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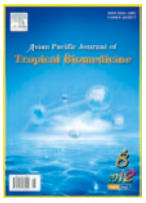

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Anti-dermatophytic activity of marine sponge, *Sigmadocia carnosa* (Dendy) on clinically isolated fungi

NB Dhayanithi[1*], TT Ajith Kumar[1], M Kalaiselvam[1], T Balasubramanian[1], N Sivakumar[2]

[1] *Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608 502, Tamil Nadu, India*

[2] *Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai-625 021, Tamil Nadu, India*

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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].

*Corresponding author: N. B. Dhayanithi, Doctoral Research Scholar, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608 502, Tamil Nadu, India.
Tel: +919965012329
E-mail: microbiodhaya@gmail.com

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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).

3. Results

The sponge spicule fragments were examined by boiling the specimen directly in a test tube with concentrated nitric acid and using light microscope. Based on the information described by Hooper[8] the specimen was identified. As the spicules were observed as sigmoid shape, matches as *S. carnosa*.

Methanol extract of marine sponge, *S. carnosa* was exhibited a moderate to high antidermatophytic effect on *T. mentagrophytes*, *T. rubrum*, *E. floccosum* and *M. gypseum*. Ethyl acetate also inhibited the growth of *T. mentagrophytes* and *T. rubrum*. But it did not show any inhibitory effect against *E. floccosum* and *M. gypseum*. Besides, acetone extract expressed weak activity against *T. rubrum* only and did not show any inhibition on *T. mentagrophytes*, *T. rubrum* and *M. gypseum*. Positive control (fluconazole 100 μg/disc concentration) also expressed significant effect against the tested fungi. Negative control was not shown any inhibition zone (Table 1).

The minimum inhibitory concentration (MIC) of sponge *S. carnosa* was performed in SDB tubes, as summarised in Table 2. The MIC of methanol extract of sponge was lower on *T. mentagrophytes*, *T. rubrum*, *E. floccosum* and *M. gypseum* and was found to 125, 250, 250 and 250 μg/mL respectively. Ethyl acetate and acetone extracts showed weak anti-dermatophytic effect against the tested fungal pathogens, with respective MIC values ranging between 500 and 2 000 μg/mL.

The extracts of sponge *S. carnosa* were subjected to dermatophytic fungal spore germination study at different concentrations (Figure 1-3). Crude methanol extracts exhibited high spore germination inhibition activity against ethyl acetate and acetone extracts. The methanol extracts has high level (100%) anti spore germination activity against *T. mentagrophytes* at 500 μg/mL (Figure 1) and *T. rubrum*, *E. floccosum* and *M. gypseum* at 1 000 μg/mL (Figure 1). Other two extracts showed weak anti spore germination activity against the tested dermatophytic fungi (Figure 2 & 3).

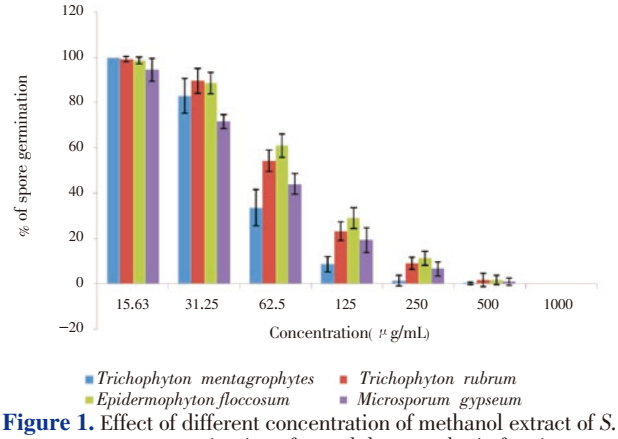

Figure 1. Effect of different concentration of methanol extract of *S. carnosa* on spore germination of tested dermatophytic fungi.

Table 1
Antidermatophytic activity of various organic extracts of marine sponge, *S. carnosa* (500 μg/disc concentration) against pathogenic dermatophytic fungi.

