

Antibacterial and Pesticidal activity of Marine Sponges *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) collected from West Coast of Mumbai, India

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Abstract

Marine sponges are on focus for their ability to produce compounds with antimicrobial activity. The aim of the current study was to screen extracts derived from marine sponge *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) for their activity against selected bacterial fungal and pesticidal pathogens. The crude extract derived from *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) were found potent against tested *E. coli* and *P. mirabilis* bacteria. The crude extract of both the sponges showed pasticidal activities against *Periplanata Americana* and *Sitopbilus oryzae*. No activity was noted against fungus when tested with crude extract of *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston). Thus confirms that the crude extract of *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) have antibacterial and anti pesticidal property.

Keywords: Bacteria, fungus, Sponge, bioactivity, bacteria, pest, fungus.

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INTRODUCTION

Sponges are simple multicellular sessile animals with no true tissue layers or organs [1]. They populate tropical and subtropical benthic marine habitats but are also found at higher latitudes and even in freshwater lakes and streams [2]. As sessile filter feeders, they pump large volumes of water through a specialized canal system, known as its aquiferous system [1]. Marine sponges are a rich source of structurally novel and biologically active metabolites [3]. Over 60% of potentially useful bioactive compounds discovered so far from living organisms have been obtained from marine fauna, 70% of which comes from sponges [4]. There are approximately 15,000 different species of sponges throughout the globe, 150 of which occur in freshwater, but only about 17 present have commercial value for traditional use, including the cosmetic industry. A total of 486 sponge species have been found in Indian waters [5]. The sponge class Demospongiae is known for producing the largest number and diversity of secondary metabolites isolated from marine invertebrates [6]. There is a worldwide interest in marine natural products as one of the few de novo sources of drug discovery [7].

The earth's oceans are the last great frontier in the search for plants and organisms with pharmaceutical

value [8]. The world health organization (WHO) approximates that 80% of the worlds inhabitants depend mainly on traditional medicine for their primary health care [9]. Medicines of natural products origin are collected from variety of sources, including terrestrial plants, terrestrial microorganism, marine animals and terrestrial vertebrates and invertebrates [10]. Natural products and other related synthetic analogues are used to treat 87% of all categorized human diseases, and the discovery of novel agents is often used to better understand targets and pathways in disease process [11].

Studies on bioactive compounds are being extensively studied in Japan, China & other western countries, but in the developing countries like India, Marine organisms have been explored for the isolation but have not been explored for its structural elucidation. The Indian sub-continent, having a coastline of above 8014km, offers potential for pursuing marine biotechnology research for discovering novel biologically active compounds that could be used in a large spectrum of human ailments and harvest bio resources for sustainable development with this context. Indian labs have concentrated on bioactive substances from marine animals such as horseshoe crab, green mussels, sponges and corals for characterization of

novel molecules. The Department of Biotechnology (DBT) has been promoting marine biotechnology in India for the last one and half decade. Many R&D programs sponsored on marine biotechnology are leading towards products and process developments and development of a viable technology for the commercial production systems.

The work carried out by NIO [12] and others consist of bioactivity of the compounds collected from the coast of south and south – east India. A very scanty work has been reported by Venkateshvaran [13]; - CIFE Mumbai, & no effort has gone into unraveling the details on the bioactive compounds. However, Zodape and Kulkarni [14] have been explored for the isolation of bioactive compounds from intertidal crab *Leptodius exaratus* of Mumbai coast and have concluded that, the Mumbai coast is under deterioration due to the presence of dinoflagellates and bacteria which are eaten by the marine animals causing the presence of toxic compounds in the body. Therefore by taking an account of these studies we have collected the sponges from west coast of Mumbai to explore the antibacterial and Pesticidal activity of *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston).

MATERIALS AND METHODS

Collection of Samples

The sponges *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) were collected during low tides from West Coast of Mumbai. Animals were taken alive to the laboratory in seawater washed under sea water and then with distilled water and sun dried.

