Isolation and Partial Purification of Bioactive Compounds from Sponge *Sigmadocia Fibulata* (Schmidt) Collected from West Coast of Mumbai, India

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The sponge *Sigmadocia fibulata* (Schmidt) was collected during low tides from West Coast of Mumbai. Crude extract was obtained by taking 10 gram of sponge samples in 10 ml of methanol. The preparative TLC (Thin Layer Chromatography) was performed by using Toluene: Ethyl acetate: Diethylamine (7:2:1) (v/v). The isolated compounds were subjected to GC-MS and FTIR analysis. The structural properties of bio active compounds were determined. From the structural determination it was confirmed that *S. fibulata* contains bioactive compounds as Triacontanoic acid, methyl ester – (Skin irritant), Hexadecanoic acid, 2-hydroxy- (hydroxymethyl) ethyl ester – (Fatty acid, Metabolite and Irritant) and 2-Nitro-1, 3-bis-oclyoxy-benzene, (A natural product found in Neolitsea daibuensis. It has a role as a plant metabolite and an algal metabolite). From their biological properties it was confirmed that *S. fibulata* contains bio active compound, which has biomedical and pharmaceutical properties.

**Keywords:** Analytical Study; Bioactive compounds; Sponge.

Sponge contains a large number of unique and diverse natural products. For medicinal use many sponges have contributed the unique bioactive compounds. The arrays of secondary metabolites of sponges are ranging from amino acids and nucleotides to peroxides and sterols. Many bioactive compounds have immense pharmacological properties, which includes anti tumor, viral, fungal, anti-bacterial and many others. Some of these properties are now in clinical and preclinical trials. Many natural products were also played important role in biological systems such as reproduction and play a crucial role as defense against predators, competitions for space, prevention of fouling and useful for the preparation of many antibiotics such as plakortin and manoalide from marine Sponges. From living marine fauna, more than 60% of potentially bioactive compounds have been obtained till 1999 of which 70% of the bioactive compounds were isolated from sponges. Throughout the globe, around 15000 different species of sponges were identified of which around 150 species were reported as freshwater sponges. Nearly 5000 different bioactive compounds were extracted from 500 different species of sponges of which 17% of sponges have commercial and traditional use including cosmetics. Amongst the marine invertebrates, around 486 different species...
of sponges were reported in Indian waters. In sponges, the class Demospongiae is well known for the production of secondary metabolites which have diverse function. The interest of Marine natural products as one of the few de novo sources of drug discovery worldwide. However, in India, the bioactive potential from marine animals has been studied very scanty. In India the work carried on bioactive compounds by NIO Goa and others were from the coast of south and south-east India. The work on bioactive compounds from west coast of India has been carried out by CIFE, Mumbai, and no efforts have gone into unraveling the details on bioactive compounds. studied the bioactive compounds from intertidal crab Leptodius exratus from Nariman point, west coast of Mumbai. Therefore an initial effort has been initiated for the isolation and partial purification of secondary metabolites from marine sponge S. fibulata (schmidt) from west coast of Mumbai.

MATERIALS AND METHODS

Samples collection
The sponges S. fibulata was collected from Nariman Point, west coast of Mumbai during low tides. The sponges were brought to the laboratory in seawater and then washed with distilled water and sun dried.

Identification of sponges
Initial Preliminary identification was done by referring the literature. The shape and size of spicules were studied to confirm the specimen. The confirmation of identification was done by Dr. P.A. Thomas, Central Marine Fisheries Research Institute (CMFRI), Thiruvananthapuram, Kerala.

Preparation of Sponge Extracts
The sponge extract was obtained by using the method as proposed by Braekman et al., 1992 with some modifications.

The extraction of sponge sample was done by following the method of with some modifications. 10 grams of sponge sample was added in 10 mL of methanol and kept standing for 24 hrs in conical flask by covering the mouth of the conical flask with stopper. After 24 hrs the sponges squeezes and made the suspension in methanol. The sample was then filtered through Whatman filter paper No.1. The sample so obtained was evaporated at low pressure by using Rotary Vacuum Evaporator at 45p C. The resultant compound so obtained was subjected to Millipore filter and finally dried in a vacuum desiccator and stored at 4p C in a refrigerator till further use.

