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EVALUATION OF ANTIFUNGAL PROPERTY OF MEDICINAL PLANTS

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This paper reports for the first time the antifungal activity of some medicinal plants used by the traditional healers for the treatment of skin infections. The leaf extract of plant species were screened against fungal pathogens *Candida albicans, Kluyeromyces polysporous, Aspergillus niger,* and *Aspergillus fumigatus.* In antifungal activity screening, methanolic extracts of plant species showed a very good activity against all the tested fungal pathogens. MIC values were determined by checking the growth after 24 and 48 h to determine the antifungal activity against the tested pathogens. MIC values of leaf extracts of *Trifolium pepens* are in the order of 0.02 to 0.48. The acetone extracts of *Swertia trichotoma* showed the highest antifungal activity against *A. fumigatus.* This probably explains the use of these plants by the indigenous people against a number of infections.

Keywords : Antifungal; Belgaum district; Herbal medicine; Traditional healers.

Introduction

In India, according to reasonable estimates, 70% of the population still rely on herbal medicines[1]. Nation witnesses 2500 species of plants from about 1500 genera which are used by traditional healers[1,2]. The traditional systems of medicine together with folklore system continue to serve a large proportion of population, particularly in the rural areas, in spite of the advent of the modern medicines[3-7]. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha[8]. In recent years, risk of opportunistic fungal infections has greatly increased in patients who are severely immunocompromised due to cancer therapy, organ or bone marrow transplantion and human immunodeficiency virus infection (HIV)[9]. Despite advances in antifungal therapies, many problems remain to be solved for most antifungal drugs available in the market. For example the use of amphotericin B, and azoles, such as fluconazole, ketoconazole and micronazole, has resulted in clinically resistant strains of *Candida* species[10]. This situation highlights the need for advent of safe, novel and effective antifungal compounds. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth[10]. The purpose of this study was to determine *in vitro* antifungal activity of few medicinal plants used by the local traditional healers of Belgaum district of Karnataka in the management of fungal infections.

Materials and Methods

Plant collections, drying and storage - Plants used in this study viz. *Trifolium pepens* (Asclepiadaceae) and *Swertia trichotoma* (Gentiaceae) were collected from Belgaum district, Karnataka, India. The leaf part of all these plants were used for various medicinal purposes by the local traditional healers. More information on these plants are presented elsewhere[6]. Leaves were seperated from stems, and dried at room temperature. Most of the workers have tended to use dried material because there are few problems associated with large scale extraction of dried plants rather than fresh plant material[4-6,11]. The dried plant material were milled to a fine powder, and stored at room temperature in closed containers in the dark until further use.

Extraction procedure- Plant samples from each species were individually extracted by weighing four aliquots of 1 g of finely ground plant material and extracting with 10 ml of acetone, hexane, dichloromethane (DCM) and, methanol in centrifuge tubes. These tubes were vigorously shaken for 3-5 min in a shaking machine at high speed. After centrifugation at 3500 rpm for 10 min the supenatant was decanted into labelled containers. This process was repeated for 2 to 3 times to exhaustively extract the plant material and extracts were combined. The solvent was

Table 1. MIC values in mg/ml of *Trifolium pepens* plant species after 24 and 48 h of incubation.

Organisms	Time (h)	MIC values (mg/ml)			
		Acetone	Hexane	DCM	Methanol
C. albicans	24	0.11	0.47	0.17	0.05
	48	0.13	0.48	0.17	0.05
K. polysporus	24	0.13	0.21	0.08	0.04
	48	0.16	0.20	0.10	0.06
A. niger	24	0.02	0.14	0.14	0.09
	48	0.02	0.11	0.15	0.06
A. fumigatus	24	0.08	0.20	0.08	0.02
	48	0.09	0.20	0.08	0.02

Table 2. MIC values in mg/ml of *Swertia trichotoma* plant species after 24 and 48 h of incubation.

Organisms	Time (h)	MIC values (mg/ml)			
		Acetone	Hexane	DCM	Methanol
C. albicans	24	0.23	0.40	0.27	0.02
	48	0.23	0.40	0.27	0.02
K. polysporus	24	0.12	0.29	0.08	0.06
	48	0.11	0.29	0.08	0.06
A. niger	24	0.25	0.19	0.06	0.02
	48	0.24	0.19	0.06	0.02
A. fumigatus	24	0.04	0.46	0.04	0.07
	48	0.04	0.46	0.04	0.07

removed under a stream of air at room temperature before dissolving extracts in acetone to a concentration of 10mg/ml, to quantify the assay.

Fungal test organisms- Four fungi were obtained from Department of Botany, Karnatak University, Dharwad, India. These fungi represent different morphological forms of fungi namely yeasts (*Candida albicans, Kluyeromyces polysporous*), and moulds (*Aspergillus niger, Aspergillus fumigatus*). These are most common and important disease-causing fungi of animals and human beings. All the fungal strains were maintained on YM agar medium in accordance with Malabadi[12], and Malabadi and Raghvendra[13,14].

