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Detection of Marine Derived Natural Products Synthesized in Marine Sponges Collected from Tuticorin, India

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Abstract: The present work describes the availability of marine derived natural products obtained from the methanolic crude extracts of the sponges such as the a.*Gelliodes fibrosa,* b. *Gelliodes carnosa,* c. *Mycale laevis* and d. *Mycale almata.* The samples were collected from Tuticorin in the Gulf of Mannar region. Natural compounds were detected and confirmed by using *HPLC* analysis. In this work many natural compounds such as the glycosides, cyclic peptides, sesterpenoids, phenolic compounds, flavones and sphingolipids were seen and identified on the marine sponges. At various concentrations, where as the sphingolipids is the compound available at all the marine sponges in large quantity. Hence it is assumed that it can be used as an effective bioactive compound and used in drug development process.

Key words: Sponges · Sphingolipids · HPLC

INTRODUCTION

Marine environment have a huge amount of the natural resources. Bioactive compounds from marine sources (flora and fauna) are used in the treatment of many diseases and serve as the compounds of interest both in their natural form and as templates for synthetic modification. One of the main reasons for studying the marine sources is because the ocean is covered more than 70% of the earth's surface and among 36 known living phyla, 34 of them are found in marine environments with more than 300000 and known species of fauna and flora [1]. Sponges are divided into three classes mainly according to the composition of their skeletons. i.e., Calcarea, Glass sponges, Demosponges. Among them demosponges have the large number bioactive compounds. More than 8,000-10,000 species of sponges were described. They are such as *Aplysina archeri, Xestospongia muta, Acanthella pulchra, Helicona simulans, Axinella dissimilis, Discodermia dissolute, Raspailia ramose* [2]. Sponges are the most primitive of multicelled animals that have existed for more than 800 million years. The marine sponges produce a variety of compounds which showed various pharmacological activities. The sponge *Reniera sarai,* crude extracts have anti-inflammatory activities. The compounds in sponges include 3-octylpyridinium salts or poly APS. It is structurally and functionally related with 3-alkylpyridinium polymer. They have a broad spectrum on biological activities [3]. Sponges are the primitive marine invertebrates which contains more natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial and anti-inflammatory activities and are often applicable for medical use. Thousands of novel compounds and their metabolites with diverse biological activities ranging from antiviral to anticancer have been isolated from various marine sponges. The sponges are known as prolific sources of bioactive compounds that could be used to treat various human diseases [4].

Pharmaceutical interest in sponges was started in the early 1950s by the discovery of the nucleosides spongothymidine and spongouridine in the marine

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sponge *Cryptotethya crypta*. Marine sponge *Dysidea avara* contains large amounts of avarol, which was determined to be a highly active cytostatic agent [5]. Sponge peptides are of microbial origin due to the presence of both d-amino acids and unusual amino acids and are rich sources of structurally unique natural compounds, several of which have shown a wide range of biological activities [6]. Many compounds previously found in sponges are biosynthesized through microorganisms associated with them or indeed produced by the crude extracts [7]. It was suggested that a number of novel compounds with bioactivity have been discovered through cultivation of sponge-associated microorganisms. To identify new bioactive compounds from marine microorganisms most of the investigation was done on the real origin of natural products from marine sponges [8].

In the present study we examined the sponges such as the *Gelliodes fibrosa, Gelliodes carnosa, Mycale laevis, Mycale almata.* This paper describes the identification of the presence of natural compounds from the marine sponges collected from the south east coast of Tamilnadu at the Tuticorin coast. It was detected using HPLC analysis.

MATERIALS AND METHODS

Collection and Maintenance of Marine Sponges: The sponges were collected using by catch method from fish nets in Tuticorin at the south east coast of Tamilnadu. The collected sponges were transferred to the laboratory in sterile containers of sea water. All the adherent fauna were removed by washing sponges with sterile distilled water. The taxonomic position of the sponge samples was identified. The marine sponges were maintained and stored in containers containing methanol for permanent preservation.

Preparation of Crude Extracts: For the preparation of crude extracts the method followed was previously described by Braekman *et al*. [8]. Sponges weighing of 250 g were cut into small pieces and extracted thrice with distilled methanol and the pooled organic solution was filtered through Whatman No.1 filter paper fitted in a Buchner funnel using suction. Solvents were removed by rotary vacuum evaporator (Buchi-type) under reduced pressure so as to get the crude methanol extract. The concentrated crude extract was collected in airtight plastic containers and kept in the refrigerator for further use.

Quantification of Marine Natural Products by HPLC: The crude extracts of each specimen were prepared quantified by HPLC. From the 1.5 ml volume of each crude extract saved for HPLC quantification, 200 μl were transferred to a vial and the solvent removed by Speed-Vac vacuum concentration. The obtained residue was dissolved in 500 μl acetonitrile: water 1:1 + 0.5% trifluoroacetic acid.and 10 μl injected by auto-sampling into a HPLC [9].