Identification of Sponges

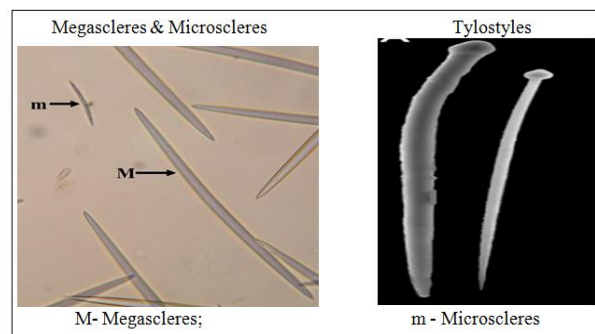
Preliminary identification was done by studying the shape and size of the spicules and by refereeing the relevant literature. The confirmation of identification was done by Dr. P. A. Thomas, Principal Scientist, Central Marine Fisheries Research Institute (CMFRI), Thiruvananthapuram, Kerala.



***Sigmadocia fibulata* (Schmidt, 1862)**



***Suberites carnosus* (Johnston, 1842)**



Preparation of Sponge Extracts

Crude extract was obtained following the method of Braekman et al., 1992 [15] with some modifications. To 10 gram of sponge samples, 10 ml of methanol was added and kept standing for 24 hrs. Solvent were then removed, by squeezing sponge samples, and filtered through Whatman filter paper No.1. The remaining solvent was evaporated at low pressure using Rotary Vacuum Evaporator at 45^o C. The resultant compound was subjected to Millipore filter system and finally dried in a vacuum desiccator and stored at 4^o C in a refrigerator till further use.



***Sigmadocia fibulata* (Schmidt, 1862)**



Suberites carnosus (Johnston, 1842)

Ethical Approval

Ethical approval is received by Maharashtra State Biodiversity Board, Nagpur for collection of sponges for research purpose. The voucher specimens of *Sigmadocia fibulata* and *Suberites carnosus*, was deposited at the repository centre at NIO Goa, India, as per the directions by Maharashtra State Biodiversity Board. The Voucher numbers of the said specimens are 1-NIO1006/18 (*Sigmadocia fibulata*) and 2-NIO1007/18 (*Suberites carnosus*).

Procurement of bacterial and fungal cultures

The pure culture of bacteria *E. coli*, *P. mirabilis*, *S. aureus*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *E. faecalis*, *S. Typhi*, *Candida albicans*, and *A. niger* were collected from The Department Of

Microbiology, Patkar –Varde College, Goregaon (w), Mumbai and APX Laboratories, Thane, Maharashtra . The culture was stored in a refrigerator at 2-8 °C. The nutrient media prepared by dissolving 5g of peptone, 3g beef extract, 8g sodium chloride and 150g agar in about 800ml of water and adjusting the pH of the solutions to 7.3 by drop wise addition of 1N sodium hydroxide. The solution heated 2-3 minutes, cooled and diluted to 1 liter with distilled water. All the apparatus such as syringes, pipettes, conical flasks, Petridishes and the nutrient media sterilized in an autoclave before their use. A basic culture medium used for growing bacterial culture under laboratory condition as proposed by Pelczar [16]. The pure culture of bacteria *E. coli*, *P. mirabilis*, *S. aureus*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *E. faecalis*, *S. Typhi*, *Candida albicans*, and *A. niger* were spread in sterile petridishes by streaking method. Total Ten petridishes were prepared. In all the petridishes, the crude extract of spong *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) was added on marked areas of all the petridishes by putting a whatman filter paper No. 40 (5 mm diameter) on the marked area of each of the petridish. 0.1 ml of each of the crude extract placed on filter paper by injection vile. Control also maintained by adding methanol to the respective petridishes. Then petridishes kept for incubation for 24 hours at 37°C.

