Evaluation of Fungal Dermophytes (Candida Albicans, Candida Tropicalis, Trichophyton Mentagrophytes) and Antifungal activities

Dr.K. S. Manjunatha

Department of Pharmaceutical Chemistry, Kuvempu University, Post Graduate Centre, Kadur-577548, Karnataka, India.

Abstract

The present investigation has focused to evaluate the antifungal activities of various solvent extracts of marine brown alga Spatoglossumasperum. The antifungal activities were evaluated against three fungaldermatophytes namely *Candida albicans, Candida tropicalis, Trichophyton mentagrophytes* and one non dermatophyte*Aspergillusflavus* using disc diffusion method. The maximum activity was recorded from chloroform extract against the non dermatophytic fungi *Aspergillusflavus*(98.83%) and the methanolic extract showed a significant antifungal activity against dermatophytic fungi *Candida albicans*(57.14%) and *Candida tropicalis*(54.75%) as compared to other solvent extracts. Thus, it can be concluded that the methanolic extract of brown seaweed *Spatoglossumasperum*is more effective on dermatophytic fungi.

Keywords: Antifungal activity, Dermatophytes, Solvent extract, *Spatoglossumasperum.*

1. Introduction

Marine macro algae are rich sources of structurally novel and biologically active secondary metabolites.

Approximately 2500 new metabolites were reported from a variety of marine organisms during the years from 1977 to 1987 [1]. There is an increasing demand in selecting therapeutic drugs from natural products, especially the seaweeds having a broad range of biological activities such as antibacterial and antifungal. Brown marine algae are mainly consumed as healthy food sources for human due to the presence of high concentration of polysaccharides, minerals, polyunsaturated fatty acids and vitamins. In recent years, there have been many reports of macro algae derived compounds that have a wide spectrum of biological activities such as antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic and antimitotic activities [2]. However, there is special attention have been focused on antiviral, antibacterial and antifungal activities against human pathogens [3-8] since fungal infections cause a high rate of mortality in human population and aquaculture organisms [4]. The present study was undertaken to investigate antifungal activities using various solvent extracts of marine brown alga *asperum.*

2. Materials and methods

2.1 Collection and preparation

*Spatoglossumasperum*was collected from the intertidal regions of the Mandapam coast (Latitude. 09˚17.417'N; Longitude. 079˚ 08.558'E) of the Gulf of Mannar and was immediately brought to the laboratory in plastic bags containing water in order to prevent deterioration. Then this alga was washed thoroughly with sterilized sea water to remove extraneous materials. The sample was shade dried until constant weight obtained and ground into powder using blender. The powdered samples were stored in airtight containers and kept in the refrigerator for future use.

2.2 Preparation of algal extracts

Seaweed powder was soaked in the polar solvents such as water, methanol, chloroform, ethyl acetate and non-polar solvent like hexane in 1:3 w/v ratio and kept in hot air oven at 60^{O}C for 24 hrs and the extracts were collected. The extract was then filtered through a Buchner funnel with Whatman No.1 filter paper. The filtrate was evaporated to dryness under pressure using a rotary vacuum evaporator at 50^{O}C and the crude extracts were weighed. The yield of powdered sample obtained was 7.5 g/100 g from methanol solvent, 5.6 g/100 g of aqueous extract, 4.8g/100g from chloroform extract, 3.3g/100g from ethyl acetate extract and 3.1 g/100 g from hexane extract. These crude extracts were then tested for their antifungal activity against selected fungal pathogens.

2.3 Pathogens used for the assay

The dematophytic fungal species such as *Candida albicans, Candida tropicalis, Trichophyton mentagrophytes*and non dermatophytic fungal species *Aspergillusflavus*were obtained from Department of Microbiology, Mohamed Sadak College, Chennai. The fungal pathogens were maintained on Potato Dextrose Agar (PDA) (Hi Media, India).

