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Antifungal Activity of Selected Indian Medicinal Plants

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Abstract

The antifungal activity of aqueous and methanolic extracts of ten different Indian medicinal plants were assessed using agar well diffusion test against four different fungi viz, Candida albicans, Aspergillus fumigatus, Aspergillus parasiticus and Cryptococcus sp.The assessment of the minimum inhibitory concentrations of the active extracts were carried out using serial dilution plate technique. A. fumigatus was found to be sensitive to eight out of the ten alcoholic extracts tested where as Cryptococus alone was found sensitive to the aqueous extracts. The alcoholic extract of the seed coat of Tamarindus indica and leaf of Allophyllus cobbe inhibited the growth of C. albicans and A. fumigatus at 12.5 mg/ml where as leaf extracts of Azadirachta indica, Annona squamosa, Mallotus phillipensis and Chromoleana odorata at the same dose rate inhibited the growth of A. fumigatus alone. From the study it could be concluded that eight out of the ten alcoholic extracts tested form promising leads for synthesis of novel antifungal agents.

Key words: Anti fungal, Minimum inhibitory concentration, Medicinal plants

I. INTRODUCTION

Fungi occur ubiquitously in nature and are well adapted for life in all climatic conditions. The skin and mucosal infections produced by fungi are a serious problem in both human and veterinary practice in tropical and subtropical regions. Most of the infections are due to fungi especially *Candida* and dermatophytes [1]. *Candida albicans* is an opportunistic pathogen causing local and systemic infections in predisposed persons, commonly affecting immunologically compromised patients and those undergoing prolonged antibiotic treatment [2] Even with the existence of potent antifungal agents, resistant or multi drug resistant strains are continuously appearing [3]. The important drugs for the therapy of dermatophytosis include terbenafine, itraconazole, ketoconazole, griseofulvin and most of them cannot be used for long term therapy due to their toxic effects. The increased incidence of immunocompromised individuals and prevalence of resistant fungi have necessitated the introduction of newer and potent antifungal agents.

Even though the synthetic drugs are a major source for the therapy of infections, recently focus has been shifted to the use of natural products for the therapy of bacterial and fungal diseases [4]. The use of plants and plant derived medicines for treatment of cutaneous infections is an age old practice. In India, many plants are used for the treatment of pruritus or infections of skin of unknown origin. Plants produce a variety of secondary metabolites with antifungal activities which include flavonoids, phenolics, unsaturated lactones, saponins etc [5].

The present investigation was carried out for the assessment of antifungal activity of the methanolic and aqueous extracts of ten different medicinal plants that are used in folklore and traditional medicine in the district of Wayanad against human and animal pathogens.

II. MATERIALS AND METHODS

2. 1. Plant material

10 different plant materials viz, leaves of *Azadirachta indica, Mallotus phillipensis, Allophyllus cobbe, Vitex negundo, Smithia sensitiva, Anona squamosa, Murraya paniculata, Chromoleana odorata, Senna alata* and seed coat of *Tamarindus indicus* were collected from different parts of the district of Wayanad, identified by a Botanist at MSSRF, Kalpetta, dried under shade and pulverized. The alcoholic extraction was carried out using methanol in a soxhlet extraction apparatus and the aqueous extract was taken as decoction. It was dried using a rotary vacuum evaporator and stored under refrigeration till further use.

2.2.Screening for antifungal activity

The fungal strains were purchased from MTCC (table 1) and were sub cultured in potato dextrose agar by incubation at 28^0C for 2-7 days.

2.2.1.Agar well diffusion tests

For the preparation of inoculum, a suspension of the culture was transferred into a sterile tube and the turbidity was measured to Mcfarland Standard of 0.5, corrected by using normal saline. Sterile MHA plates were prepared and fungal cultures were swab cultured on to the surface of the agar. Wells of 6 mm diameter were bored using a sterile well borer and 25µL of the extract diluted in 10% dimethyl sulphoxide (DMSO)/ tween-80 to obtain concentrations of 500, 250, 100, 50, 25 and 12.5 mg/ml were added to the wells. After incubation for 7 days at 28°C, the plates were examined for the presence of growth/ inhibition of growth and the diameter of zone of inhibition were measured in millimeters. Hexadisc containing Amphotericin B 100 units, Clotrimazole 10 mcg, Fluconazole 25 mcg, Itraconazole 10 mcg, Ketoconazole 10 mcg and Nystatin 100 units were used as positive control. The tests were done in triplicates [6].

2.2.2.Minimum Inhibitory Concentration

Minimum inhibitory concentration was determined by a serial dilution plate technique where solutions of the reconstituted extracts at based on the results of agar dilution technique were added into the pre-sterilized and pre-cooled SDA, mixed well, media poured and allowed to set. The plates were then inoculated with the test fungi and incubated at 28°C for 2-7 days. Control plates which contained no extract were also prepared along with the extract. The MIC of each plant was determined after incubation, being the lowest concentration with no visible growth [7].

III. RESULTS

Ten plant extracts were tested for their antifungal activity against 5 fungi (Table 1). The different extracts were tested for their yield and the % yield was recorded (Table 2).