RESULTS AND DISCUSSION

Table-1: Effect of crude extract of *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) showing zone of inhibition in Bacterial and Fungus Strains

Sr. No	Name of bacteria	Presence/Absence	Zone of Inhibition(Diameter inmm)	
			<i>Sigmadociafibulata</i>	<i>Suberites carnosus</i>
1.	<i>Streptococcus pneumoniae</i>	Absent	-	-
2.	<i>Coryne bacterium</i>	Present	2.5	4.5
3.	<i>Bacillus cereus</i>	Absent	-	-
4.	<i>E.coli</i>	Present	6.5	3.5
5.	<i>Pseudomonasaeruginosa</i>	Absent	-	-
6.	<i>Salmonellatyphi</i>	Absent	-	-
7.	<i>Proteusmirabilis</i>	Present	7.5	3.5
9.	<i>S. aureus</i>	Absent	-	-
10.	<i>Vibro cholera</i>	Absent	-	-
11	<i>E. facalis</i>	Absent	-	-
12.	<i>A niger</i>	Absent	-	-
13.	<i>Candida albicans</i>	Absent	-	-

Table-2: Effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on mortality of *Periplaneta Americana*

Sr. No	Concentration of crude extract added in μ l	No. of <i>Periplaneta Americana</i> (cockroach) taken in each jar	Mean % Mortality at 24 hrs duration <i>Sigmatocia fibulata</i> (Schmidt)	Mean % Mortality at 24 hrs duration <i>Suberites carnosus</i> (Johnston)
24 Hrs. % Mortality				
1	100	10	70	70
2	200	10	90	80
3	300	10	100	80
4	400	10	100	80
5	500	10	100	90
6	600	10	100	90
7	700	10	100	90
8	800	10	100	90
9	900	10	100	100
10	1000	10	100	100
control	00	10	00	00

Each experiment were perform by five replicates

Table-3: Effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on mortality of Rice weevil *Sitophilus oryzae* (By Coating Method)

Sr. No	Concentration of crude extract added in μ l	No. of Rice weevil <i>Sitophilus oryzae</i> taken in each jar	Mean % Mortality at 24 hrs duration <i>Sigmatocia fibulata</i> (Schmidt)	Mean % Mortality at 24 hrs duration <i>Suberites carnosus</i> (Johnston)
24 Hrs. % Mortality				
1	100	10	40	30
2	200	10	60	50
3	300	10	50	40
4	400	10	80	70
5	500	10	80	80
6	600	10	70	60
7	700	10	90	90
8	800	10	100	90
9	900	10	100	100
10	1000	10	100	100
control	00	10	00	00

Each experiment were perform by five replicates

Table-4: Effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on mortality of Rice weevil *Sitophilus oryzae* (By sprinkling Method)

Sr. No	Concentration of crude extract added in μ l	No. of Rice weevil <i>Sitophilus oryzae</i> taken in each jar	Mean % Mortality at 24 hrs duration <i>Sigmatocia fibulata</i> (Schmidt)	Mean % Mortality at 24 hrs duration <i>Suberites carnosus</i> (Johnston)
24 Hrs. % Mortality				
1	100	10	50	40
2	200	10	50	40
3	300	10	60	50
4	400	10	70	70
5	500	10	80	50
6	600	10	60	60
7	700	10	80	80
8	800	10	100	90
9	900	10	100	100
10	1000	10	100	100
control	00	10	00	00

Each experiment were perform by five replicates

DISCUSSION

Nowadays people are more preferable use natural origin remedies than the synthetic drugs for curing illness because of their less side effects. Sponges are the amazing animals that develop strong immune system which resembles to that of other invertebrates.

Table-1 showing the effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) showing zone of inhibition in bacterial and fungus Strains.

In the present study Table-1 showing the effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) showing zone of inhibition in bacterial and fungus Strains. showing the effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) showing zone of inhibition in bacterial and fungus Strains. In our study we found that the crude extract of sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) showed zones of inhibition as *Coryne* bacterium, 2.5mm and 4.5mm, *E.coli*, 6.5 mm and 3.5 mm, *Proteus mirabilis*, 7.5 mm and 3.5mm in sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) respectively. Whereas in case of *Streptococcus pneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. aureus*, *Vibro cholera*, *E. facalis*, no bioactivity noted in respect of zone of inhibition. In case of fungal strains *A. niger* and *Candida albicans* no activity was noted against fungus when tested with crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston). We found the crude extract of sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston), more potent bioactivity was noted against *Coryne* bacterium, *E.coli*, *Proteus mirabilis*. It was evident from our study that, the crude extract of sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston), showed antibacterial activity. Our results were found contradictory to the studies carried out by Perveen et al., 2002 [17] stated that, the gram positive bacteria showing more activity than the gram negative bacteria. In another study, Beesoo et al., 2017 [18]; Gopi et al., 2012 [19]; Ines et al., 2007 [20] gives an evidence of the marine bacteria stating that marine gram positive bacteria has more sensitivity than gram negative bacteria. According to them gram negative bacteria are more resistant to sponge extract. The antimicrobial metabolic defense in sponges is well established [21, 22]. This could be because of natural marine environment Gupta et al., 2012 [23]; Mora et al., 2008 [24]; Nazemi et al., 2014 [25]. Many bioactive metabolites and compounds isolated from sponges displayed potential bioactivity such as antimicrobial, anti-inflammatory and cytotoxic activities [26, 27].