Antifungal assay-A serial dilution assay of Eloff[15]. was used to determine the minimum inhibitory concentration (MIC) values for plant extracts using tetrazolium violet reduction as an indicator of growth. This method had previously been used only for antibacterial activities[3-5,15,16]. To apply it for measuring antifungal activities, a slight modification was made to suit fungal growth conditions[10]. Residues of the different extracts were dissolved in acetone to a concentration of 10mg/ml. The plant extracts (100 µl) were serially diluted 50% with water in a 96-well microtitre plates[15]. Fungal cultures were transferred into fresh YM agar broth, and 100 µl of this added to each well. Amphotericin B was used as the reference antibiotic and positive control, and appropriate solvent blanks were included as negative control. As an indicator of growth, 40 µl of 0.2 mg/ ml of p-iodonitrotetrazolium violet (INT) (Sigma, USA) dissolved in water, was added to each of the mircroplate wells. The covered microplates were incubated for 2 to 3 days at 35°C. The MIC was recorded as the lowest concentration of the extract that inhibited fungal growth after 24 and 48h. MIC values are recorded in the Table 1 and 2. All the experiments were repeated for three times and the readings in the table represents the avarage of three independent experiments. When cells from wells showing no growth after 48 h were incubated in fresh growth medium, however, fungal growth resumed.

Results and Discussion

In the present study, the plant material was extracted with four different solvents (acetone, hexane, DCM and methanol). Among all the solvents, methanol was the best extractant. Of the four solvents used, methanol extracted

more chemical compounds from the leaves of the plants tested, but the extract probably contained highly polar compounds and tannins, that may not be of much interest for clinical applications. Further, on the basis of information provided by the local traditional healers, the leaf juice of *T. pepens* and *S. trichotoma* were used for skin infections diseases. Skin infections of deep and superficial wouds are common in tropical developing countries like India[5,6]. Among the infectious diseases, diseases caused by fungal infections account for a larger proportion of health problems in human particularly among children and women[5]. MIC values were determined by checking growth after 24 and 48 h to determine the antifungal property (Table1 and 2). On the basis of results, it was observed that, the MIC values of leaf extracts of *T. pepens* are in the order of 0.02 to 0.48 (Table 1). Among all the solvants, the methanol extracts of *T. pepens* showed the highest antifungal activity against all the tested pathogens, with the MIC values in the range of 0.02 to 0.09 (Table 1). Another interesting result was obtained with the acetone extracts of *T. pepens* which also exihibited antifungal activity against *A. niger* with the MIC values of 0.03. Rest of the leaf extracts of hexane and DCM showed a moderate antifungal activity with the MIC values of 0.08 to 0.48. These results of *T. pepens* are significant in terms of high antifungal activity particularly against *C. albincans* with a MIC values of 0.05 (Table 1). Hence, methanol extract was found to be the best solvant showing the highest antifungal properties against all the tested pathogens. The MIC values of *S. trichotoma* are in the range of 0.02 to 0.46. The methanolic extracts of *S. trichotoma* showed the highest antifungal activity against all the tested fungal pathogens with MIC values of 0.02 to 0.07. The DCM extracts of *S. trichotoma* showed maximum antifungal activity against *K. polysporous*, *A. niger* and *A. fumigatus*, respectively. The acetone extracts of *S. trichotoma* showed the highest antifungal activity against *A. fumigatus*.

Our present study, to some extent, confirmed the medicinal properties of these plants. Some other workers determined antifungal activities and MIC using different plant species. Their MIC values were generally high. Delaporte[17] used *Tillandsia streptocarpa* to test antimicrobial activity on *C. albicans* (MIC> 0.5 mg/ml), Chandrasekaran and Venkatesalu[16] have found that seed extracts of *Syzgium jambolanum* were effective against different pathogens, *C. albicans*, *C. neoformans*, *A. fumigatus* and M. *gypseum* with the MIC values of 0.62, 0.25, 0.125 and 0.25 mg/ml, respectively. Chamundeeswari[18] found antifungal activity of *Trewia polycarpa* root extracts on *C. albicans*, *A. niger*, *C.neoformans* and *Penicillum* sp. Alcoholic extracts had mild antifungal activity with MIC values of 0.25, 0.25, 0.125 and 0.13 mg/ml, respectively. The ethanol extract of the whole plant of *L. inermis* showed antifungal activity against *T. mentagrphytes*, *C. albicans*, *A. niger*, *C. neoformans* and *M. Canis*[19]. When comparing the MIC values with our data, it is clear that extracts of *T. pepens* and *S. trichotoma* have a strong activity against fungal pathogens.

Result of the present study suggest a fairly good correlation between traditional therapeutic use and *in vitro* antifungal activity. These results corroborate the importance of ethanobotanical surveys for screening plants as source for bioactive compounds. Hence, this could result in the discovery of novel antifungal agents. Further study of bio-guided fractionation is very much necessary to characterize the active constituents.

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