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ORIGINAL RESEARCH ARTICLE

Antimicrobial Activity of Sponges Endemic to Uchipully Coastal Line, Near Rameswaram, Tamilnadu

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ABSTRACT

The study reports the *in-vitro* screening of methanolic extracts of nine marine sponges (porifera) collected from Uchipully coastal line, near Rameswaram, Tamilnadu, India, in search for novel pharmaceuticals. In vitro antimicrobial activity for nine sponge species against pathogenic strains of bacteria *viz Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus epidermis, Staphylococcus aureus* and pathogenic strains of fungi *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* was tested using agar well diffusion assay method. The results revealed that the sponge extracts inhibited the growth of majority of the bacterial as well as fungal pathogens. The maximum growth inhibition of *Escherichia coli* was recorded with all the extracts of sponges.

Keywords: Antimicrobial activity, Bacteria, Sponges.

INTRODUCTION

Marine sponges constitute an important component of benthic communities throughout the world, with regard to its biomass as well as their potential to influence benthic or pelagic processes (Dayton1974; Dayton1989; Gili 1998; Maldonado 2005). Sponges exist in oceans for about 580 million years, and diversified with 8,000 species today (Bergquist 1978; Van Soest *et al*., 2008). They inhabit a wide variety of marine and freshwater ecosystems and are found throughout tropical, temperate and polar regions (Hooper *et al*., 2002). Marine sponges, especially those found in tropical ocean areas, continue to represent the single most prolific source of structurally novel natural products of marine origin (Blunt *et al*., 2009). Sponges (Phylum: Porifera) are among the oldest multicellular animals (Metazoa) and show relatively little differentiation and tissue coordination (Bergquist, 1978; Simpson, 1984; Leys and Meech, 2006). Sponges, which are the most primitive invertebrates, represent an important constitutive group of the coral reef fauna with a wide range of species (Van Soest, 1994). Sponges are known to produce large number and vast variety of secondary metabolites.

Sponge-derived secondary metabolites have also attracted considerable attention because of their relevant and diverse pharmacological actions (Keyzers and Davies-Coleman, 2005). Some of the products showing antiviral, antitumor, antimicrobial or general cytotoxic properties. Certain marine sponges also contain amino acid derivatives. Biologically active peptides are the axinellins (Maldonado *et al*., 2005), kapakahines (Bergquist, 1978), microsclerodermins (Simpson, 1984), polytheonamides (Leys and Meech, 2006), papuamides (Hooper *et al*., 2002), stylisins (Blunt *et al*., 2009), hymenamides (Kobayashi *et al*., 1993), wainunuamide and dominicin (Williams *et al*., 2005). Additional noteworthy examples include the laxaphycins (Bonnard *et al*., 2007) and discodermins (Matsunaga *et al*., 1984; Matsunaga *et al*.,1985) they inhibit tumor promotion), calyculin15 (tumor promoting), and the antithrombin cyclotheonamides. Some of the isolated substances from sponges have striking structural similarities to metabolites of microbial origin (Proksch *et al*., 2002). Over 5000

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secondary metabolites were isolated from 500 species of marine sponges (Rifai *et al*., 2005)

Over 800 antibiotics have been isolated from marine sponges (Touati *et al*., 2007). Many of these compounds likely to serve as chemical weapons that protect the immobile animals from being overgrown or ingested (Pawlik, 1992; Paul and Ritson-Williams, 2008). The first report of antimicrobial activity of sponge extract was by Nigrelli et al. (Newbold *et al*., 1999). The present study was carried out to test the antimicrobial activity of the methanol extracts of sponges, against bacterial and fungal species.

MATERIALS AND METHODS

Sample Collection

Sponges sample were collected from Uchipully near Mandapam coast, Rameswaram, Tamilnadu, India. Samples were collected through normal diving at the depth of 5-6 m during September – October 2010 and immediately kept in methanol and transferred to lab for further study. Nine sponge types (A, B, C, D, E, F, G, H, I) were collected for this study.

Extract Preparation

The shade dried sponges powdered in an electric blender and was extracted separately to exhaustion in a soxhalet apparatus using methanol solvent system. Sponges extracts were filtered through a cotton plug followed by Whatmann filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C). Extracts were preserved in air tight container and kept at 4-5°C until further use. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to study the antimicrobial activity.

Test organisms

The bacterial spp used for the test were *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Klebsiella pneumoniae* (*K. pneumonia*), *Bacillus subtilis* (*B.subtilis*), *Staphylococcus epidermis* (*S.epiderms*), *Staphylococcus aureus* (*S.aureus*) and of the Fungal spp were *Aspergillus niger* (*A.niger*), *Aspergillus flavus* (*A.flavus*), *Aspergillus fumigates* (*A.Fumigatus*)

Antimicrobial susceptibility test by Agar well diffusion method

In order to determine the antimicrobial spectrum, the antibacterial activity and antifungal activity was performed by agar well diffusion method. The inoculum suspension of each bacterial strain was swabbed on the entire surface of Mueller Hinton Agar (MHA). On the surface of the medium, wells were made by using sterile cork borer (6 mm size). Each well was filled with 100 µl of sponges extracts. The diameter of inhibition zones were measured in mm after incubation at 37°C for 24 hours.

