

Full Length Research Paper

Antifungal activity of some species of marine sponges (class: Demospongiae) of the palk bay, southeast coast of India

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Nine species of sponges (Orders: Keratosida, Hadromerida, Haplosclerida and Poecilosclerida) of the class Demospongiae were tested for antifungal activity. In general, only trace and moderate activity was observed against the fungal pathogens tested. However, the encrusting Keratosida sponges depicted strong antifungal activity than the other sponges. *In vitro* antifungal activity of sponge extracts was determined against six species of pathogenic fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* sp., *Candida albicans*, *Cryptococcus* sp.). Stock cultures of fungi were maintained in Czapek Dox agar. On the surface of the medium, discs inoculated with the extract (20 µl/6 mm disc) were placed. The inhibition zone was measured after 72 h of incubation and the antifungal activity has been expressed as inhibition zone in mm. In the present investigation, four solvents viz; ethanol, methanol, acetone and chloroform were used to obtain crude extracts from sponges. Of these, only chloroform and ethanol extracts showed activity against the fungi. There was higher activity against *Cryptococcus* sp. (10 mm) in the crude extracts of chloroform and minimum activity against *A. fumigatus* (5 mm) in the crude extract of ethanol. From the present study, it could be understood that the sponges might be used for the extraction of useful drugs that have antifungal activity against the important human pathogenic fungal species. Besides, the result of the present study is providing baseline data for the future researches in this line of work and is also throwing more light on the use of sponges by the pharmaceutical technologies for the extraction of useful drugs.

Key words: Demospongiae, solvents, antifungal activity, Palk Bay.

INTRODUCTION

Incidence of fungal infection is emerging worldwide, and despite treatment, mortality remains high (Dannaoui et al., 2003). Many studies have shown an increasing frequency of systemic infections within the last decades in advanced human immunodeficiency virus infected patients and other patients with deficient immune systems (Thiebaut, 2002). Currently, very few antifungal agents are available and their use may be limited by dangerous side effects (Lorthoraly et al., 1999; Andriole, 1999). Furthermore, with the emergence of new triazol-

resistant strains of fungi, new compounds must still be screened in search of fungicidal effect, with a broad spectrum of activity and without side effects. In the marine environment, sponges (Porifera) are one of the richest sources of both biologically active secondary metabolites and chemical diversity (Kijjoo and Sawangwong, 2004; Proksch et al., 2003). These natural products may play a role in warding off predators, and perhaps they also repel fouling organisms. Marine sponges are also a well known source of a unique class

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Table 1. List of marine sponges tested for antifungal activity.

S/No	Order	Family	Species
1		Aplysillidae	<i>Psammaplysilla purpurea</i> (Carter)
2	Keratosida	Spongiidae	<i>Spongia officinalis</i> Linnaeus var. <i>ceylonensis</i> Dendy
3		Spongiidae	<i>Hyattella cribriformis</i> (Hyatt)
4		Dysideidae	<i>Dysidea fragilis</i> (Mantagu)
5	Hadromerida	Spirastrellidae	<i>Spirastrella inconstans</i> var. <i>digitata</i> (Dendy)
6		Spirastrellidae	<i>Spirastrella inconstans</i> (Dendy)
7	Haplosclerida	Haliclonidae	<i>Haliclona tenuiramosa</i> (Burton)
8		Callyspongiidae	<i>Callyspongia diffusa</i> (Ridely)
9	Pocelosclerida	Psammascidae	<i>Desmapsamma anchorata</i> (Carter)

of metabolites collectively known as C21 bisfuranoterpenes (Fontana et al., 1996; Rochfort et al., 1996; Capon et al., 2001). Hence, the present study was undertaken to investigate the antifungal properties of nine species of sponges (Table 1).

MATERIALS AND METHODS

Collection and preparation of samples

Nine species of marine sponges (class: Demospongiae) were collected from the shallow waters of the Palk Bay region (Lat. 9° 17' N, Long. 79° 17' E) at Gandhi Nagar, southeast coast of India. These samples were rinsed with sterile seawater to remove associated debris and salt. The specimens were weighed (5 g) and preserved separately in ethanol, methanol, acetone and chloroform (1:2) and brought to the laboratory. Afterwards, these specimens were soaked in the above mentioned solvents respectively for 48 h. The extracts were later obtained from the soaked samples by grinding, using pestle and mortar and filtering through Whatman No.1 filter paper. The filterates were centrifuged at 3,000 rpm. The solvent was evaporated under reduced pressure using desiccator. After the evaporation of the solvents, the extracts became brown, yellow, black and pink in colour and were thick; the residue was weighed and dissolved in the same solvents for antifungal testing. Six strains of human pathogenic fungi were obtained from Raja Muthiah Medical College, Annamalai University, India. The characteristics and symptoms of test fungi are summarized in Table 2.

