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Antimicrobial properties of sea anemone *Stichodactyla mertensii* and *Stichodactyla gigantea* from Mandapam coast of India

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ABSTRACT

Objective: To investigate the antimicrobial activities of the methanol and aqueous extract of sea anemone *Stichodactyla mertensii* (*S. mertensii*) and *Stichodactyla gigantea* (*S. gigantea*). **Methods:** The sea anemone *S. mertensii* and *S. gigantea* were subjected to extraction by using methanol and distilled water. They were evaluated for antimicrobial activity against bacterial and fungal pathogens. **Results:** In antibacterial activity, *S. gigantea* exhibited significantly inhibitory activity against *Pseudomonas aeruginosa* than the *S. mertensii* of butanolic extract. In antifungal activity, the *S. mertensii* extract showed good activity against *Aspergillus niger* (*A. niger*)compared with other strains. Whereas *S. gigantea* recorded maximum and minimum zone of inhibition against *Botrytis cinerea*, *A. niger* and *Cladosporium cucumerinum* respectively. **Conclusions:** The results support that the sea anemones *S. mertensii* and *S. gigantea* extracts for treatment of some bacterial and fungal diseases as an ethanomedicinal source.

1. Introduction

Marine organisms have proven to be rich sources of organic compounds with interesting biological activities[1]. The marine environment provides novel substances to control bacterial, fungal and viral diseases and cancer chemotherapy. The cnidaria is a large, diverse and ecologically important phylum. It includes about 9 400 species, of which 68% are members of the class Anthozoa[2]. In common with all animals, anthozoans need to protect themselves against the lethal or debilitating consequences of microbial or parasitic invasion. Indeed, a recent reports offers evidence that bleaching, one of the most destructive pathological conditions affecting reef corals may be caused by bacterial infection[3]. Concern about the health of ecologically important anthozoans, mainly scleractinian corals[4]. has stimulated interest in the way these animals overcome or avoid opportunistic or pathogenic infection. However, unlike their coelomate relatives, anthozoans have received little attention with respect to their anti-microbial and anti-parasitic defenses.

Sea anemones produced various biologically active polypeptides[5]. In recent years, cytolytic toxins of sea anemone attracts a great interest of researchers, because they exhibit antitumor, antiparasitic, antimicrobial, dermatonectrotic and other types of biological activities due to the powerful membranolytic action[6-7]. Sea anemones have tentacles that surround a central mouth opening and these are used to catch and transfer food items such as crustaceans, molluscs and small fish to their mouth. The nematocysts present on the edges of the tentacles expel specific toxins[8]. The present study was aimed at determining the antimicrobial activity of tropical sea anemones such as *Stichodactyla mertensii* (*S. mertensii*) and *Stichodactyla gigantea* (*S. gigantea*) from Mandapam coast of India.

2. Materials and methods

2.1. Study site

The study was performed at Mandapam area, Ramanadhapuram district of Tamilnadu, India, situated at the Southeast coast of Bay of Bengal (Latitude 09° 16′ N and Longitude 72 ° 12′ E) (Figure 1).

2.2. Sampling

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The two sea anemones *S. mertensii* Brandt, 1835 and *S. gigantea* Forsskal, 1775 were collected in the summer of February and March, 2009 by scuba diving at the depth ranging from 3 to 4 meter. The samples were thoroughly washed with sea water and brought to the laboratory with sterilised sea water in an air tightened pack for extraction and the specimens were identified by following the standard literature of Indo-Pacific coral reef field guide[9].

Figure 1. General view of Mandapam coastal region.

2.3. Preparation of organic extracts

2.3.1. Methanolic extract

Crude extract was prepared according to the method of Sunahara *et al*[10]. The sea anemone *S. mertensii* was fully immersed in methanol and maintained for five days. The methanol extract was decanted and the anemone material was extracted at room temperature. The methanol extract was filtered through Whatman®No.1 Filter paper and the solvent was concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30 ℃) with reduced pressure to give predominantly an aqueous suspension and stored at 4 ℃ for further use.