Fungal pathogens	Zone of inhibition (mm) at different extracts			
	Methanol[a]	Ethyl acetate[a]	Acetone [a]	Flucanazole[b]
T. mentagrophytes	15.7 ± 1.5	7.5 ± 0.7	–	21.0 ± 1.4
T. rubrum	12.0 ± 1.0	12.5 ± 2.1	7.5 ± 0.7	20.0 ± 1.4
E. floccosum	17.0 ± 1.5	–	–	20.0 ± 1.4
M. gypseum	17.7 ± 2.8	–	–	21.5 ± 0.7

Values are given as Mean ± SD of three experiments, [a]Test extracts (250 μg/disc concentration), [b]positive control (Flucanazole at 100 μg/disc concentration)

Table 2
Determination of minimum inhibitory concentration (MIC) of various rganic extracts of marine sponge, *S. carnosa* against pathogenic dermatophytic fungi.

Extracts in Different solvents at different concentration (μg/mL)		Minimum inhibitory concentration			
		Trichophyton mentagrophytes	Trichophyton rubrum	Epidermophyton floccosum	Microsporum gypseum
Methanol	67.5	+	+	+	+
	125	–	+	+	+
	250	–	–	–	–
	500	–	–	–	–
	1000	–	–	–	–
	2000	–	–	–	–
Ethyl acetate	125	+	+	+	+
	250	+	+	+	+
	500	–	–	+	+
	1000	–	–	+	+
	2000	–	–	–	–
Acetone	125	+	+	+	+
	250	+	+	+	+
	500	+	+	+	+
	1000	+	–	+	+
	2000	–	–	–	–

No growth (–); presence of growth (+)

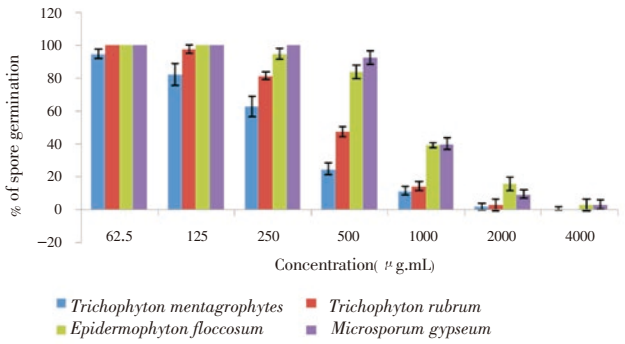

Figure 2. Effect of different concentration of ethyl acetate extract of *S. carnosa* on spore germination of tested dermatophytic fungi.

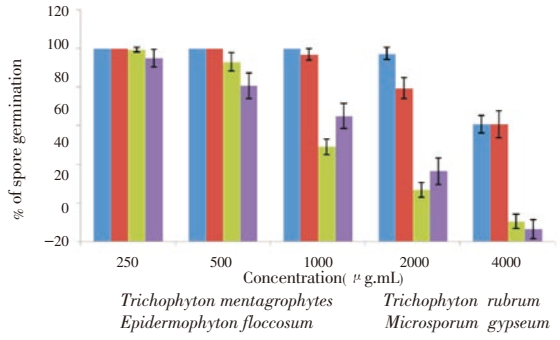

Figure 3. Effect of different concentration of acetone extract of *S. carnosa* on spore germination of tested dermatophytic fungi.

Table 3
Qualitative analysis of majority of secondary metabolites of sponge extracts

Bio active compounds	Organic extracts		
	Methanol	Ethyl acetate	Acetone
Terpenoids	+	−	−
Steroids	+	+	−
Alkaloids	+	+	+
Saponins	+	+	+
Glycosides	+	+	+

(+) present, (−) absent

The results obtained from qualitative analysis of bioactive components of sponge extracts are shown in Table 3. Methanol extracts showed presence of terpenoids, steroids, alkaloids, amino acid derivates, saponins and glycosides. Ethyl acetate extract has bioactive components except terpenoids and acetone extract lack terpenoids and steroids. Based on the previous reports, these are the active metabolites present in sponge for their adaptation and protecting themselves from other organisms.

4. Discussion

The marine sponges are belongs to invertebrate and it could be produced some secondary metabolites to protect themselves from foreign organisms[14]. Marine sponges have been used as an alternative source of therapeutics recently. Researchers have recently concentrated the effects on the search of active natural products from marine sponges[9, 13].