Antifungal susceptibility assays

In vitro antifungal activity of sponge extracts was determined against six species of pathogenic fungi. Stock cultures of fungi were maintained in Czapek Dox agar. Inoculum for *Candida albicans* was prepared by spread plating of 0.2 ml of 24 h old cultures grown on Czapek Dox broth. For *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*, well-drained spores were distributed uniformly on the surface of the agar plates with the help of a sterile cotton swab. Other fungal strains (*Cryptococcus* sp. and *Penicillium* sp.) were inoculated by taking a piece of fungal colony using a sterile cotton swab and gently swabbed on the surface uniformly. Agar was used as the medium for antifungal assay. On the surface

of the medium, discs inoculated with the extract (20 µl/6 mm disc) were placed. The inhibition zone was measured after 72 h of incubation and the antifungal activity has been expressed as inhibition zone in mm (Sithrangaboopathy, 2003).

RESULTS AND DISCUSSION

The results of antifungal activity of sponges are shown in Figure 1A to D. In the present study in general, only trace and moderate activity of sponges was observed against the human pathogenic fungi tested. Four species of the order Keratosida (*Psammaplysilla purpurea*, *Spongia officinalis* var. *ceylonensis*, *Hyattella cribriformis* and *Dysidea fragilis*) exhibited antifungal activities. In the other Orders, Poecilosclerida, Haplosclerida and Hadromerida, (*Commelina diffusa*, *Stenalia inconstans*, *Stenalia inconstans* var. *digitata*, *Haliclona tenuiramosa* and *Desmapsamma anchorata*) did not exhibit any antifungal activity. Further, only ethanol and chloroform sponge extracts showed antifungal activity while others (extracts of methanol and acetone) did not affect the growth of the test fungi.

The metabolites extracted from the species of *Hyposmocoma communis* showed activity only against *C. albicans* and *A. fumigatus* (Rifai et al., 2004). The sponges examined in the present investigation come under the Orders: Keratosida, Poecilosclerida, Haplosclerida and Hadromerida and only a few members of these orders have been reported to possess a broad spectrum of biological activities (Amade et al., 1982). Though studies (Lazarua and Anita Mary, 2000) reveal that the chemical composition varies considerably from family to family in the Keratosida sponges, many 'wonder' compounds have been isolated from *Spongia officinalis*, *Psammaplysilla purpurea*, *Dysidea fragilis* and *Dendrilla* sp. Thus, Keratose sponges appear to be good sources than the other orders of the Phylum: Porifera in possessing 'wonder drug' potentials. In the present

Table 2. List of fungal pathogens used for assay and their characteristics.

S/No	Fungal pathogen	Disease	Symptom
1	<i>Aspergillus niger</i> Van Tieghem	Aspergillosis	Infection in blood vessels
2	<i>Aspergillus flavus</i> (Raper and Fennell)	Liver cancer	Anomalous growth of liver cells
3	<i>Aspergillus fumigatus</i> (Fresenius)	Fungus ball	Lung problem
4	<i>Penicillium</i> sp.	Penicilliosis	Opportunistic in human infection and opportunistic in HIV infected persons
5	<i>Candida albicans</i> (C.P.Robin) Berkhout	Candidiasis	Superficial infection in skin, mouth and throat and genital lesions
6	<i>Cryptococcus</i> sp.	Cryptococcosis	Pneumonitis and cryptococcal meningitis