Table 1: List of fungal strains

Sl No.	Name of fungus	MTCC no
1	*Candida albicans*	1637
2	*Aspergillus fumigates*	8234
3	*Aspergillus parasiticus*	9164
4	*Cryptococcus sp.*	7841

Table 2: Per cent yield of extract from different plants

Plant	Part tested	Type of extract	% yield
Azadirachta indica	Leaf	Alcoholic	15.17
		Aqueous	13.12
Tamarindus indicus	Seed coat	Alcoholic	32.98
		Aqueous	16.30
Mallotus phillipensis	Leaf	Alcoholic	12.53
		Aqueous	10.49
Allophyllus cobbe	Leaf	Alcoholic	17.08
		Aqueous	10.00
Vitex negundo	Leaf	Alcoholic	44.10
		Aqueous	14.08
Smithia sensitive	Leaf	Alcoholic	12.12
		Aqueous	8.34
Anona squamosa	Leaf	Alcoholic	10.11
		Aqueous	14.70
Murraya paniculata	Leaf	Alcoholic	14.69
		Aqueous	13.01
Chromoleana odorata	Leaf	Alcoholic	13.20
		Aqueous	13.88
Senna alata	Leaf	Alcoholic	16.89
		Aqueous	14.78

The aqueous as well as the methanolic extracts were tested separately for the antifungal activity. Initially the dose at which the growth was inhibited was found out using agar well diffusion method. The minimum inhibitory concentrations were found out using serial dilution plate technique and the results for the yield and antifungal activity for methanolic and aqueous extracts are shown in table 3 and 4 respectively.

Table 3: Antifungal activity of different methanolic extracts (MIC mg/ml)

Plant	*A. indica*	*T. indicus*	*M. phillipensis*	*A. cobbe*	*V. negundo*	*S. sensitiva*	*A. squamosa*	*M. paniculata*	*C.odorata*	*S. alata*
Candida albicans	-	12.5	-	12.5	-	-	-	-	-	-
Cryptococcus sp.,	500	12.5	25	12.5	-	-	250	-	-	50
Aspergillus fumigates	12.5	-	12.5	100	25	100	12.5	-	12.5	12.5
Aspergillus parasiticus	25	-	-	-	-	-	-	-	250	-

Table 4: Antifungal activity of different aqueous extracts (MIC mg/ml)

Plant	*A. indica*	*T. indicus*	*M. phillipensis*	*A. cobbe*	*V. negundo*	*S. sensitiva*	*A. squamosa*	*M. paniculata*	*C.odorata*	*S. alata*
Candida albicans	-	-	-	-	-	-	-	-	-	-
Cryptococcus sp.,	-	100	12.5	25	250	-	-	-	-	-
Aspergillus fumigates	-	-	-	-	-	-	-	-	-	-
Aspergillus parasiticus	-	-	-	-	-	-	-	-	250	-

IV. DISCUSSION

The plants for the study were selected based on their use in folklore and traditional medicine of the district of Wayanad, Kerala for the treatment of cutaneous infections and pruritus of unknown origin. In traditional folklore medicines, combinations of plant extracts are used than a single plant extract whose activity will be usually weak under clinical infections [8]. The results of the present study show that the methanolic extract of the plants were more active against the fungi tested whereas the aqueous extract of *M. phillipensis* and *A. cobbe* were equally effective against *Cryptococcus sp*. The MIC values were between 500 to 12.5 mg/ml. It was interesting to note that none of the extracts of *Murraya paniculata* showed activity against the organisms tested.

It was proposed by [9] that those plant extracts showing MIC above 1.6 mg/ml can be considered as weak inhibitors of fungal growth. Plant extracts being crude fusions containing much number of components show high minimum inhibitory concentrations and those extracts that show MIC's below 50 mg/ml can be considered to be having antifungal property as in the case of clove, sweet flag and eugenol against *Candida albicans*. [10,11]. Bibliography [12] reported the efficacy of hydroalcoholic extract of 15 various plants of Mexico against different strains of *Candida.* There are several reports of the antifungal activity of *Cassia alata* against dermatophytes, but there was no activity against candida and non dermatophytic fungi [13,14]. In our study, the alcoholic extract of *Senna alata* inhibited the growth of *Cryptococcus* and *Aspergillus fumigatus* with an MIC of 50 and 12.5 mg/ml respectively, which may be due to the difference in geographical area and phytochemical constituents of the *Senna alata* collected for the present study. The MIC values of Neem leaf extract against different dermatophytes were below 1mg/ml [15,16] of 12.5 mg/ml in the case of *A. fumigatus* and higher MIC's for other fungi. This may be due to difference in the susceptibility as well as cell wall structure of the fungi taken for the present study. There are several reports that phytochemicals like Saponins and tannins have potent antifungal property. [17,4]. Saponins enter the cells and damage their cell membrane leading to leakage of cellular contents and death of the cells [18].

In the present study, it is seen that the methanolic extract of *A. cobbe* is active against almost all the organisms tested except *A. parasiticus*. The higher MIC values may be due to the crude nature of the extract, however the results substantiate the use of these crude plants for skin infections suggestive of fungal infestation. The fact that these plants show antifungal property makes them suitable for further investigations into the constituents and isolation of the active ingredient.

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