl apigenin (64.1) and 1, 6-Anhydro-beta,-Ethyl Palmitate (62.4). Among the nine compounds 2-Methoxy-1, 4-Benzenediol recognized as peak compound then secondary level maximum peak phytocompound is 3-dimethylocta-1, 5-dien-3, 7-diol-dimethyltryptamine (Figure-2.) (See the Table-2).

4. Discussion

Research has indicated that the secondary metabolites of sponges play an important role in their defense against infectious microorganisms [30]. Specific programs directed towards the collection and characterization of marine natural products such as various types of sponges and evaluations of their biological activity have been established. This systematic investigation of marine environments is reflected in the large number of novel compounds especially reported in the literature over the past decade [31]. Marine natural products especially sponges could yield new drugs to cure the severe diseases. The quest for drugs from the sea has yielded an impressive list of natural products mostly from invertebrates such as sponges that are either in the late stages of clinical trials, or have already entered the market. Some of the Sponge-derived bioactive compounds presently available in the market are Ara-A (antiviral), Ara-C (anticancer) and Manoalide (phospholipase A2 inhibitor), while IPL512602 (anti-inflammatory), KRN 7000 (anticancer), LAF389 (anticancer), Discodermolide (anticancer) and HTI286 (anticancer) are under clinical trial [28, 32]. Besides their pharmaceutical potential, sponges are an important to explain classification patterns and phylogenetic relationships [22]. During the year 1998 Hattori demonstrated new ceramide compound from marine sponge. Possible Biogenetic Relevance with Manzamine portrayed novel Lipid contents of the sponge *Haliclona* sp. Sponges produce a wide array of secondary metabolites ranging from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols [12]. Consistently, [13] proposed the new antifouling hexapeptide from a Palauan sponge, *Haliclona* sp. The antinociceptive and anti-inflammatory effects were investigated against different experimental models in mice described by [4] also this author explained the sponge *Caissera* possessed antinoceptive and anti-edematogenic effects.

Our knowledge, this is the first study that described the marine sponge extract could be resist them propagation of microbiota present in CSF shunt infection. Since the structurally derived chemical compounds were [11] explained marine natural products halitoxin, toxic complex of several marine sponges of the genus *Haliclona.* Furthermore, [5] demonstrated similar findings with other pathogenic microbes and its constructive valuable pharmacological compounds such as Araguspongines-K and L, New Bioactive Bis, Oxaquinolizidine N-Oxide Alkaloids from Red Sea Specimens of *Xestospongia exigua.* This study explained the eighth experimental sponge possessed the peak bioactive compound named as 2-Methoxy-1, 4-Benzenediol is act as an antimicrobial compounds so this particular sponge activity is high compared with other seven sponges. This kind of similar findings also published by [8].

Pharmaceutically important Phytochemicals originated from sponge (Blunt JW, Groud TV *et al.*) [6, 19] reported inhibition of HIV by two bis-quinolizidine alkaloids petrosins isolated from the Indian marine sponge *Petrosia similis*. The extensive investigation determined that both petrosins inhibited HIV-1 replications. It has been reported the Cribrostatin-6 showed antibacterial activity against Gram-positive bacteria, and it was most active against *S. pneumoniae* [7] and [22] investigated a series of kalihinols, diterpenes isolated from the Philippine marine sponge *Acanthella cavernosa*, as potential bacterial folate biosynthesis inhibitors. The present study also confirmed by the [25] with the biological activities of aqueous extracts from marine sponges and cytotoxic effects of 3 alkylpyridinium polymers from *Reniera sarai* and also be of the same opinion depicted by [10] in the marine sponge of the genus *Ircinia* against few peculiar human affected pathogenic organisms.

Potential studies of a larger patient population will be needed to determine the relationships between the diverse CSF affected microbiota and clinical outcomes, particularly the response to treatment and occurrence of reinfection [13, 15, 36]. Marine sponges are a rich source of bioactive compounds, and many species can be useful for the development of new antimicrobial drugs. Since the reason such a peculiar pathogenic organism's growth and it other performances were suppressed by these kinds of oceanic natural product of marine sponges. The composition of the microbiota changed over the course of infection treatment, probably in response to antibiotic use and surgical removal of the CSF affected microbiota especially this study focusing pathogenic organisms [24].

5. Conclusion

Marine sponges are potential sources of unique bioactive metabolites and many of these compounds are valuable for medicinal uses. The present study was undertaken to assess the potential antibacterial spectrum of the eight various marine sponges against CSF affected pathogenic organisms. The evaluation of wide array of marine sponges for potential use in chemotherapy poses problems of procurement of materials and of techniques of screening for significant drug activity. From the present result clealrly expressed the maximum peak anti-bacterial activity was noted on two experimental sponges such as *I. fusca* and *S. carnosa* crude extract showed that the peak activity 21±3.64 and 20±3.15 bioactivity against the pathogens of *H. influenzae* and *N. meningitides* respectively. Subsequently another maximum zone of inhibition (17±2.32) formed with the sponge-5 extract on *K. pneumoniae*. In this study also concluded the dominant bioactivity denotes on the marine sponge of *Ircinia fusca* possessed an important antimicrobial compound such as 2-Methoxy-1, 4-Benzenediol identified through a GC-MS analysis. Hence, the present results profounded the promising antibacterial effect on eight active marine sponges against seven CSF affected pathogenic strains so this study concluded all the eight marine sponges possessed excellent source of antibacterial bioactive compound thus they act as a excellent peculiar antibacterial agent.

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