

Antimicrobial Potential of the Marine Sponge *Sigmadocia pumila* from the South Eastern Region of India

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Abstract: The crude methanolic extracts of the sponge *Sigmadocia pumila* collected from kanyakumari region were tested for antibacterial activity using the agar well diffusion method. The extracts were tested against Gram positive and Gram negative organisms. The antifungal activity was examined using *Sigmadocia pumila* against various fungal strains such *Trichoderma viride*, *Fuzarium spp*, *Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum* and *Aspergillus flavis*. Hence it is assumed that the sponge exhibited high antimicrobial activity.

Key words: *Sigmadocia pumila* % Antibacterial % Antifungal

INTRODUCTION

Sponges are the most primitive of multicelled animals that have existed for more than 800 million years. The sponges (Porifera), being evolutionarily ancient, multicellular, sessile organisms, inhabit every type of marine benthic environment [1]. Sponges are divided into three classes mainly according to the composition of their skeletons i.e. Calcarea, Glass sponges and Demo sponges. Among them demo sponges have the largest number of bioactive compounds. More than 8,000-10,000 species of sponges were described. Such as *Aplysina archeri*, *Xestospongia muta*, *Acanthella pulchra*, *Heliconia simulans*, *Axinella dissimilis*, *Discodermia dissolute*, *Raspailia ramose* [2].

Pharmaceutical studies on sponges have been started in the early 1950s by the discovery of the nucleosides spongothymidine and spongouridine in the marine sponge *Cryptotethya crypta*. The nucleosides were the basis for the synthesis of ara-C, the first marine-derived anticancer agent and the antiviral drug ara-A. Ara-C is currently used in the routine treatment of patients with leukemia and lymphoma. More than 15,000 marine products have been described so far [3]. The bioactive substances from sponges the terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides and amino acid derivatives (which are frequently halogenated) have been described from their associated microorganisms [4].

The methanolic extract of the marine sponge *Haliclona exigua* has showed promising antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Candida parapsilosis*. The marine sponges including *Amphimedon viridi* and *Neopetrosia* sp. possess antileishmanial activity [5]. The crude extracts from *Clathria gargonoids* and *callyspongia diffusa* have larvicidal activity against culex and insecticidal properties. *Sigmadosia carnosia* has higher toxic effect on *Aedes egypti*. *Haliclona pigmentifera* and *Petrosia similis* are considered as good source for exhibiting pesticidal activity [6].

The major leading dreadful diseases such as tuberculosis, aids, malaria and cancers are controlled with the new lead molecules discovered from marine organisms especially sponges. The lead molecules are discovered from the *Axinyssa* sp. *Halichondria* sp. and *Chondrosia reticulate*. These sponge extracts has displayed potent anti-M tuberculosis activity. *Phakellia ventilabrum* extract exhibits antimalarial activity [7]. Marine sponges are known as chemical factories because they produce hundreds of unique chemical compounds that have been isolated and their structures are determined. *Ircinia felix*, *pandaros acanthifolium*, *Topsentia ophiraphidites* and *Verongula rigida* are the abundant source of chemical compounds. The compounds from *Topsentia* sp. have strong hemolytic activity on fresh bovine erythrocytes.

An aqueous extract of *Myrmekioderma styx* exhibits hemagglutination activity. *Spongia* sp. and *Spirastrella* sp. release the spongistatins compound, which exhibit antiproliferative activity [8]. From the marine sponge *oceanapia sagittaria* the pyridoacrydine alkaloids kuanoniamines A and C were isolated. The kuanoniamines A and C were evaluated against the growth of five human tumor and non-tumor cell lines on the proliferation of human lymphocytes. kuanoniamines A was shown to be a potent inhibitor of DNA synthesis and it was also found to cause an extensive reduction of the MCF-7 cells in G-2 phase [9].

The marine sponge *Aplysina caissara* an endemic Brazilian species of the order Verongida, have bromotyrosine compounds such as Fistyralin-3 and 11-hydroxyaerolithionin which have displayed moderate antibiotic activity against *E. coli*, *P. aeruginosa* and *S. aureus* [10]. The biotechnological potential of *Cliona varians* sponge based proteins first showed that lectins purified from *Cliona varians* displayed a cytotoxic effect on Gram positive bacteria, such as *Bacillus subtilis*, *Staphylococcus aureus* and agglutinated *Leishmania chagasi* promastigotes [11].

Bioactive compounds from marine sponges have extensive use in the treatment of many diseases and these compounds act as the templates for synthetic modification. Several molecules isolated from various sponges are currently involved in the advanced stage of clinical trials. From the sponge *Ircinia* sp, the biologically active molecules show strong antibiotic, analgesic and anti-inflammatory properties [12]. The present study describes the antimicrobial ability for the crude extracts of *Sigmadocia pumila* against the Gram positive and Gram negative organisms.