2.4 Antifungal assay

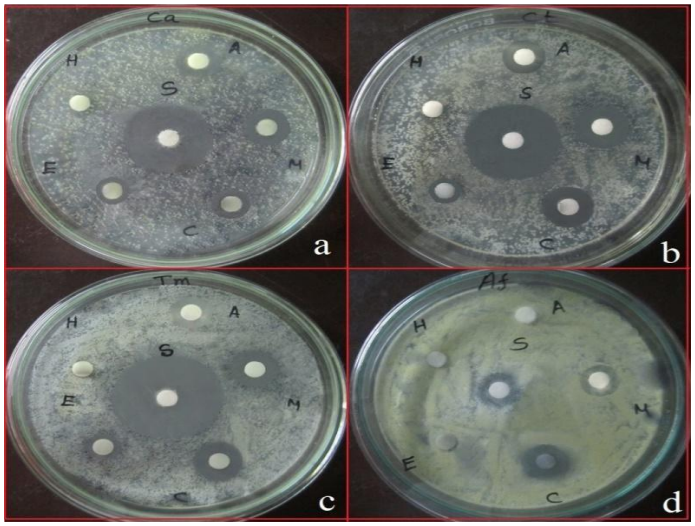
Antifungal activity was evaluated using the disc diffusion technique in sterilized petriplates [9]. Sterile filter paper discs 6 mm in diameters (Whatman No. 1) were loaded with different extracts (100 µg/mL) and air- dried. Discs containing flucanozole was used as control (100 µg/mL). The discs were placed on Potato Dextrose Agar (PDA). Plates were swabbed inoculated using sterile cotton buds with each of the previously mentioned fungal pathogens. Plates were incubated for 48 hrs at room temperature, for each algal extract zone of inhibition was recorded in millimeters and it was compared with control and the results were expressed in percentage of inhibition. The antifungal assay was done in triplicates.

3. Results and Discussion

The antifungal activity of various solvent extracts (A-aqueous/water, M-methanol, C-chloroform, E-ethyl acetate and H-hexane) of *S. asperum*was tested against fungal dermatophytes and non dermatophyte. The antifungal activities of various solvent extracts of *S. asperum*are shown in the Figure 1. The results of the antifungal activity are summarized in Table 1 and Figure 2. In the present study the highest inhibition was recorded in chloroform

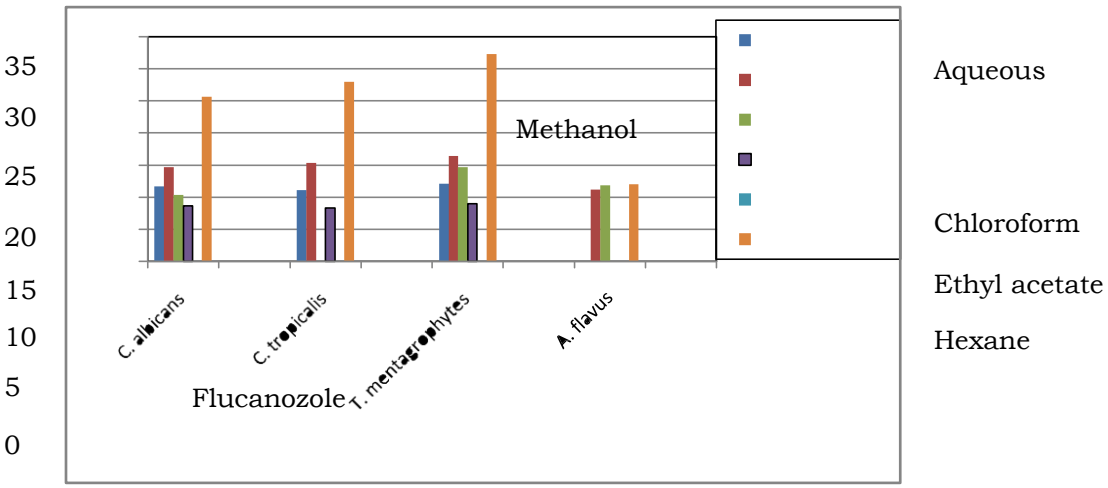
98.83% (11.86 ± 0.61 mm) and methanolic extract 92.91% (11.15 ± 0.882 mm) against *A. flavus*, in the same way [10] reported that the chloroform extract showed 10mm zone of inhibition against *A. flavus*and no inhibition observed by them in the methanolic extract of marine brown alga *Turbinariaconoides*. In the present study, no inhibition zone wasobserved in aqueous, ethyl acetate and hexane extract against *A. flavus,* and it was agreed with the reports of [10] as they observed no inhibition in the aqueous, methanol and ethyl acetate extract of marine brown algae *T. conoides*and *Sargassumwightii.*