2.3.2. Aqueous extract

Aqueous extraction of *S. gigantea* was carried out according to the method of Yano *et al*[11]. The live animal was kept inside the glass bowl along with some amount of distilled water in an ice container for 15 minutes. During stress condition the nematocysts were collected from the tentacles and same procedure was repeated for thrice. The collected nematocysts containing toxins were filtered by Whatman No.1 filter paper. Residues were centrifuged at 5000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilisation and stored at 4 ℃ until further use.

2.4. Microorganism

Antimicrobial activity of sea anemone extract was determined against ten bacterial stains *viz.*, *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*), *Salmonella paratyphi* (*S. paratyphi*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella oxytoca* (*K. oxytoca*), *Klebsiella pneumonia* (*K. pneumonia*), *Vibrio cholerae* (*V. cholerae*), *Escherichia coli* (*E.coli*) and *Proteus mirabilis* (*P. mirabilis*) and 10 fungal stains *viz.*, *Aspergillus niger* (*A. niger*), *Aspergillus fumigatus* (*A. fumigatus*), *Botrytis cinerea* (*B. cinerea*), *Cladosporium cucumerinum* (*C. cucumerinum*), *Penicillium expansum* (*P. expansum*), *Rhizopus oryzae* (*R. oryzae*), *Trichoderma harzianum* (*T. harzianum*), *Trichoderma koningii* (*T. koningii*), *Pneumocystis jirovecii (P. jirovecii)* and *Stachybotrys chartarum* (*S. chartarum*) were the pathogenic microorganisms included in this study. The bacterial cultures were obtained in pure form from the Rajah Muthiah Medical College, Annamalai Nagar and fungal strains were obtained from the ESMA Institute of Technology, Karur.

2.5. Antimicrobial assay

The antibacterial and antifungal activity was carried out by disc diffusion method[12-15]. The extracts were applied to 6 mm sterile discs in aliquots of 30 μL of solvent, allowed to dry at room temperature and placed on nutrient agar plates seeded with microorganisms and incubated at 32 ℃ for 4 h. Zones of growth inhibition were measured in mm using vernier caliper or a scale and recorded.

3. Results

The results of the antibacterial and antifungal activity against the tested pathogens of the two species of sea anemones are given in Table 1 and 2.

3.1. Antibacterial activity

In the present study *S. mertensii* and *S. gigantea* were showed to be a promising source of antibacterial compound and were screened to evaluate antibacterial activity in crude, methanol, dichloromethane, ethanol, butanol and acetone. It showed moderate antibacterial activity against 9 bacterial pathogens. From the bacteria tested, inhibition zones were showing maximum of (8.3±0.8) mm against *E. coli* and *P. mirabilis* of butanol and acetone extract of *S. mertensii* and the minimum was noticed at (1.3±0.4) mm against *V. cholerae* in methanolic extract. In the case of *S. gigantea* the inhibition zone was maximum at (12.0±0.8) mm against *P. aeruginosa* in butanol and the minimum was recorded at (2.3 ±0.4) mm in butanol and acetone extract against *S. typhi* and *K. oxytoca* (Table 1).

3.2. Antifungal activity

In vitro antifungal activity of *S. mertensii* and *S. gigantea* extracts was active against ten species of fungal pathogens represented in Table 2. The different solvent extract of *S. mertensii* was active against pathogenic fungal. Among them the MeOH extracts active against nine species. The maximum zone of inhibition was noticed against *A. niger* and minimum was observed against *P. expansum*. Similarly, dichloromethane and acetone extracts showed the maximum inhibition of (4.2±0.2) mm against *R. oryzae*, *T. koningi*, *A. fumigatus*, *P. expansum* and *T. harzianum*.

