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 Czapex 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 (Kijjoa 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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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).

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

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

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 filtrates were centrifuged at 3,000 rpm. The solvent was evaporated under reduced pressure using desiccator.

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 spreading 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 (Psammamysilla purpurea, Spongia officinalis var. ceylonensis, Hyattella cribriformis and Dysidea fragilis) exhibited antifungal activities. In the other Orders, Poecilosclerida, Haplosclerida and Hadromerida, (Commeлина 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, Psammamysilla 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.

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

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*
In this 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. fumicatus and A. niger. Antimicrobial activities of S. officinalis, Crambe crambe and Ircinia fasciculate 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.

Ethanolic 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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REFERENCES