Figure1: Antifungal activity of various solvent extracts of *Spatoglossumasperum*



A-Aqueous, M-Methanol, C-Chloroform, E-Ethyl acetate, H-Hexane, S-Standard (flucanozole) (a) *Candida albicans*(b) *Candida tropicalis*(c) *Trichophyton mentagrophytes*(d) *Aspergillusflavus*

Figure 2: Antifungal activity of various solvent extracts of *Spatoglossumasperum*



The methanolic extract showed the highest percentage of inhibition against all the pathogens studied, *Candida albicans*57.14% (14.67 ± 0.33 mm), *Candida tropicalis*54.75% (15.33 ± 0.33 mm); *Trichophyton mentagrophytes*50.85% (16.44 ± 0.577 mm), chloroform and aqueous extract of *S. asperum*showed moderate activities against the fungal strain were studied. Whereas ethyl acetate and hexane extract doesn't show any activity against the above pathogens studied [10]. Lavanya and Veerappan reported the methanolic extract of *S. wightii*and *T. conoides*exhibited highest activity against *C. albicans*, similarly the methanolic extract of *S. asperum*showed the highest activity against *C. albicans*. In the present study, aqueous extract of *S. asperum*showed moderate activity against *C. tropicalis*and it was correlated with the study of [11] they also reported the aqueous extract of *T. conoides*showed moderate activity against *C. tropicalis*.

Chloroform extract of marine brown algae *S. wightii*and*T. conoides*exhibited moderate activity[10] against *Candida albicans*, as in the present study, it was also found to be moderate activity against *Candida albicans*.[12] Selvaraj*et al.*, reported, the chloroform extract of *Stecheospermummarginatum*showed moderate antifungal activity against *Trichophyton mentagrophytes*, *Aspergillusflavus*and *Candida albicans*, in the same way, the present study it was observed that the chloroform extract of *S. asperum*exhibited moderate activity against *Candida albicans, Candida tropicalis*and *Trichophyton mentagrophytes*and higher activity against *Aspergillusflavus*[10]. Lavanya R and Veerappan reported ethyl acetate and hexane extract of *S. wightii*and *T. conoides*showed no activities against *Candida albicans,* whereas, in the present study ethyl acetate extract showed lesser activities and no activity observed for hexane extract.

The aqueous extract of *S. asperum*against *Aspergillusflavus*shows no activity and it was supported by [10] Lavanya and Veerappan reported the aqueous extract of *Caulerpadecorticatum*and *Caulerpascalpelliformis*showed no activity against *A. flavus*[13]. Aruna*et al.*, reported among the seaweeds tested the high rate of antifungal activity was noticed in the brown alga *S. wightii*and followed by red alga *K. alvarezii.* The methanolic extract of red seaweeds *Asparagopsistaxiformis, Laurenciabrandenii, Laurenciaceylanica*and *Hypneavalentiae*showed higher activity against *Candida albicans*[13]. It is correlated with the present study that the methanolic extract of brown seaweeds *S. asperum*showed the highest activity against *Candida albicans.* Many earlier reports

have shown the antifungal potential of seaweeds [12], though the present study was well correlated with the study of [10, 14].

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities, compounds with cytostatic, antiviral, anthelmenthic, antifungal and antibacterial and antifungal activities have been detected in green, brown and red algae [15,16]. The production of antifungal activities was considered to be an indicator of the capability of the seaweed to synthesize bioactive secondary metabolites. The search for new antifungal drugs is important because there are few existing therapies for life-threatening infections. The present investigation clearly demonstrates that the antifungal activities and the greatest inhibition zone were recorded from the marine brown alga *S. asperum*.

4. Conclusion

In the present study, the methanolic extract of *S. asperum*exerted good antifungal activity against different dermatophytic fungal species and chloroform extract showed higher activity against non dermatophytes. Fungal mycelial growth was strongly inhibited in the methanolic extract. Hence, further work is required to identify the bioactive molecules that are responsible for the antifungal activity (phenolic compounds, polysaccharides or fatty acids) and to assess the skin protective role of seaweed extract.

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