In the case of *S. gigantea*, the maximum zone of inhibition

Table 1
Antibacterial activity of *S. mertensii* and *S. gigantea* against human pathogens.

Pathogens	Zone of inhibition (mm)									
	S. mertensii					*S. gigantea*				
	M	DCM	E	A	B	M	DCM	E	A	B
S. aureus	5.0 ± 0.8	4.0 ± 0.8	–	–	–	2.6 ± 0.4	6.0 ± 0.8	–	–	7.3 ± 0.4
S. typhi	3.3 ± 0.4	3.3 ± 0.4	2.3 ± 0.4	–	7.0 ± 0.8	3.3 ± 0.4	3.3 ± 0.4	–	6.0 ± 0.8	2.3 ± 0.4
S. paratyphi	–	–	5.0 ± 0.8	5.0 ± 0.8	6.3 ± 0.4	7.3 ± 0.4	5.0 ± 0.8	–	5.0 ± 0.8	3.3 ± 0.4
K. pneumonia	–	7.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	–	3.3 ± 0.4	6.0 ± 0.8	–	4.3 ± 0.4	5.0 ± 0.8
K. oxytoca	–	–	5.0 ± 0.8	5.0 ± 0.8	3.3 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	2.3 ± 0.4	5.0 ± 0.8
P. aeruginosa	–	–	4.0 ± 0.8	–	6.3± 0.4	4.7 ± 0.2	8.3 ± 0.8	5.0 ± 0.8	4.3 ± 0.4	12.0 ± 0.8
V. cholerae	1.3 ± 0.4	3.3 ± 0.4	5.0 ± 0.8	4.0 ± 0.8	6.3 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	5.0 ± 0.8	4.3 ± 0.4	4.3 ± 0.4
E. coli	–	4.0 ± 0.8	6.0 ± 0.8	4.0 ± 0.8	8.3 ± 0.8	3.3 ± 0.4	6.0 ± 0.8	–	–	–
P. mirabilis	–	–	–	8.3 ± 0.8	6.0 ± 0.8	–	–	–	4.3 ± 0.4	4.3 ± 0.4

M= Methanol; DCM= Dichloromethane; E= Ethanol; A= Acetone; B= Butanol.

Table 2
Antifungal activity of *S. mertensii* and *S. gigantea* against fungal pathogens.

Pathogens	Zone of inhibition (mm)							
	S. mertensii				*S. gigantea*			
	M	DCM	A	B	M	DCM	A	B
A. niger	10.3 ± 1.2	2.1 ± 0.6	2.8 ± 0.2	–	1.1 ± 0.2	1.1 ± 0.2	–	1.1 ± 0.2
B. cinerea	6.2 ± 0.5	1.5 ± 0.4	1.5 ± 0.4	–	7.3 ± 0.2	1.1 ± 0.2	2.3 ± 0.6	–
C. cucumerinum	4.8 ± 0.2	2.1 ± 0.6	3.2 ± 0.5	–	1.1 ± 0.2	3.8 ± 0.6	–	1.1 ± 0.2
P. expansum	1.5 ± 0.4	2.1 ± 0.6	4.2 ± 0.2	–	2.3 ± 0.6	1.1 ± 0.2	1.1 ± 0.2	2.3 ± 0.6
R. oryzae	8.2 ± 0.5	4.2 ± 0.2	2.1 ± 0.6	–	2.3 ± 0.6	1.1 ± 0.2	–	3.8 ± 0.6
T. harzianum	6.9 ± 0.1	3.2 ± 0.2	4.2 ± 0.2	–	3.8 ± 0.6	3.8 ± 0.6	2.3 ± 0.6	4.4 ± 0.4
T. koningi	2.1 ± 0.6	4.2 ± 0.2	3.2 ± 0.5	–	2.3 ± 0.6	1.1 ± 0.2	1.1 ± 0.2	2.3 ± 0.6
A. fumigatus	2.1 ± 0.6	4.2 ± 0.2	–	–	2.3 ± 0.6	4.4 ± 0.4	2.3 ± 0.6	–
P. jirovecii	5.2 ± 0.5	2.1 ± 0.6	3.2 ± 0.5	–	3.8 ± 0.6	3.8 ± 0.6	2.3 ± 0.6	2.3 ± 0.6
S. chartarum	–	–	–	2.1 ± 0.6	2.3 ± 0.6	–	–	–