RESULTS

The marine sponge samples were collected from the fish nets during the active fishing season in Tuticorin using by catch method.The taxonomic position of the sponges were analyzed. Then the sponges were stored in sterile containers containing the solvent methanol. The crude methanolic extracts were prepared from the sponges a. *Gelliodes fibrosa*, b.*Gelliodes carnosa,* c.*Mycale laevis,* d. *Mycale almata.* It was then subjected to HPLC analysis for the detection of the synthesized natural compounds present on the methanolic crude extracts. Fig. 1 shows the detection of marine derived natural products from the sponge *Gelliodes fibrosa* such as glycosides, sphingolipids and cyclic peptides based on the retention

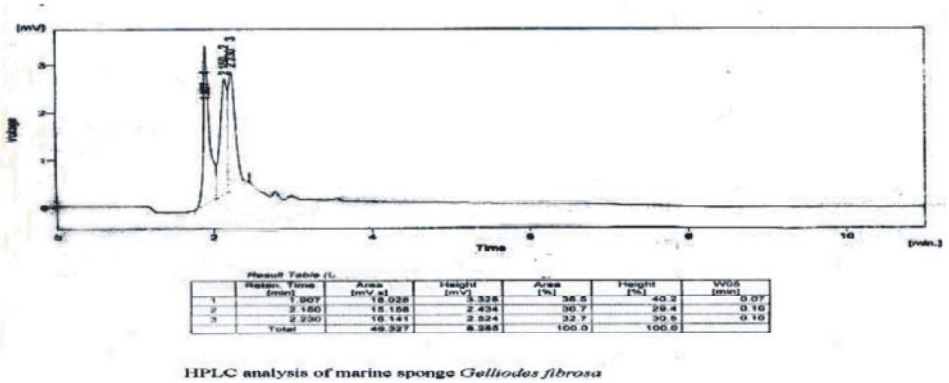
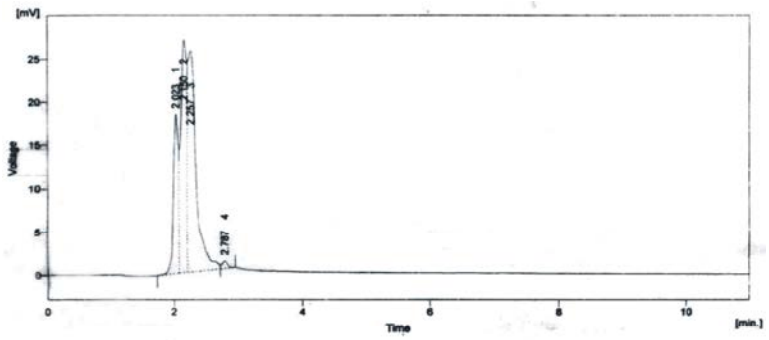


Fig 1: HPLC analysis of *Gelliodes fibrosa*

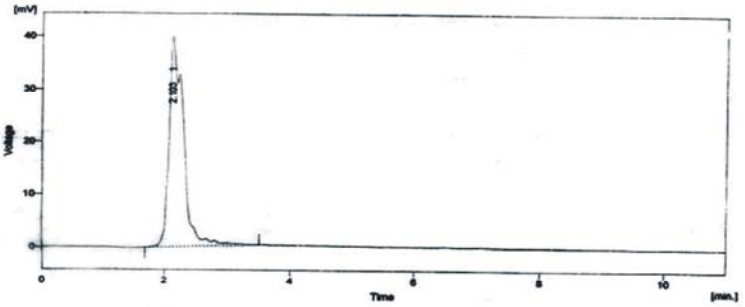


Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.023	98.431	18.411	18.6	25.6	0.09
2	2.150	174.854	26.965	33.7	37.5	0.13
3	2.257	242.739	25.601	46.7	35.6	0.13
4	2.787	5.511	0.895	1.1	1.2	0.11
	Total	519.535	71.871	100.0	100.0	

HPLC analysis of marine sponge *Gelliodes carnosa*

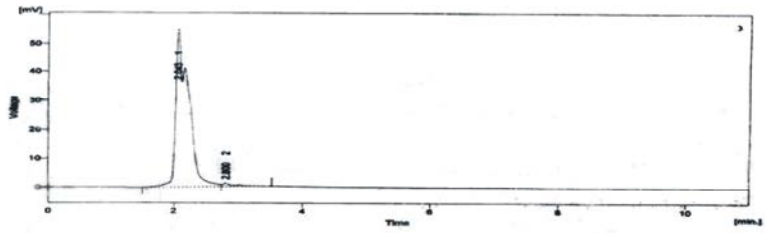
Fig 2: HPLC analysis of *Gelliodes carnosa*



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.103	642.942	40.118	100.0	100.0	0.25
	Total	642.942	40.118	100.0	100.0	

HPLC analysis of marine sponge *Mycale laevis*

Fig 3: HPLC analysis of *Mycale laevis*



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.043	760.564	54.970	97.9	97.9	0.24
2	2.800	16.946	1.157	2.2	2.1	0.14
	Total	777.510	56.127	100.0	100.0	

HPLC analysis of marine sponge *Mycale almata*

Fig 4: HPLC analysis of *Mycale almata*

Fig 5: Structure of Sphingolipid

time. Fig. 2 analyses the detection of marine derived natural products from the sponge *Gelliodes carnosa* such as sesterpenoids, sphingolipids, phenolic compounds and flavones glycosides based on the retention time. Fig. 3 explains the detection of marine derived natural products from the sponge *Mycale laevis* such as galacto glycosphingolipids based on the retention time. Fig. 4 reveals the detection of marine derived natural products from the sponge *Mycale almata* such as sphingolipids and epidioxy sterols based on the retention time. The structure of Sphingolipids is mentioned in Fig 5.