MATERIALS AND METHODS

Collection and Preparation of Extracts of Sponges:

Specimens of the marine sponge *Sigmadocia pumila* were collected from the coast of Kanyakumari, Tamilnadu, India "by-Catch method" during active fishing season. The sponges which were detached at a depth ranging from 10 to 25m and entangled in the fishing nets while fishing and were segregated after completion of fishing in the morning hours. Sponges were cut into small pieces and extracted thrice with distilled methanol and the pooled organic solution was filtered through Whatmann 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.

Antibacterial Activity: The antibacterial activities of the methanol extracts of sponge *Sigmadocia pumila* were determined by the standard agar well diffusion assay Perez *et al.* [13]. using Muller Hinton agar (Hi Media). The bacterial cultures were obtained from the Microbial type culture collection and gene bank (MTCC), Institute for microbial technology, Chandigarh, India. The Gram positive bacterial strains included *Bacillus thuringiensis* MTCC 4714, *Enterococci fecalis* MTCC 439, *Listeria monocytogenes* MTCC 1143, *Staphylococcus aureus* MTCC 737 and *Proteus vulgaris* MTCC 426. Gram negative strains such as the *E. coli* MTCC 443, *Klebsiella pneumonia* MTCC 109, *Pseudomonas putida* MTCC 1688 and *Serratia liquefaciens* MTCC 3039 were used in the test. Wells of 6.0 mm diameter were punched using sterilized cork borer. Culture of each microbial pathogenic strain was swabbed with sterile cotton on the surface of medium. The crude extracts were suspended in methanol at concentrations (1, 5 and 10 %) of 1mg/ml for antimicrobial studies. Sponge *Sigmadocia pumila* extracts were tested with different aliquots 50, 100 and 150 μ l in each well. The plates were incubated for 24 hrs at 37°C and solvent control was performed in each case. Areas of inhibited microbial growth were observed as clear zone around the well after 24 hours. Antimicrobial activity was measured as diameter of zone of inhibition, excluding the well diameter.

Antifungal Activity: Antifungal activity of the crude extract of marine sponge *Sigmadocia pumila* was determined by using the standard method [14]. Fungal isolates were obtained from the Microbial type culture collection and gene bank (MTCC), Institute for microbial technology, Chandigarh, India. They included *Trichoderma viride* MTCC 2047, *Fuzarium spp* MTCC 284, *Aspergillus niger* MTCC 1344, *Candida albicans* MTCC 183, *Penicillium chrysogenum* MTCC 5108 and *Aspergillus flavis* MTCC 873. The fungal cultures were maintained in 0.2% dextrose medium at 5.6 pH and the optical density 0.10 at 530 nm was adjusted using spectrophotometer. Each fungal inoculum was applied on plate and evenly spread on potato dextrose agar using a sterile swab. To each well, 100 μ l of the samples were added and the solvent was used as the control. Agar diffusion assay was followed to evaluate the antimicrobial activity. The Petri plates were incubated at 30°C for 2 days. At the end of the 48 hrs, inhibition zones formed in the medium were measured in millimeters.

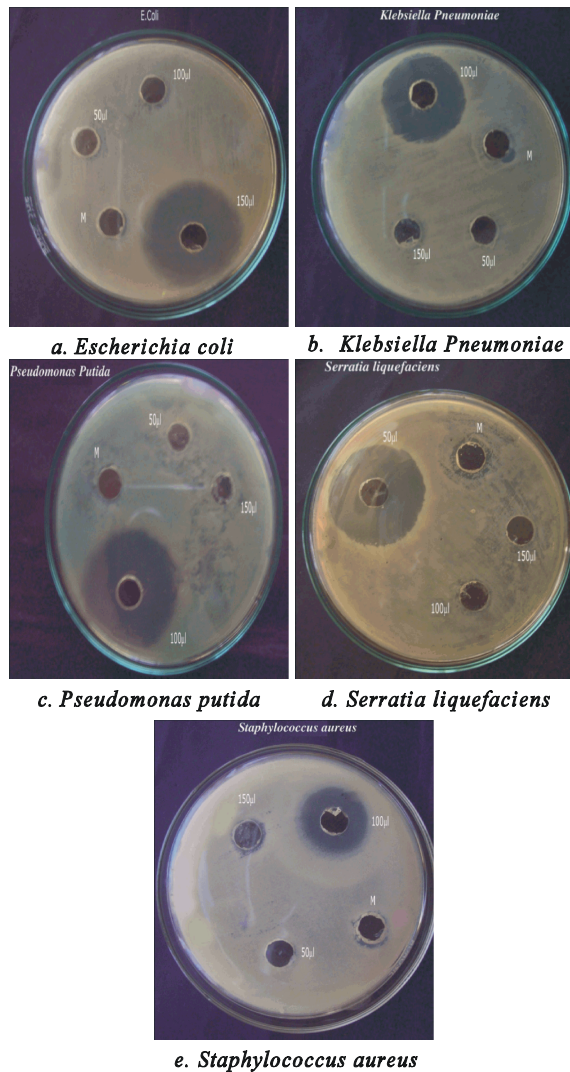


Fig. 1: Antibacterial activity of the methanolic crude extracts of the sponge *Sigmadocia pumila* by well diffusion method.