M= Methanol; DCM= Dichloromethane; A= Acetone; B= Butanol.

was recorded against *B. cinerea* (7.3±0.2) mm and minimum (1.1±0.2) mm against *A. niger* and *C. cucumerinum* respectively.

4. Discussion

The antibacterial and antifungal activities were prominated in the crude extract of the *S. mertensii* and *S. gigantea*. The butanol and acetone extract of *S. mertensii* showed roughly 8 mm zone of inhibition against *E. coli* and *P. mirabilis* in methanolic extract. Similar results were found in *P. indicus*, *P. sinensis*, *Heteractis magnifica* and *Stichodactyla haddoni* (*S. haddoni*) reported by Rajiv Chandra Rajak and Balaji[16,17]. In the case of *S. gigantea*, it showed about 12 mm zone of inhibition against *P. aeruginosa* of butanol extract. Yano *et al*[11] have showed that some spieces and herbs' extract such as *Coriandrum sativum* and *Cuminum cyminum* inhibit the growth against *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *E. coli*.

As an earlier report has been made, the crude extract of *S. haddoni* showed good activity against Gram-negative bacteria by Sureshkumar *et al*[18]. Prakash Williams *et al*[19] reported that the tissue extract of sea anemone showed highly inhibition activity (20 mm) against *K. pneumonia* and 24 mm with the hexane tissue extract against fish pathogen. Burholder *et al*[20] isolated 2 bromo-compounds from some sponge extracts *Verongi fistularies* and *Verongi vauliformis* that inhibited the growth of Gram-positive and Gram-negative bacteria.

Boobathy *et al*[21] have reported moderate activity from the aqueous and methanolic extracts of sponge *Callyspongia diffusa* against 4 human pathogens, *P. aeruginosa*, *E. coli*, *V. parahaemolyticus* and *V. cholera*. Patterson Edward and Murugan[22] have reported broad-spectrum antibacterial activity of aqueous ink extract of the cephalopods *Loligo duvaucelii* and Sepia pharaonis against nine human pathogens. Murugan *et al*[23-32] have reported the antibacterial activity from *Rapana rapiformis* against eight human pathogens. Murugan and Santhana Ramasamy[14] have reported the crude methanol extract of ascidian *Didemnum psammathodes*, respond the range of inhibition varied from 6 to 10 mm with an average of 7.1 mm.

Touati *et al*[33] have studied the antibacterial activity of seven marine sponge from Tunisian coast against Gram positive and Gram negative bacteria. Ethyl acetate extract of *Axinella damicornis* exhibited significant activity against all bacterial strains. Rifai *et al*[34] have studied antibacterial and antifungal activity from ten specie of sponges *Hippospongia communis*, *Ircinia variabilis*, *Penares candida*, *Spongia officinalis*, *Stella dorigena*, *Geodia cydonium*, *Haliclona offoculata*, *Paratetilla bacca*, *Spongia ceylonica* and *Tetilla japonica* from Gulf of Thailand.

Marine organisms collected from the Southeast coast of India have been shown to possess a number of biological activities. In our studies, the most interesting species

are that *S.mertensii* and *S.gigantea*. To the best of our knowledge, this is the first report demonstrating the antimicrobial activity of most of the sea anemones taken up in this study, with few exceptions. These organisms are currently undergoing detailed investigations with the objective of isolating biologically active molecules along with the search for novel compounds. Furthermore, the encouraging biological activities seen in this study show that the Indian coastline is a potential source of variety of marine organisms worthy of further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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