

# Production of Novel Bioactive Compounds by the Bacteria Associated with Some Marine Sponges of Gulf of Mannar and Palk Bay, Southeast Coast of India

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## Abstract:

**S**ponges, exclusively are aquatic and mostly marine, are found from the deepest oceans to the edge of the sea. There are approximately 15,000 species of sponges in the world, of which, 150 occur in freshwater, but only about 17 are of commercial value. A total of 486 species of sponges have been identified in India. In the Gulf of Mannar and Palk Bay a maximum of 319 species of sponges have been recorded. It has been proved that marine organisms are excellent source of bioactive secondary metabolites and number of compounds of originated from marine organisms had been reported to possess in-vitro and in-vivo immuno stimulatory activity. Extracts from 20 sponge species were tested for bacterial symbionts and bioactive compounds were isolated from such associated bacterial species in the present study.

**Keywords-** Gulf of Mannar & Palk Bay - Marine sponges – Associated bacteria – bioactive compounds

## I. INTRODUCTION

Sponges, exclusively are aquatic and mostly marine, are found from the deepest oceans to the edge of the sea. There are approximately 15,000 species of sponges in the world, of which, 150 occur in freshwater, but only about 17 are of commercial value [1]. A total of 486 species of sponges have been identified in India. In the Gulf of Mannar and Palk Bay a maximum of 275 species of sponges have been recorded. The distribution of sponges in other areas of India is in Gulf of Kutch – 25 species; and Orissa coast – 54 species [2]. Technologies have been developed to produce novel products from marine sponges; which could contribute to human healthcare (e.g. bioactive compounds that can be used for new medicines) to the food and feed industries (eg. Antioxidants) and to the energy industries with the applications of new biofuels. A variety of natural products from the marine sponges have been found to exhibit remarkable antitumour and anti-inflammatory activities ([3]; [4]; [5]; [6]; & [7]). It has been proved that marine organisms are excellent source of bioactive secondary metabolites and number of compounds of originated from marine organisms had been reported to possess in-vitro and in-vivo immuno stimulatory activity ([8]; [9]; [10]; & [11]) The world database on marine sponges is complex and exhaustive. A similar comprehensive and systematic inventory on marine sponges is required for India. Though two-thirds of the total Indian marine habitat have been covered, remote islands still remain untouched. To build a systematic inventory system requires systematic inventorying of marine invertebrates, which continues to be a limiting factor. Marine biotechnology aims to develop methods for producing novel products originating from marine organisms. Because of the diversity in ocean life many new potentially interesting compounds are continuously being discovered but their application relies on the development of technologies for their sustainable large-scale production. Many sponges produce bioactive compounds that have potential for being developed into drugs. Progress in the field of metagenomics, production of symbiotic microorganisms from sponges and sponge cell culture are essential.

## II. MATERIAL AND METHODS

### Description of the study area

The present investigation was based on sample specimens of sponges collected from the Gulf of Mannar and Palk Bay waters. Gulf of Mannar and Palk Bay are located in the vicinity of Mandapam (Lat. N. 9° 16' - 79° 9' E. Long.) a coastal town situated on a narrow strip of land leading to Rameswaram Island (Lat. N. 9° 17' - 79° 19' E. Long) (Fig.1a & 1b) on the southeast coast of peninsular India.

Palk Bay is on the northern side of Mandapam and to its south is the Gulf of Mannar. The waters of both sides meet at Pamban Pass (Lat. N. 9° 17' - 79° 12' E. Long) and Adam's Bridge. The Gulf of Mannar and Palk Bay area adjacent to each other and are connected throughout the year by the Pamban Pass. Palk Bay water flows from north to south during northeast monsoon and Gulf of Mannar water flows from south to north during southwest monsoon.