Ethical approval
Ethical approval for the collection of S. fibulata was sought from the Maharashtra State Biodiversity Board, Nagpur and the voucher specimen of S. fibulata was sent to the repository center at NIO Goa, India, to deposit the sample. The Voucher numbers of the said specimen is 1-NIO1006/18 (S. fibulata).

TLC Analysis
Model Chem. Tech, HPTLC (High Performance Thin Layer Chromatography) available at Ancrom test lab. Mulund, Mumbai was used for the analysis of samples.

The aluminum TLC plates pre-coated with silica gel (60 F254) with 0.5 mm thickness were used for the experiment. The dried plates were charged and 10 mL of sponge crude extract was loaded on the plate and allowed the sample to dry at 100oC. The loaded plate then emerged in the saturated twin trough chamber containing Toluene: Ethyl acetate: Diethylamine (7:2:1) (v/v) as a mobile phase. The sample spots were run up...
to 9 cm length in the mobile phase. The plate was removed from twin trough chamber and dried at 110°C and dragendorff reagent was sprayed to develop the color spots. The spots were so obtained were observed under Deutorium lamp at 254 nm of the densitometric scanner. The spots scrapped off and dissolved in methanol. The pure samples were so obtained were filtered through Millipore filter and evaporated to dryness to get the pure compounds. The pure samples further processes for analytical studies.

**GC-MS (Gas chromatography – Mass Spectrometry) Analysis**

The dried powdered form samples were analyzed at IIT (SAIF) Powai, Mumbai. Analysis was performed with Jeol model Accu TOF GCV, GC MS (EI +ve or –ve) was used. The mass range of the spectrometer was ranges from 10 - 2000 amu at a mass resolution of 6000.

**FTIR (Fourier Transform Infrared Spectroscopy) Analysis**

The dried isolated powdered sponge samples analyzed at IIT (SAIF) Powai, Mumbai. Analysis was performed with Bruker instrument corporation Germany make, 3000 Hyperion Microscope with Vertex 80 FTIR model was used. The scanning was done in the range of 4000-900 cm⁻¹.

Analytical grade solvents and chemicals were used from M/S. S.D. fine chemicals, Thane, India.

**RESULTS AND DISCUSSION**

Many marine sponges harbor a large number of secondary metabolites possessing a broad-spectrum of pharmacological applications have not yet been discovered. There are few sponges such as *Hyrtios* species, *H .erectus*, *H. reticulatus*, *H gumminae*, and *H.tubulatus* discovered bioactive compounds which have promising antifungal, antibacterial and antitumor properties¹⁴. The study carried out by ¹⁵ have obtained many compounds as Sterols (22ee)-24a-methyl-cholest-48(9), 22(23)-triene-3a,713-diol(1) and (22e)-24 amethylergost-6, 22(23)-diens-5a, 8a-epidioxy-3/3-ol(2) a fatty-acid nonadecanoic acid and aoily ester methyl nonadecanoate are the

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**Photograph No.1.** TLC of crude extract of Sponge *S. fibulata*

**Photograph No. 2.** Graph showing isolated compounds of Sponge *S. fibulata*
leab compounds where as complex compounds as (22e)-ergost-6, 22(23), 24(28)- triene-5a, 5a-epidioxy-3/3-o1 (3) and ergosterol (4) are the extra compounds present in sponge *Suberites carnosus*. 16 have identified a major parasite class of sponge called dàmospongiaá is well-known for generating the diverse and biggest number secondary metabolites amongst the marine invertebrates. Many sponges derived bioactive compounds as ara-a (anti-viral), ara-c (anti-cancer) and manoalide (phospholipids a 2 inhibitor), IPL512602 (anti-inflammatory), KRN 7000

![Fig. 3. Gas Chromatogram of the Isolated compound G1 to G3, of *S. fibulata*](image1.png)

![Fig. 4. Compound G1](image2.png)

![Fig. 5. Compound G2](image3.png)
Fig. 6. Compound G3

Fig. 7. FTIR spectra of isolated compound 1 of *S. fibulata* at Rf values G1- 0.029

Fig. 8. FTIR spectra of isolated compound 2 of *Sigmadocia fibulata* at Rf values G2- 0.137

Fig. 9. FTIR spectra of isolated compound 3 of *S. fibulata* at Rf values G3-0.773
The study carried out by 19 found the antinocicapta and anti-inflammatory compounds from marine sponge Caissera. The structurally promising secondary metabolites of different genus of sponge Haliclonal have been derived by 20. The important pharmaceutical compounds have been identified by 21, 22 and they have identified the inhibition of HIV by two bis-quinolizidine alkaloids petrosins from Indian sponge Petrosia similis which inhibited HIV-1 replication. The study carried out by 23, reported the gorgoster type sterol lacking methyl substitution of the cyclopropane ring system, namely 22,23-methylene (22S, 23S) cholesterol 3 along with known compounds have been identified from marine sponge S. fibulata. The previous work have also been carried out on same sponge by 24, 25 determined the mixture of steroids and peptides for the first time.