Table-2 Showing the effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus*(Johnston)on mortality of *Periplaneta americana*.

In control group 100% survival of the cockroach *Periplaneta americana* and Rice weevil *Sitopbilus oryzae* recorded after 24 hours. The experiments were conducted to find the effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on cockroach *Periplaneta americana*. Data represented in Table-2. Show the mortality of cockroach *Periplaneta americana* after exposure to *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston). It was found that, mortality of cockroach *Periplaneta americana* was noted 100% after 24 hrs exposure in the crude extract of *Sigmatocia fibulata* (Schmidt) was recorded at 300 µl concentration and *Suberites carnosus* (Johnston) recorded at 900 µl concentration. Cockroaches showed cleaning behavior at the initial stages of poisoning by rubbing their bodies with their legs or sides of the cages. Marentric movements of the mouth parts were also observed and at the later stages of poisoning tremors, incoordination and convulsion leading to paralysis were noted. Cockroaches in moribid condition were found to be dead after 24hrs.

Table 3 and 4 Showing the Effect of crude extract of *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on mortality of Rice weevil *Sitopbilus oryzae* (By Coating Method and by sprinkling Method).

Two kinds of experiments were conducted to find the effect of crude extract of marine sponges *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on rice weevils *Sitopbilus oryzae*. Data represented in Table 3 & 4 show the mortality of rice weevils *Sitopbilus oryzae* after 24 hours of exposure to crude extract of *Sigmatocia fibulata* and *Suberites carnosus*. It was evident that bioactive compounds present in the crude extract shows bioactivity by inducing 100% mortality of the rice weevils after 24 hours exposure.

By coating method it was found that, mean mortality of rice weevils was noted 100% in *Sigmatocia fibulata* (Schmidt) 700 µl and *Suberites carnosus* at 800 µl concentration. From the above data it was evident that *Sigmatocia fibulata* and *Suberites carnosus* contains bioactive compounds.

By sprinkling method the effect of crude extract of *Sigmatocia fibulata* and *Suberites carnosus* on mortality of rice weevils *Sitopbilus oryzae*. Data represented in Table-3, show the mean mortality of rice weevils *Sitopbilus oryzae* after 24 hours of exposure to

crude extract of *Sigmatocia fibulata* noted 100% mortality in 800 µl and in *Suberites carnosus* at 900 µl concentration. In the initial stage of the experiment on *Sitophilus oryzae*, after 2 hrs of exposure, schooling behavior was noted, and after 2 hrs the weevils were dispersed there after towards the corners of the petridishes, and after 6 hour the locomotary movement of the weevils was restricted and these weevils could not be moved afterwards. From the above data it was evident that the *Sigmatocia fibulata* and *Suberites carnosus* contains bioactive compounds. The pesticidal activity of the marine sponges *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) on mortality are being reported for the first time against cockroach *Periplaneta americana* and weevil *Sitophilus oryzae*.

CONCLUSION

More potent bioactivity was noted against *P. mirabilis* than *E. coli*. It was evident from our study that, the crude extract of sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston), showed effect on gram negative bacteria. Thus it concludes that sponge *Sigmatocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) have antibacterial and pesticidal activity. This finding is significant because multi-drug resistant strains of the organism are on the increase in both hospital and community environments against orthodox antibiotics and its control is very difficult by therapeutic means. Therefore it is necessary to screen the crude extract of sponge for its structural elucidation to find the new drugs for pharmaceutical industry.

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Conflict of Interest: There is no conflict of interest.

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