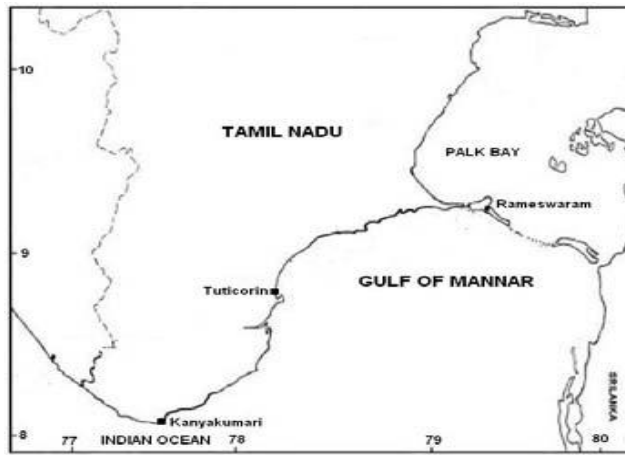


Figure-1a. Showing the Palk Bay and Gulf of Mannar waters in the vicinity of Mandapam

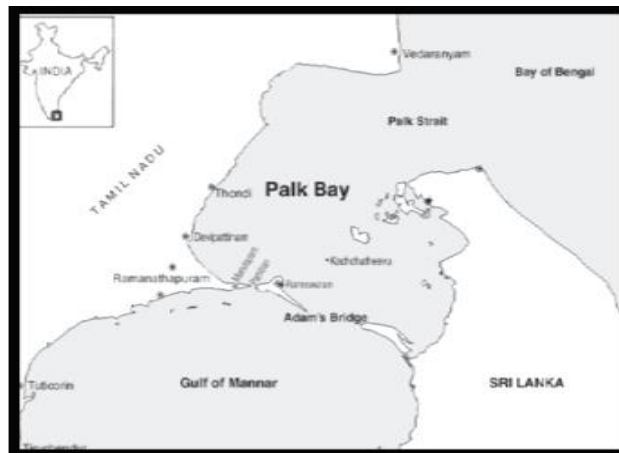


Figure-1b. Showing the Palk Bay and Gulf of Mannar waters in the vicinity of Mandapam

**Gulf of Mannar (GOM)**

Lying close together, along south of Mandapam Coast in the Gulf of Mannar, there are a few islands (Between 79° 3' and 79° 14' E. Long., and 9° 11' and 9° 18' N. Lat.), which are actually a part of a long chain of 21 small islands found along the 140 Km stretch between Rameswaram and Tuticorin [12].

They include Shingle, Krusadai, Pullivasal, Poomarichan, Manoliputti, Manoli and Hare Island. The Physiographic and Geographical location of these islands were given in Figure – 2 and Table -1.

The main importance of these islands lies in the fact that they have fringing coral reefs rising from shallow areas of seashore ([13]; [14] & [15]). These islands support sand cays and the entire complex is a discontinuous barrier reef system. Fringing reefs are located at a distance of 100 to 150 m from the islands and are narrow ([13]; [14]; [16] & [17]). Patch reefs arise from depths of 3 to 5 fathoms and are 1 to 2 Km in length with widths of 100 m. In the pearl bank beds, some colonies are seen at a depth of 12 to 27 m. The Gulf of Mannar has three distinct marine ecosystems namely corals, seagrass and mangroves. Around 3,600 species of fauna and flora have been identified in the Gulf of Mannar area including approximately 108 species of sponges [18].

