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Full Length Research Paper

In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds

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The methanol extract of 9 Indian medicinal plants belonging to 9 different families were evaluated for *in vitro* antifungal activity against some yeasts including *Candida albicans* (1) ATCC2091, *C. albicans* (2) ATCC18804, *Candida glabrata* NCIM3448, *Candida tropicalis* ATCC4563, *Cryptococcus luteolus* ATCC32044, *Cryptococcus neoformans* ATCC34664, *Trichosporon beigelli* NCIM3404, and some moulds such as *Aspergillus candidus* NCIM883, *Aspergillus flavus* NCIM538, *Aspergillus niger* ATCC6275 and *Mucor heimalis* NCIM873. The *in vitro* antifungal activity was evaluated at three different concentrations by agar disc diffusion method and the activity obtained was not concentration dependent. *A. flavus* was the most susceptible fungal strain while *C. glabrata* was the most resistant one. *Saussurea lappa* showed the best antifungal activity. The results were compared with the standard antifungals.

Key words: medicinal plants, antifungal activity, methanol extracts, yeast, mould, *Saussurea lappa.*

INTRODUCTION

Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. The subcontinent is rich in medicinal plants and is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Krishnaraju et al., 2005). Several plants have been used in folklore medicine (Premanathan et al., 2000). The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare.

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents (McNeil et al., 2001).

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Candida albicans, the agent of candidiasis, is an increasingly important disease that has a world wide distribution due to the fact that it is a frequent opportunistic pathogen in AIDS patients (De Pavia et al., 2003). It is a common commensal of the gastrointestinal and urogenital tracts of human (Black, 1996) and is also the cause of Candiasis in women (Demarch et al., 1995). *C. albicans* is a major concern worldwide (Nolte et al., 1997). *Candida tropicalis* is one of the non-albicans candida strains currently emerging in fungal infections (Powderly et al., 1999). *Cryptococcus neoformans* is the cause of the most common life-threatening meningitis in HIV-positive patients (Michaels et al., 1999).

Since strains of *C. albicans* with multiple antibiotic resistance is increasing worldwide, it is of great importance to find effective treatments for these pathogens. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Srinivasan et al., 2001). To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. The available antifungal drugs produce many

Table 1. Ethnomedical information of the screened plants

Plant species	Family	Common name	Part used	Therapeutic use (Anjaria et al. 2002; Sriram et al. 2004)
Caesalpinia pulcherrima (L.) Swartz.	Caesalpiniaceae	Sandhesharo	Aerial parts	Abortifacient, antiperiodic, astringent, cathartic, emmenagogue, purgative, stimulant, tonic, ulcers, asthama, bronchitis, cholera, malaria, tumors,
Cyperus rotundus L.	Cyperaceae	Moth, Shaiyo	Whole plant	Anthelmentic, aromatic, astringent, diaphoretic, diuretic, emmenagogue, stomachic, diarrohea, dysentry, inflammations
Euphorbia tirucalli L.	Euphorbiaceae	Dandilyo thor	Stem	carminative, purgative, stomachic, asthama, dropsy, dyspepsia, gonorrhoea, leprosy, neuralgia, syphilis
Holarrhena antidysenterica (Heyne. ex Roth.) A. DC.	Apocynaceae	Kada chaal	Bark	Anthelmentic, aphrodisiac, astringent, carminative Coolant, febrifuge, asthma, biliousness , boils, bronchitis, diarrohea, diabetes, dropsy, dyspepsia, fever, headache, leprosy, inflammation, piles, skin disease, swelling, ulcers, wounds
Mangifera indica L.	Anacardiaceae	Ambo	Leaf	Anthelmentic, asthma, aphrodisiac, emetic, laxative, tonic, anorexia, constipation, diarrohea, diphtheria, dysentry, inflammation, rheumatism, syphilis, ulcers, worms, vomiting
Mesua ferrea L.	Guttiferae	Nagkesar	Seed	aromatic, astringent, coolant
Saussurea lappa Costus.	Compositae	Kuth	Root	asthma, bronchitis, flatulence, leprosy
Trapa natans L.	Trapaceae	Singara	Rind	Antipyretic, aphrodisiac, apetiser, astringent, coolant, diuretic, tonic, bronchitis, burns, diarrohea, dysentry, dyspepsia , fatigue , fever, haemmorrhage , inflammation, leprosy, pharyngitis
Vitex negundo L.	Verbenaceae	Nagod	Leaf	Antipyretic, astringent, carminative, digestive, emmenagogue, expectorant, febrifuge, stomachic, tonic, arthritis, chlorea, cough, dysentry, dysamenorrhoea, dyspepsia, fever, flatulence , headache, haemorrhoids, inflammation, leprosy, rheumatism, sciatica, ulcers, vermifuge, wounds