**Characterization of isolated extracts of Sponge by HPTLC**

The extracts isolated from the S. fibulata (Schmidt) collected from Nariman Point Mumbai coast are spotted on HPTLC plates and the plates were developed in a twin-trough chamber using I - butanol: methanol: water in the proportion of 3:1:1 (v/v), as a mobile phase. The plates were dried and sprayed with the dragendorff reagent. The photograph of the HPTLC plate of S. fibulata (Schmidt) and is shown in Photograph No.1, and 2. The spraying reagents gave positive tests for the presence of amides. The distinct and well-separated three spots were at Rf values of S. fibulata are G1- 0.029; G2- 0.137and G3-0.773.

The densitometric scanning of these spots resulted in the quantification of these substances are found to be S. fibulata (67.69 %); (2.64 %); and (29.67 %) respectively. The pure compounds were isolated by scraping the spots into methanol. Pure compounds were obtained by evaporating the solvent methanol by vacuum desiccators. These compounds are then characterized by FTIR analysis.

**Characterization of isolated extracts of sponge on GC-MS**

GC-MS of the extracts isolated from the S. fibulata have been performed and the results are presented in Fig. No.1. the gas chromatograms of the extracts of the S. fibulata shown in Fig No. 1 G1 to G3, indicate that there are a large number of peaks. However the peaks at Rt value of S. fibulata

### Table 1. Correlation of IR spectra of the compound isolated from S. fibulata at Rf value 0.029 on TLC

| Sr. No. | Wave number: cm⁻¹ intensity | Type of IR vibrations | Probable group Assignment |
|---------|-----------------------------|-----------------------|---------------------------|
| 1       | 3826.10                     | N-H- streatching vibration | O-H, N-H group |
| 2       | 3750.62                     | N-H- streatching vibration | O-H, N-H group |
| 3       | 3445.73                     | N-H- streatching vibration | O-H, N-H group |
| 4       | 2956.73                     | N-H -streatching vibration | O-H, N-H group |
| 5       | 2920.15                     | N-H -streatching vibration | O-H, N-H group |
| 6       | 2850.82                     | N-H -streatching vibration | O-H, N-H group |
| 7       | 1739.03                     | C=N -streaching         | C=0,C=C, C=N group |
| 8       | 1632.18                     | C=N- streatching        | C=0,C=C, C=N group |
| 9       | 1464.50                     | C=N- streatching        | C=0,C=C, C=N group |
| 10      | 1383.22                     | C=N -streatching        | C=0,C=C, C=N group |
| 11      | 1217.97                     | C-O - stretching        | C-O group |
| 12      | 1119.42                     | C-N stretching          | C-N group |
| 13      | 1022.33                     | -                      | C-C group |
| 14      | 924.02                      | -                      | C-C group |
| 15      | 861.17                      | C-H bending            | Rock |
| 16      | 418.68                      | -                      | Rock |
Table 2. Correlation of IR spectra of the compound isolated from *S. fibulata* at Rf value 0.137 on TLC

| Sr. No. | Wave number: cm⁻¹ | Type of IR vibrations | Probable group Assignment |
|---------|-------------------|-----------------------|---------------------------|
| 1       | 3434.32           | N-H stretching        | O-H aliphatic primary amine |
| 2       | 2919.55           | C-H stretching        | C-H Alkyl stretch          |
| 3       | 2849.99           | C-H stretching        | C-H group                 |
| 4       | 1736.87           | C=O stretching        | C=O group                 |
| 5       | 1631.94           | C=C stretching        | C=C alkene group          |
| 6       | 1466.40           | C-H bending           | C=N group                 |
| 7       | 1413.15           | C-H bending           | C=N group                 |
| 8       | 1388.86           | C-O                  | C-O group                 |
| 9       | 1312.04           | C-O                  | C-O group                 |
| 10      | 1259.82           | C-O stretching       | C-O alky aryl ether group |
| 11      | 1199.07           | C-O stretching       | C-C group                 |
| 12      | 1023.97           | C-C                  | C-C group                 |
| 13      | 921.70            | C-C                  | C-C group                 |
| 14      | 862.66            | C-C                  | C-C group                 |
| 15      | 15                | Rock                 |                           |