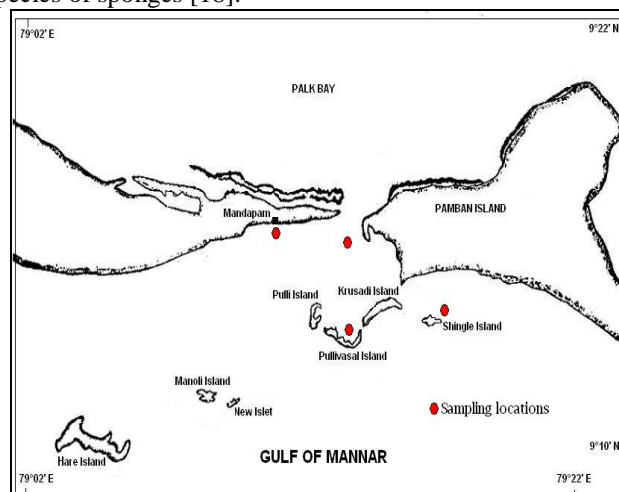


Figure-2. Showing the Physiographic and Geographical Location of Mandapam group of Islands

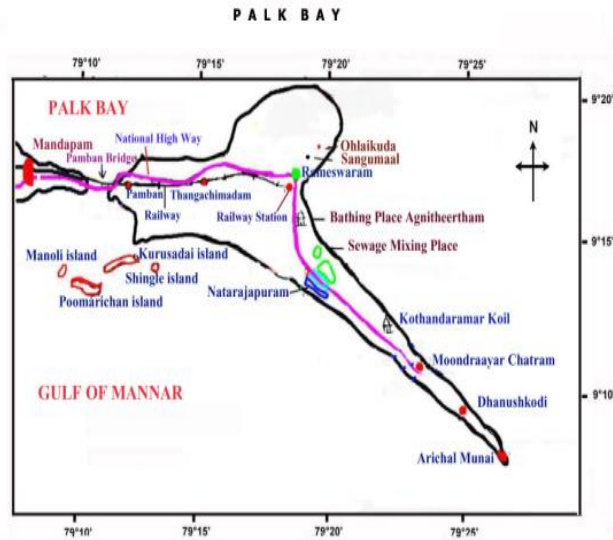


Figure-3. Showing Palk Bay region in the vicinity of Mandapam

### Palk Bay (PB)

This is a shallow basin with an average depth of 9 m and has mainly muddy bottom at inshore regions [14]. The depth at which reefs occurs ranges between 1 and 4 m. Coral reefs in the Palk Bay are of fringing type. They lie in an east-west direction along the northern side of Mandapam Peninsula and extend up to, and along the eastern side of Rameswaram Island. The reefs are 25 to 30 Km long and generally less than 200 m wide and extend from 79° 8' to 79° 20' E. Long along the 9° 17' N. Latitude and are discontinuous at Pamban.

Table 1. Physiographic and Geographical location of Coral Reef Islands of Mandapam Coast in the Gulf of Mannar

Serial No.	Name of Island	Approximate Distance from Mainland (Km)	Island Area (ha)	Geographical Location
1	Shingle	4 Km from Pamban	13	Lat.N 9° 15' – 79° 14' E.Long
2	Krusadai	3 Km from Pamban	67	Lat.N 9° 15' – 79° 12' E.Long
3	Pullivasal	5 Km from Mandapam Camp	30	Lat.N 9° 14' – 79° 11' E.Long
4	Poomarichan	5 Km from Mandapam Camp	17	Lat.N 9° 14' – 79° 11' E.Long
5	Manoliputti	6 Km from Mandapam Camp	2.5	Lat.N 9° 13' – 79° 07' E.Long
6	Manoli	6 Km from Mandapam Camp	26	Lat.N 9° 13' – 79° 07' E.Long
7	Hare	7 Km from Mandapam Camp	129	Lat.N 9° 12' – 79° 05' E.Long

[Source: Deshmukh and Venkataramani (1995), and Kumaraguru (1997)]

The reef which runs parallel to the northern side of Mandapam coast alone is about 7 Km in length and is found distributed at a distance of 200 to 600 m away from the shore at different places (Fig.1). A narrow navigational channel, almost at the mid-length of the reef, separates it into eastern and western reefs. The eastern sector of the reef terminates at Pamban Pass and is called 'Kadhuvaalimunai reef' and the western sector is called the 'Vellaperkkumunai reef'. Palk Bay is rich in biodiversity having all the important groups of flora and fauna in its environment. A total of 275 sponge species have been recorded including 31 endemic form in the Palk Bay region (Figure-3).