RESULTS AND DISCUSSION

The results revealed potential antimicrobial activity in extract sample B, C, D, E, F, G, H and I (**Table 1 & 2**). The antimicrobial effect of sample D was recorded higher on *P.aeuroginosa, E.coli, K. pneumoniae, B. subtilis, A. niger, A. fumigatus, S. aureus and A.flavus* when compared to that of other samples. In case of sample B, *E.coli* inhibited maximally compared to that of *P. aeruginosa, B.*subtilis, *K. pneumoniae and S. aureus.* Sample E revealed significant zone inhibition for *E.coli* to that of *P. aeruginosa, B.*subtilis, *K. pneumoniae* and *S. aureus.* In case of antifungal effect, sample D showed maximum activity on *A.fumigatus, A.niger* and *A.flavus* compared to that of other samples.

Similar studies revealing potent antimicrobial activity of marine sponges were recorded elsewhere, Rifai *et al.,* 2005, reported that ten marine sponges extract collected from Atlantic coast of Morocco and from the Gulf of Thailand were tested against four bacterial pathogens and five fungal pathogens. Touati *et al.,* 2007, reported that marine sponge extracts collected from Tunisian coast was tested against eight human pathogenic bacteria and six human pathogenic fungi using the agar disk diffusion method. Galeano and Martrnez, 2007, reported that twenty-four sponge species (Poriferae), collected from the Uraba Gulf reefs (Colombian Caribbean region), against certified strains of bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 25922) and yeast (*Candida albicans* 10231). Safaeian *et al*., 2009 studied that, *in vitro* antimicrobial activity of six sponge species (Porifera), collected from offshore zone of Nay Band Bay, Iran, against pathogenic strains of bacteria (two Gram-positive: *Bacillus subtilis* ATTC 6633, *Staphylococcus aureus* ATCC 25923 and two Gram negative: *Pseudomonas aeroginosa*

ATCC 27853, *Escherichia coli* ATCC 25922) and pathogenic fungi (*Candida albicans* ATCC 10231, *Aspergillus spp*. PTCC 5266, *Penicillium spp*. PTCC 5251).

Table 1: Antibacterial activity of methanolic extracts (100 µl sample) of various marine sponge types

Sample ID	*E.coli*	*B.subtilis*	*S.aureus*	*P. aeruginosa*	*K. pneumoniae*
A	-	-	-	9 mm	-
B	16 mm	14 mm	13 mm	15 mm	14 mm
C	-	-	10 mm	-	-
D	18 mm	17 mm	15 mm	19 mm	18 mm
E	15 mm	13 mm	12 mm	14 mm	13 mm
F	14 mm	12 mm	10 mm	11 mm	14 mm
G	12 mm	10 mm	10 mm	12 mm	11 mm
H	-	-	-	-	10 mm
I	-	-	-	10 mm	-

*(Zone of inhibition in mm)

Table 2: Antifungal activity of methanolic extracts (100 µl sample) of various marine sponge types

Sample ID	*A.niger*	*A.fumigatus*	*A.flavus*
A	-	-	-
B	13 mm	15 mm	13 mm
C	-	9 mm	-
D	17 mm	19 mm	16 mm
E	12 mm	13 mm	14 mm
F	10 mm	12 mm	10 mm
G	9 mm	10 mm	9 mm
H	-	-	-
I	-	10 mm	-

*(Zone of inhibition in mm)

Figure 1: Different sponges

Figure 1: A

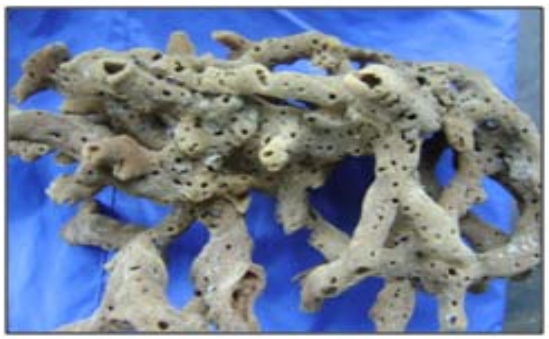


Figure 1: B

Figure 1: C

Figure 1: D

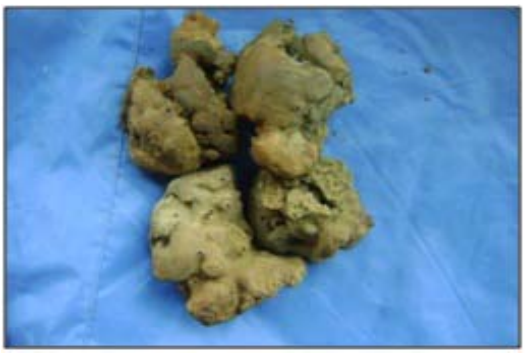


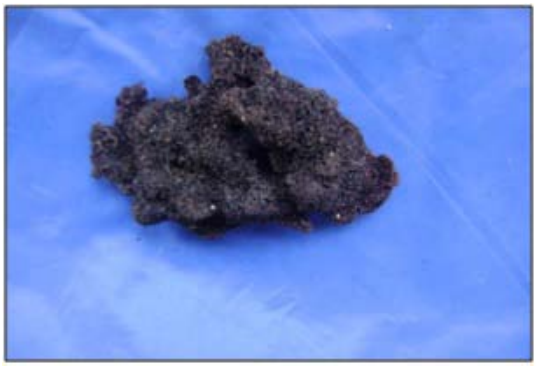
Figure 1: E

Figure 1: F

Figure 1: G

Figure 1: H

Figure 1: I



CONCLUSION

The present studies envisage exploiting potent antimicrobial agents from marine sponges of Uchipully coastal line.

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