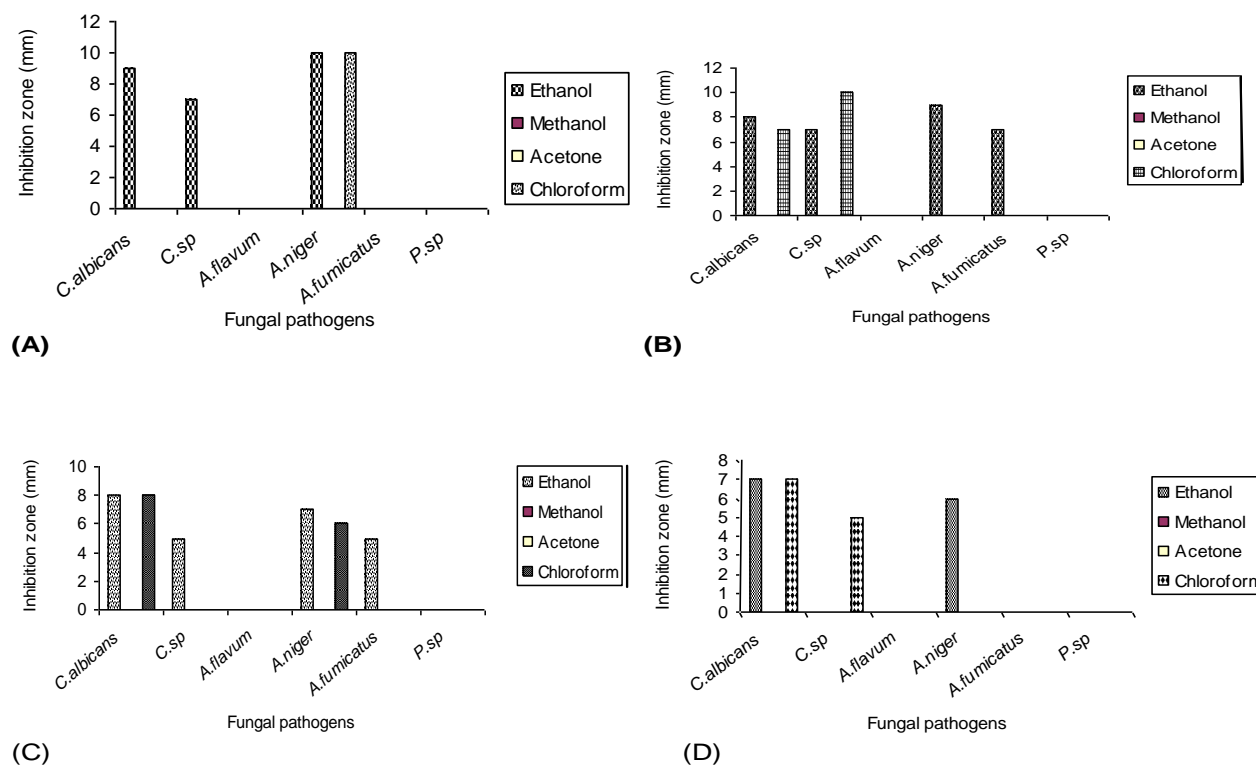


Figure 1. Antifungal activity of some species of marine sponges collected from the Palk Bay. A. Antifungal activity of *S. officinalis* B. Antifungal activity of *P. purpurea* (C) Antifungal activity of *H. cribriformis* (D) Antifungal activity of *D. fragilis*

investigation, it was found that all the sponge species of the Order, Keratosida possessed good activity against *C. albicans*, *A. niger*, *A. fumigatus* and *A. flavus*. This could be attributed to the fact that the Keratosida sponges have elasticity, more water retention capacity and more bioactive substances than the other sponge orders (Lazarus and Anita, 2000).

In an earlier study (Amade et al., 1987), out of the 7 species of Brittany sponges, only two (*D. fragilis* and *Phakellia ventilabrum*) showed slight inhibition on some bacteria and fungi. However, in the present study, *D. fragilis* showed the inhibition against *C. albicans*, *Cryptococcus* sp. *A. fumigatus* and *A. niger*. Antimicrobial activities of *S. officinalis*, *Crambe crambe* and *Ircinia fasciculata* have also been studied (Amade et al., 1987; Burkholder and Ruetzler, 1969; Uriz et al., 1992). In this regard, present study is significant in that the antifungal activity of *S. officinalis* var. *ceylonensis* was higher than that of the earlier reports.

Ethanollic extracts of 19 species of sponges collected from Polynesia were tested against bacteria and fungi. Among these, 8 had no activity, 4 had very weak activity and 7 showed significant activity against bacteria and fungi (Amade et al., 1982). In the present study, out of the ethanolic extracts of 9 species of sponges collected from the Palk Bay region, only 4 species (*S. officinalis* var. *ceylonensis*, *P. purpurea*, *D. fragilis* and *H. cribriformis*) extracts were slightly active against fungi and 5 species (*H. tenuiramosa*, *D. anchorata*, *S. inconstans* and *S. inconstans* var. *digitata*) extracts showed no fungal inhibition. Thus, the antimicrobial activity of sponges may vary from species to species as determined by the biochemical and physiological synthesis of antimicrobial compounds. When the chemical defense and anti-fouling activity were analysed, strong antimicrobial activity was found in the dichloromethane extract of *Ircinia spinosula* (against marine fungi and bacteria) and the ethanol extract of *Ircinia oros* (against diatoms) (Tsoukatou et al., 2002). Such studies including the present one may thus be useful in the prevention and/or control of biofilm formation of microbes.

Though petroleum ether, chloroform and methanol extracts of the sponge *Tethya* sp. were tested, only the petroleum ether extract was very active against mosquito larvae. But the petroleum ether extract showed lesser haemolytic activity whereas the chloroform extract showed maximum lytic activity, indicating the presence of toxicity in the chloroform extract (Madhavi and Sujala, 1998). In the present study, in the chloroform extracts activity was noticed only against *C. albicans*, *A. niger* and *Cryptococcus* sp. But the acetone extracts of the 9 species of sponges had no activity against all the fungal species tested. Of the different species of sponges, only 4 species viz *P. purpurea*, *S. officinalis* var. *ceylonensis*, *H. cribriformis* and *D. fragilis* exhibited inhibitory activity against the pathogenic fungi viz *C. albicans*, *A. niger*, *Cryptococcus* sp. *A. fumigatus* and *A. flavus* and 5

species namely *S. inconstans* var. *digitata*, *S. inconstans*, *C. diffusa*, *D. anchorata* and *H. tenuiramosa* showed no activity against all the fungal pathogens tested in the present investigation.

Thus, it can be inferred that the fungi are more resistant to the sponge extracts. This could be attributed to the fact that the cell walls of the fungi are composed of chitin, a nitrogen containing polysaccharide. The hard cover of the crabs and exoskeletons of arthropods are also composed of this substance chitin, which is relatively resistant, including for microbial decomposition (Ronald, 1997).

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