Table 3. Correlation of IR spectra of the compound isolated from *S. fibulata* at Rf value 0.773 on TLC

| Sr. No. | Wave number: cm⁻¹ intensity | Type of IR vibrations | Probable group Assignment |
|---------|-----------------------------|-----------------------|---------------------------|
| 1       | 1500-1600                   | C-C                   | O-H, N-H group            |
| 2       | 1050-1150                   | N=O                   | O-H,N-H group             |
| 3       | 2190-2140                   | C-O                   | C-H group                 |
| 4       | 1000-1350                   | C=C                   | C-H group                 |
| 5       | 2880-2900                   | C-N                   |                           |
| 6       | 3300                        | C-H                   |                           |
Table 4. Showing Name of the compound Molecular Weight, Molecular formula, and Structure of the compounds isolated from *S. fibulata*

| S. No. | Name of the compound | Molecular Weight | Molecular formula | Structure |
|--------|----------------------|------------------|-------------------|-----------|
| 1      | Triacontanoic acid, methyl ester | MW-466 | C_{31}H_{62}O_{2} | ![Image](image1.png) |
| 2      | Hexadecanoic acid, 2-hydroxy 1-(hydroxymethyl)ethyl ester | MW-330 | C_{19}H_{38}O_{4} | ![Image](image2.png) |
| 3      | 2-Nitro-1,3-bis-octyloxy-benzene | MW-379 | C_{22}H_{37}NO_{4} | ![Image](image3.png) |

that a large number of components are present in each of the isolated extracts *S. fibulata* (Schmidt). However, for the present study we have selected the components of GC peaks at Rt values of *S. fibulata* (Schmidt) are 28.18; 28.20 and 28.21 only, because they gave some conclusive evidence to identify the nature of the components on the database library. Therefore, the respective eluents at these Rt values were irradiated into the Electron impact (EI') source of the mass spectrometer by which the molecules lose one electron and form positively charged molecules. Thus the molecules separate in ion trap mass spectrometer. The MS spectra shown in FigNo, 2 to 4, indicate that the mass peaks are due to the compounds having molecular weight of *S. fibulata* is 466, 330, and 374 respectively. These mass spectra are correlate to the Compounds as Triacontanoic acid, methyl ester – (Skin irritant), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester – (Fatty acid, Metabolite and Irritant) and 2-Nitro-1, 3-bis-octyloxy-benzene, (A natural product found in Neolitsea daibuensis. It has a role as a plant metabolite and an algal metabolite) of *S. fibulata*. The structures of these molecules are as shown in Table No. 4.

The FTIR studies are carried out in KBr pallets. Each of the compounds isolated at Rf values at G1- 0.029; G2- 0.137 and G3-0.773 on TLC for each of the isolated compounds of *S. fibulata* extracts gave the IR spectras as shown in Fig No 5 to 7. All the IR spectra’s of the compounds at the respective Rf values mentioned above are found to be dissimilar and different of the extracts isolated from *S. fibulata*. The wave numbers of some of the important IR peaks along with their intensity, type of vibration and probable groups present in the respective compounds isolated at Rf values G1- 0.029; G2- 0.137 and G3-0.773 are as shown in table 1 to 3.

**CONCLUSION**

From the above results it is concluded that, the compounds extracted from sponge *S. fibulata* showed antibacterial, pesticidal, and biomedical properties as we have studied it well. Our findings
are significant for the development of multi-drug therapy for both pharmaceuticals and biomedical applications. Therefore we have screen the crude extracts of sponges for its structural elucidation to find the new drugs for pharmaceutical industry. The study further suggests that, *Sigmadocia fibulata* further screening is required for molecular level to understand the physiology and mode of action of the compound. The clinical study is also required which may be useful for pharmaceutical industry to manufacture the new drugs for safe performance and safety indexes to be studied to eradicate the diseases from mankind in future.

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**Conflict of Interest**

There is no conflict of interest.

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