### Diversity of Sponges in Gulf of Mannar and Palk Bay.

India has a long coastline (6100 km) which may house new deep-water species assemblages not previously known to science. Our knowledge of the sponge fauna has marginally increased in this decade as a result of enhanced collection efforts driven by pharmaceutical interests. Very few taxonomic papers have been published from Indian waters. A total of 486 species of marine sponges have been described in India. The Gulf of Mannar and Palk Bay region have the highest diversity (319 species), followed by Andaman and Nicobar Islands (95 species), Lakshadweep (82 species), Orissa coast (54 species), and Gulf of Kachchh (25 species).

### Collection of sponges and their identification

Water in the Gulf of Mannar side becomes turbid due to southwest monsoon winds and currents during the months from May to August. On the other hand, water in the Palk Bay side gets turbid due to northeast monsoon winds and currents during the months from September to April. Hence, fishing activities are intense in the Gulf of Mannar side during northeast monsoon season and in the Palk Bay side during the southwest monsoon season. Thus fishing activity is carried out around Mandapam throughout the year. Hence specimens of sponges can be collected from GOM from September and April and in Palk Bay from May to August.

**Underwater collection of sponges using SCUBA Diving Technique.**

Sample specimens of the sponges were directly collected from the Gulf of Mannar and Palk Bay waters. This was done by snorkeling, skin diving and SCUBA diving techniques. *In-situ* underwater observations were made to locate the habitat of sponges in the Gulf of Mannar and Palk Bay waters of Mandapam coast. Snorkeling and skin diving techniques were used to observe shallow waters up to 3m, and beyond this depth SCUBA diving, technique was used.

Underwater equipment such as, lycra suit, face mask, snorkel, underwater watch, underwater knife, full-foot fins and gloves were used for snorkeling and skin diving operations/observations. SCUBA cylinder / aqua lung, SCUBA buoyancy control device, full-foot fins, face mask, neoprene/lycra suit, underwater slate, underwater pencil, underwater still camera, underwater video camera and other related accessories were used for underwater-diving operations/observations.

A team of three members (including 2 certified SCUBA divers) was involved in the operation. Team members used snorkeling and skin diving techniques up to 3 m depth to observe and collect the sponges underwater. Spot diving was carried out in and around the northern most seven islands (Shingle, Krusadai, Pullivasal, Poomarichan, Manoliputti, Manoli and Hare) in the Gulf of Mannar and also in the Palk Bay.

There were 21 spots (3 in each of the seven islands) in the Gulf of Mannar (from September -2013 to April 2014) and 4 spots in the Palk Bay (May-2013 to August-2013). The diving spots were fixed using Global Positioning System (GPS - Garmin Model 12 XL) and indicated in Table-2. Between May 2013 and April 2014, about 50 SCUBA (2 in each of the 25 spots) dives were done, all during daytime between 0730 and 1130 hours.

**Collection of sponges from fishing boats**

The specimens of sponges were also collected from the fishing boats that are using trawl net, since the sponges were also caught in the trawl net along with fishes, Therefore, the fishermen of Mandapam region (fishing both in the GOM and Palk Bay) were requested to collect them for the present study. The collected specimens were brought to landing centres of the Gulf of Mannar and Palk Bay along with other edible reef fishes caught in the trawl nets. These landing centers included Mandapam

Table-2. Location of various diving spots selected for *in-situ* observations of habits and habitats of *C.collare* in the Gulf of Mannar and Palk Bay