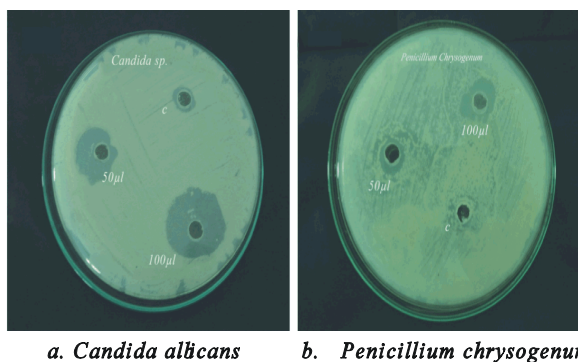


Fig. 2: Antifungal activity of the methanolic crude extracts of the marine sponge *Sigmadocia Pumila*

RESULTS

The trend of antibacterial activity for *Sigmadocia pumila* is given in Fig. 1. In the agar well diffusion method, the methanol extracts of *Sigmadocia pumila* exhibited high inhibitory activity towards human pathogens of Gram negative organisms such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas putida* and *Serratia liquefaciens*. They were not active against the Gram positive organisms except *Staphylococcus aureus*. In *E. coli* the zone formed at 50µl was 2mm and at 150 µl it was 16mm diameter. Then *Klebsiella Pneumoniae* formed 14mm zone at 100 µl concentration of extract, *Pseudomonas putida* formed the zone of 15mm at 100 µl of extract. In *Serratia liquefaciens* 16mm zone was formed at 50 µl concentration of the extract. Then in *Staphylococcus aureus* 2mm and 12mm diameter zones were formed at 50 and 100 µl of extract. In the rest of the concentrations the extract was not effective.

Fig. 2 suggests the antifungal activity for the methanolic extracts of *Sigmadocia pumila* showing moderate effects on some organisms such as *Candida albicans* and *Penicillium chrysogenum* and no activity was seen on organisms such as *Trichoderma viride*, *Aspergillus niger*, *Aspergillus flavis* and *Fusarium spp.* In *Candida albicans* the diameter of the zone was 6mm at 50 µl and 10mm at 100 µl. Then in *Penicillium chrysogenum* the inhibitory zone measured was 3mm and 6mm for 50 and 100 µl of the extract.

DISCUSSION

In the present study the extracts of the sponge *Sigmadocia pumila* showed antimicrobial action against the Gram negative organisms effectively. Organisms such as *E. coli*, *pseudomonas putida* and *staphylococcus aureus* were inhibited effectively by the extracts. Sponges are primitive marine invertebrates which contain more natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial, larvicidal, hemolytic and anti-inflammatory activities and are often applicable for medical use [15]. Marine sponge *Aplysina cavernicola* produces aerophysinin and aerthionin and other dibromo and dichlorotyrosine derivatives, with some antibiotic activity against *Bacillus subtilis* and *Proteus vulgaris* [16].

Antimicrobial activities of marine bacteria associated with sponges at the south east coast of India have showed a variety of secondary metabolites. They are the compounds such as amino acids, sugars, fatty acids,

terpenes, etc. Recently it was reported that sponge associated *Streptomyces* synthesizes various marine natural products [17]. A 27 kDa lectin purified from Demosponge *Suberites domuncula* displayed antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* [18].

In the present study, it was confirmed that the sponge *Sigmatocia pumila* had the ability to inhibit the growth of *E. coli* and *Staphylococcus aureus* even at low concentrations of 50 µl and 100 µl of the extracts. Kanagasabhapathy *et al.* [19] studied the antimicrobial activity of several marine sponges, which were collected from the gulf of Mannar, India. They showed that they are very active against the human pathogenic bacteria. They found that the sponge *psammaphysilla purpurea* has a remarkable antimicrobial activity against different human pathogenic organisms. Jimenez *et al.* [20] reported the use of marine natural products as an alternative source for detecting the larvicidal, hemolytic and anticancer activities.

Marine organisms represent a valuable source of new compounds. The *Sigmatocia pumila* crude extracts showed the ability to induce various *in vitro* activities especially significant antimicrobial activity. It may be due to the presence of bioactive compounds. Hence *Sigmatocia pumila* can be considered as an effective organism to develop drugs.

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