Infectious diseases represent a serious public health problem and they remain the major cause of death all over the world[14]. Diseases, particularly those involving the skin and mucosal surfaces represent a severe problem, especially in tropical and subtropical developing countries, dermatophytes being the most common pathogen[1]. Currently, several antifungal drugs have been used, but the prolonged duration of treatment, drug toxicity and resistance are encountered difficulties[15, 16]. For these factors render the development of new more efficient and safe antifungal drugs from natural resource is the urgent requirement.

The broad range of antimicrobial activity shown by the sponge species might be reflecting the differences in the secondary metabolites among the species. In this study, an attempt was made to determine the organic extracts of marine sponge *S. carnosa* that inhibit the growth of four clinical isolates of dermatophytes and its minimum inhibitory concentrations. Previous studies reported that aqueous and organic extracts from four marine sponges[9] against *Candida albicans* and *Cryptococcus neoformans*. Earlier study reported the *in vitro* screening of 10 marine sponges and organic and aqueous extracts were tested for anticancer, antibacterial, antifungal and anti chemotactic activities[17].

The present study indicated that methanol extract of the sponge *S. carnosa* produced minimum inhibitory concentration (Table 2) at 125 μg/mL against *T. mentagrophytes* followed by *T. rubrum* (250 μg/mL), *M. gypseum* (250 μg/mL) and *E. floccosum* (250 μg/mL). Ethyl acetate and acetone extract produced MIC range from 500−2 000 μg/mL against at the four fungal pathogens. It indicated that the sponge extracts possess highly polar based bioactive compounds. Organic solvents are generally able to extract multivariable types of polar and non-polar hydrophobic compounds[9]. In the current study, the methanol extract of sponge showed potential effect on fungal spore germination and exhibited a wide range of antidermatophytic activity. But other two extracts like ethyl acetate and acetone showed low activity. Earlier reports revealed that the organic extract from *S. carnosa* exhibited weak activity against the tested bacteria and fungi except *Fusarium* sp.[18-21].

In this study, the methanol extract of sponge showed potential effect on fungal spore germination and exhibited a wide range of anti dermatophytic activity. Limited literatures are available for antidermatophytic potentials of marine sponges. Previous study reported that the essential oil of *M. glyptostroboides* showed potential antidermatophytic effect and spore germination of the tested skin fungal pathogens[10].

This anti dermatophytic effect of the sponge might be the presence of several bioactive compounds[22-24]. Previous reports has showed initial inhibitory bioactivities of a marine sponge and isolated two new bioactive alkaloids such as N-methyl-epi-manzamine D1 and epi-manzamine D2[25]. Seven lactone metabolites, namely the known plakortones B-D was isolated previously[23]. In the present investigation number of different phyto-compounds has been qualitatively identified from the organic extract of marine sponge *S. carnosa*. Methanolic extract has more bioactive compounds compare with other two extracts. The present study indicated that the presence of terpenoids, steroids, alkaloids, saponins and glycosides in methanol extract. It might be the reason that methanol extract showed good activity against *T. mentagrophytes*, *T. rubrum*, *E. floccosum* and *M.*

gypsum than other solvents. It indicated that presence of terpenoids, steroids, alkaloids are responsible for control the growth of dermatophytic fungal pathogens. Terpenoid phenols, including carvacrol, are components of oregano and other plant essential oils that exhibit potent antifungal activity against a wide range of pathogens[26]. The anti spore germination effect was higher in methanolic extract may be the presence of trepenoids and sterols. Trapenoid was not extracted during ethyl acetate and acetone extraction. Earlier studies also reported that steroids, terpenoids, amino acid derivatives, saponins, steroids, glycosides and alkaloids were present in marine sponge[4]. Terpenoids exhibits anti fungal activity[27].

The results obtained in this study potentially supported that sponge *S. carnosa* can be used as a source for wide range of new antidermatophytic agents to control superficial human fungal infections. The results proposing that the sponge, *S. carnosa* could be a valuable source of novel substances for future drug discovery. Further research also need in this line.

Conflict of interest statement

We declare that we have no conflict of interest.

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