DISCUSSION

The sponge and their products have strong bioactivities including anticancer, antimicrobial, larvicidal, haemolytic and anti-inflammatory activities and are often applicable for medical use [10] Thousands of novel compounds and their metabolites with diverse biological activities ranging from antiviral to anticancer have been isolated from various marine sources. The sponges are known as prolific sources of bioactive compounds that could be used to treat various human diseases [11]. It clearly showed that the discovery of the nucleosides spongothymidine and spongouridine in the marine sponge *Cryptotethya crypta*. Marine sponge *Dysidea avara* contains large amounts of avarol, which was determined to be a highly active cytotoxic agent. Sponge peptides of microbial origin due to the presence of both d-amino acids and unusual amino acids are rich sources of structurally unique natural compounds, several of which have shown a wide variety of biological activities [12].

Studies showed that the marine sponges of the class demospongia such as *Dendrilla, Axinella, Clathria* having the wide range of biological activity. They shows potent activity in antibacterial, brine shrimp cytotoxicity, antifouling and ichthyotoxic assay.It includes various marine natural products such as terpenes, phospholipids, alkaloids and sterols [13]. It was clear that many bioactive natural products from marine invertebrates have striking similarities to metabolites because of their associated microorganisms including bacteria. It is to highlight the possible role of marine bacteria associated with sponges in providing solution to the problem of infection by pathogenic bacteria [14]. Marine sponge associated bacteria have been shown to produce many natural bioactive agents, including polyketides and many of the sponge-derived compounds that have entered clinical and pre-clinical development are believed to be ultimately microbial in origin [15].

Recent investigation showed that sponge-associated bacteria are important resources of marine natural products and have been proved to synthesize polyketides and nonribosomal peptide compounds. Marine invertebrates harbour microorganisms that include bacteria, cyanobacteria and fungi within their tissues where they reside in the extra- and intracellular space [16]. Marine sponge *Aplysina cavernicola* produces aeroplysinin and aerthionin and other dibromo and dichlorotyrosine derivatives, with some antibiotic activity against *Bacillus subtilis* and *Proteus vulgaris*. The sponges *Ircinia ramose* have antiviral, CNS stimulatory and antialgal properties. Sponge associated bacteria are capable of producing antibacterial metabolites. Surface associated bacteria with sponge *Ircinia ramosa* have shown Antibacterial activity [17].

Many natural compounds found in sponges are biosynthesized. It showed a great deal of interest in studying the bioactivities from sponges and in recent years, a number of novel compounds with such activities have been discovered through cultivation of sponge-associated microorganisms. Recent fact suggests that marine microorganisms, especially marine microbial symbionts, have become one of the research hotspots of marine microbiology and marine natural products [18].

Sponge-symbiont relationship is categorized as obligatory mutualism (i.e., the symbionts plays an essential role in the metabolism of their host), facultatively mutualism the sponge host provides a sheltered habitat for their symbionts. A further distinction is made between *epibionts* (microorganisms living on the sponge surface) and endosymbionts [19]. Marine invertebrates develop highly specific relationships with numerous associated microorganisms and these associations are of recognized ecological and biological importance [20]. Sponges harbour extraneous microorganisms on their surface, in their canal systems and in the intercellular matrix, which constitute a large part of the body, as much as up to 40% of the total biomass [21]. New pharmacologically active leads from the sponge *Theonella swinhoei* showed that macrolide displayed potent *in vitro* cytotoxicity against KB cells (human nasopharynx cancer) with an IC50 value of 6 nM [22]. In the present work it is revealed that all the marine sponges *Gelliodes fibrosa, Gelliodes carnosa, Mycale laevis, Mycale almata* contains various natural products such as the glycosides, cyclic peptides, sesterpenoids, phenolic compounds and sphingolipids. The sphingolipids is prevalent in all the four sponges and it is highly significant. Hence it could act as an effective bioactive compound.

CONCLUSION

The current study suggests that the crude methnolic extracts of marine sponges collected from coast of Tuticorin such *Gelliodes fibrosa, Gelliodes carnosa, Mycale laevis, Mycale almata* showed the presence of marine sponge derived natural compounds. The HPLC analysis using the crude extracts of sponges was done to evaluate and identify the presence of natural compounds. It is identified that the sphingolipids is seen in all the sponges. The present work emphasizes the role of marine sponges as an important source of leads for drug discovery. It is assumed that the sphingolipid acts as an effective bioactive compound. Thus the synthetic chemistry is required to develop high yield synthetic methods which are able to produce sufficient compounds for a broad biological screening in pharmacotherapeutic areas.

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