S.No.	Name of Island	Diving Location (using GPS)
1	Shingle Island	NO9° 14' 43.2" EO79° 13' 46.9"
2		NO9° 14' 32.8" EO79° 13' 48.2"
3		NO9° 14' 43.2" EO79° 14' 20.3"
4	Krusadai Island	NO9° 15' 02.5" EO79° 13' 26.5"
5		NO9° 15' 10.5" EO79° 12' 35.0"
6		NO9° 14' 50.8" EO79° 13' 10.8"
7	Pullivasal Island	NO9° 14' 32.0" EO79° 10' 54.0"
8		NO9° 14' 32.4" EO79° 12' 03.7"
9		NO9° 14' 18.9" EO79° 12' 02.3"
10	Poomarichan Island	NO9° 14' 54.7" EO79° 10' 47.1"
11		NO9° 15' 07.0" EO79° 10' 45.7"
12		NO9° 15' 00.4" EO79° 10' 40.6"
13	Manoliputti Island	NO9° 12' 29.2" EO79° 08' 30.0"
14		NO9° 13' 02.7" EO79° 09' 20.0"
15		NO9° 13' 23.1" EO79° 09' 14.5"
16	Manoli Island	NO9° 11' 43.0" EO79° 05' 46.1"
17		NO9° 12' 12.5" EO79° 07' 35.1"
18		NO9° 13' 24.6" EO79° 08' 17.0"
19	Hare Island	NO9° 12' 45.4" EO79° 05' 00.6"
20		NO9° 12' 30.9" EO79° 04' 34.1"
21		NO9° 11' 28.8" EO79° 03' 46.7"
22	Palk Bay	NO9° 17' 27.4" EO79° 10' 39.8"
23		NO9° 17' 32.2" EO79° 10' 39.0"
24		NO9° 17' 36.7" EO79° 09' 18.0"
25		NO9° 17' 47.9" EO79° 08' 40.0"

South (behind Hotel Tamil Nadu), Mandapam Jetty (INP Jetty), Thonithurai South, and Pamban South in the Gulf of Mannar side, and Theedai, Mandapam North, Thonithurai North, and Pamban North in the Palk Bay side. Detailed information about the landing centres, crafts (number of boats selected for collection of specimens), gears, distance and depth of collection, and area covered were given in Table-3. The samples sponge species collected from the GOM and PB were placed inside sterile ethyl polythene bags underwater and transferred to the lab aseptically in ice boxes. They were stored in laboratory in sterile conditions. They were identified based on their taxonomic position. They were identified by comparing with specimen available in the Museum of CMFRI, Mandapam and the voucher specimens have been deposited in the department laboratory.

#### Isolation of bacteria from sponges

The sponge sample was homogenated and serially diluted up to 10<sup>-6</sup> dilutions and plated on the surface of Zobell marine agar. The plates were incubated at room temperature for 24-48 hrs. The isolates were identified using taxonomic, physiological and biochemical methods based on Bergey's manual of systematic bacteriology [19].

#### Isolation of marine bacteria

Sponge associated bacteria were isolated by following the method outlined by [20]. Initially, the sponge samples were washed with jets of filtered and autoclaved seawater until they were visibly free of debris. Then the sponge surface was sterilized by a rapid wash of 70% ethanol and immediately immersed in autoclaved and filtered seawater and then aspirated. One gram of sponge tissue was removed with a sterile scalpel and the tissue was immediately transferred to 99 ml sponge dissociation medium (2.7% NaCl, 0.008% KCl, 0.01% Na<sub>2</sub>SO<sub>4</sub>, pH 8).

The samples were soaked for 20 minutes and then the tissue and diluents were macerated and the homogenate was plated on Zobell marine agar 2216 (Himedia, Mumbai), using a dilution series of 10<sup>-5</sup>. The plates were incubated at room temperature (approx. 27–30<sup>o</sup> C) for 7 days and isolation of bacteria with different colony characteristics was carried out from the third day onwards up to the seventh day. Day 7 counts were used for the calculation of colony forming units (CFU). The isolated colonies were repeatedly streaked to obtain pure cultures and stored in Zobell agar slants at 4<sup>o</sup>C for further studies.

