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Anti-dermatophytic activity of marine sponge, *Sigmadocia carnosa* (Dendy) on clinically isolated fungi

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ABSTRACT

Objective: To screen the anti-fungal effects and find out the active metabolites from sponge, *Sigmadocia carnosa (S. carnosa)* against four dermatophytic fungi. **Methods:** The methanol, ethyl acetate and acetone extract of marine sponge, *S. carnosa* was examined against *Trichophyton mentagrophytes (T. mentagrophytes), Trichophyton rubrum (T. rubrum), Epidermophyton floccosum (E. floccosum)* and *Microsporum gypseum (M. gypseum)* and qualitative analysed to find out the active molecules. **Results:** The methanol extract of sponge was expressed significant activity than ethyl acetate and acetone. The minimum inhibitory concentration (MIC) of methanol extract of sponge that resulted in complete growth inhibition of *T. mentagrophytes, T. rubrum, E. floccosum* and *M.* gypseum were found to 125, 250, 250 and 250 μg/mL respectively. But, 100 % inhibition of fungal spore germination was observed in *T. mentagrophytes* at 500 μg/mL concentration followed by *T. rubrum, E. floccosum* and *M.* gypseum at 1 000 μg/mL concentration. Other two extracts showed weak anti spore germination activity against the tested dermatophytic fungi. Methanol extracts showed presence of terpenoids, steroids, alkaloids, saponins and glycosides. **Conclusion:** Based on the literature, this is the first study which has conducted to inhibit the growth and spore germination of dermatophytic fungi with *S. carnosa*. Further research also needs to purify and characterize the secondary metabolites from the sponge, *S. carnosa* for the valuable source of novel substances for future drug discovery.

1. Introduction

Dermatophyte infections in humans and animals are among the most common forms of skin diseases in worldwide[1]. This superficial fungal infection damages the keratinized tissues such as the stratum corneum, nail, and hair and dominantly caused by three genuses such as *Microsporum*, *Epidermophyton* and *Trichophyton*[2].

The drugs used against dermatophytosis exhibit several side effects and have limited efficacy[3]. So there is a distinct need for the discovery of new safer and more effective antifungal agents. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world[4]. Given the diversity of marine organisms and habitats, marine natural products encompass a wide variety of chemical classes such as terpenes, polyketides, acetogenins, peptides and alkaloids of varying structure representing biosynthetic schemes of stunning variety[5]. A small number of marine plants, and animals have already yielded more than 12 000 novel chemicals with hundred of new compounds. But, majority of these chemicals have been identified from marine invertebrates of which sponges are predominate[6]. Marine sponges have a rich source of structurally unique natural compounds of which several have shown a wide variety of biological activities[7]. The present study was carried out to prove inhibition of growth and spore germination activity of marine sponge *Sigmadocia carnosa (S. carnosa)*.

2. Material and methods

2.1. Collection and identification of sponge

The marine sponge sample was collected from the Tuticorin, Tamil Nadu, India. It was packed with sterile polythene bags with sea water and transported to the laboratory. Further, it was identified based on the spicules observed by light microscope[8].

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2.2. Preparation of sponge extraction

A total of 50 g of shade dried sponge powder was mixed with 100 mL of ethyl acetate/ methanol/ acetone and homogenized. This was kept in shaker at 100 rpm for 24 hours, and centrifuged at 12 000 rpm for 20 minutes. Followed, the supernatant was filtered through Whatman No. 1 filter paper and syringe filter. The filtrate was dried to evaporate the solvents at room temperature. The sediment extracts was weighed and dissolved in 5% dimethyl sulfoxide (DMSO) used for further study[9].

2.3. Collection of dermatophytes

Medically pathogenic dermatophytic fungi such as, *Trichophyton mentagrophytes (T. mentagrophytes)*, *Trichophyton rubrum (T. rubrum)*, *Epidermophyton floccosum (E. floccosum)* and *Microsporum gypsum* (*M. gypsum)* were obtained from the Department of Microbiology, Rajah Muthiah Medical College, Annamalai University and they were inoculated into Sabouraud dextrose broth (SDB) and incubated at 25 − 30 ℃ for 7 days.

2.4. Determination of antidermatophytic activity of sponge

Disc diffusion method was followed for anti − dermatophytic activity. 21 days fresh culture of *T. mentagrophytes*, *T. rubrum*, *E. floccosum* and *M. gypsum* were spreaded on Sabouraud dextrose agar. Whatmann No.1 filter paper discs (5 mm) were loaded with 500 μg/disc concentration of different extracts (ethyl acetate, methanol and acetone) of sponge. After the evaporation of solvent, the discs were placed on the SDA plates. Commercially available fluconazole (100 μg/disc) and DMSO were used as a positive and negative control respectively. They were incubated at 30℃ for 7 − 14 days in an incubator and were looked for the development of clearance/inhibition zones around the disc. The zone of inhibition was measured by making use of antibiotic zone scale and the results were recorded.

2.5. Minimum inhibitory concentration assay

The susceptibility of dermatophytes was determined by minimum inhibitory concentration determination method[10]. Stock concentration of sponge extracts was prepared in Sabouraud dextrose broth (SDB) and it serially diluted at final concentration of 31.25, 62.5, 125, 250, 500, 1 000, 2 000, 4000 μg/mL. 10 μL spore suspension (1.0×10^8 spores/mL) of each test pathogens was inoculated in the test tubes in SDA medium and incubated at (28 ± 2) ℃ for 2 − 7 days. The minimum concentrations at which no visible growth was observed were defined as the MICs, which were expressed in μg/mL. The control tubes containing SDB medium were inoculated only with fungal spore suspension.

2.6. Preparation of the spore suspension

The medically important dermatophytic fungi were cultured on Sabouraud dextrose agar (SDA) plates in dark at (28 ± 2) ℃ for 7 − 9 days, and then the spores were collected from sporulating colonies and suspended in sterile distilled water containing 0.1% (v/v) Tween 20. The concentrations of spores were adjusted up to 1.0×10^8 spores/mL using hematocytometer. The same were used for spore germination assay[10].

2.7. Spore germination assay

Spore germination assay was performed by previously described method[11]. Different concentration of sponge extract was dissolved in test tube with appropriate solvents and serially diluted to get 31.25, 62.5, 125, 250, 500, 1 000, 2 000 and 4000 μg/mL concentrations. The tubes were inoculated with spore suspension of each fungal pathogen containing 1.0×10^8 spores/mL. From this, 10 μL spore suspension from each were placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moisture chamber at 28 ℃ for 4 h. Each slide was then stained with lactophenol−cotton blue and observed under the microscope for spore germination. The spores generated germ tubes were enumerated and percentage of spore germination was calculated. The control different solvents were tested separately for spore germination of different fungi.

2.8. Qualitative analysis of active metabolites from sponge extract

Terpenoids, steroids, alkaloids, saponins and glycosides were screened from marine sponge by adopting the method[12].

2.8.1. Terpenoid and steroid

Four milligrams of extract was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

2.8.2. Alkaloid

The extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer′s reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

2.8.3. Saponins

Frothing test was performed to identify the presence of saponins. 100 milligrams of extract was added in 5 ml distilled water. Frothing persistence indicated presence of positive result.

2.8.4. Glycoside

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

2.9. Statistical analysis

All data were expressed as Man±SE of triplicate measurements in the Excel program (MS office − 2007).

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