HPLC fractionation of the ethyl acetate extract Constituents in the crude ethyl acetate extract were separated on a semi-preparative reverse phase C-18 (VYDAC) column (10\_250 mm), attached to a LKB-BROMA 2150 HPLC system, using a water-methanol gradient (10–90%) and at flow rate of 1.5 mL. Constituents eluting from the column were detected at 254 nm using a Shimadzu SPD-6AV UV–VIS detector. The different fractions were collected, pooled and concentrated in vacuo. The concentrated fractions were identified.

Table-3. Details field of sponges collected from fishes boats and fish catch area in the Gulf of Mannar and Palk Bay

Landing centers		Craft used	Number of Boats Selected	Gears used	Distance from Mainland	Depth of Fish Catch	Area covered
<b>No.</b>	<b>Gulf of Mannar</b>	Plank Built Boat	7	Encircling Nets t	8 to 10Km	1 to 2m	Hare and Manoli Island
1	Mandapam South (Behind Hotel Tamil Nadu)						
2	Mandapam Jetty						
3	Thonithurai South						
4	Pamban South	Plank Built Boat	5	Encircling Nets	5 to 8 Km	1 to 2m	Shingle, Krusadai and Pullivalal Island
<b>No.</b>	<b>Palk Bay</b>	Plank Built Boat	3	Encircling Nets	300 to 800 m	1 to 2m	Entire western Sector of Palk Bay Reefs
1	Theedai						
2	Mandapam North						
3	Thonithurai North						
4	Pamban North	Plank Built Boat	5	Encircling Nets	300 to 900m	1 to 2m	Eastern Sector of Palk Bay Reefs

### III. RESULTS AND DISCUSSION

List of bacterial species isolated from the marine sponge species have been indicated in the Table- 1. There are about 20 sponge species such as, *Sigmatocia fibulatus*, *Sigmatocia carnosa*, *Spongia officinalis*, *Spongia sp. Stellata tenui*, *Spirastrella inconstans var moeandrina*, *Spirastrella inconstans var digitata*, *Spirastrella inconstans var globosa*, *Spirastrella vagabunda*, *Spirastrella pachyspira*, *Carinella australiensis*, *Callyspongia diffusa*, *Callyspongia fibrosa*, *Callyspongia reticulis var solomonensis*, *Cinachyra cavernosa*, *C.lobata*, *Hyattella cribriformis*, *Haliclona exigua* and *Haliclona cribricutis*, have been collected from Gulf of Mannar and Plak Bay waters (Table-1). The availability of all the 20 species of sponges have already been reported by ([13]; [14]; [15]; [16] & [17])

#### Bioactive compounds

The concentrated fractions were obtained through HPLC were identified. *Pseudomonas aeruginosa*, *Streptococcus aureus*, *Vibrio cholera*, *Vibrio parahaemolyticus* and *E.coli* were isolated from marine sponge *Sigmatocia fibulatus* and bioactive compounds such as lauric acid, myristic acid, stearic acid and Oleic acid were separated from the bacterial species respectively. Similarly Lauric acid has been separated from the *Pseudomonas aeruginosa* isolated from *Callyspongia diffusa*, *Sigmatocia carnosa*, *Pertosia nigricans*, and *Ircinia fusca*. Likewise, Oleic acid has also been separated from the *E.coli* isolated from the sponges *Dysidea avara*, *Plakortis simplex* and *Spongia officinalis*. The results of the study corroborates with study reports of ([21] – [32]).

### IV. CONCLUSION

Sponges exhibited secondary metabolite producing-marine bacteria with antibacterial potential against pathogenic strains. Harnessing the chemical potential of sponge-associated microbes could overcome the acknowledged supply problem with marine natural products and create an essential inexhaustible source of novel drugs from the sea. Further works are needed to clarify the antibacterial compounds responsible for the growth inhibition toward pathogenic strains.

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