adverse effects, show recurrence, or lead to the development of resistance. There is general consensus that new antifungal agents without these disadvantages are strongly needed (Selitrennikoff, 2001).

MATERIAL AND METHODS

Plant material

Fresh plant/plant parts were collected randomly from the semi-arid region of Rajkot Gujarat, India. The details of plant/plant parts screened, their families, vernacular names and their therapeutic uses are given in Table 1. The taxonomic identities of these plants were confirmed by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot and the voucher specimen numbers of the plants were preserved. Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of plant material

The air-dried and powdered plant material (10 g of each) was extracted with 100 ml of methanol, kept on a rotary shaker for 24 h. Thereafter it was filtered and centrifuged at 5000 g for 15 m. The supernatant was collected and evaporated to dryness to give the crude dried extract. The extractive yield (%) of all the extracts is shown in Table 2b.

Fungal strains used

The test fungal strains investigated include 7 yeasts; *C. albicans* (1) ATCC2091, *C. albicans* (2) ATCC18804, *Candida glabrata* NCIM3448, *Candida tropicalis* ATCC4563, *Candida luteolus* ATCC32044, *Candida neoformans* ATCC34664, *Trichosporon beigelli* NCIM3404, and 4 moulds; *Aspergillus candidus* NCIM883, *Aspergillus flavus* NCIM538, *Aspergillus niger* ATCC6275 and *Mucor heimalis* NCIM873. All the fungal strains were obtained from National Chemical Laboratory (NCL), Pune, India.

Table 2a. Screening of some Indian medicinal plants for antifungal activity against some strains of yeast.

Plant species	Inhibition zone (mm)*																				
	Candida albicans			*Candida albicans*			*Candida glabrata*			*Candida tropicalis*			*Cryptococcus luteolus*			*Cryptococcus Neoformans*			*Trichosporon begelli*		
	500[a]	250	125	500	250	125	500	250	125	500	250	125	500	250	125	500	250	125	500	250	125
Caesalpinia pulcherrima (L.) Swartz.	-	-	10	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	11	10	11
Cyperus rotundus L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
Euphorbia tirucalli L.	-	-	9	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	9	10	-
Holarrhena antidysenterica (Heyne. ex Roth.) A. DC.	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	10	12	11
Mangifera indica L.	-	-	10	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	12	9	-
Mesua ferra L.	-	-	9	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	9	11	10
Saussurea lappa Costus.	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	9	10	12
Trapa natans L.	-	-	-	-	-	-	-	-	-	-	-	11	-	11	9	-	-	-	-	-	11
Vitex negundo L.	-	9	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	9
Fluconazole (10 mcg/disc)	-			17			22			-			20			-			28		
Amphotericin B (100 units/disc)	12			17			17						20			16			14		

[a]Concentration of plant extract (µg/disc).
Values are mean of three replicates.

The fungal strains were grown in Sabouraud broth and maintained on MGYP slants (yeast) and potato dextrose agar slants (mould) at 4°C.

Antifungal assay

To evaluate the antifungal activity, sterile agar plates were used according to the disc diffusion assay (Bauer et al., 1966). Activated cultures of fungal strains in Sabouraud's broth were adjusted to 1x 10^8 cfu/m as per Mcfarland standard. 100 µl of the inoculum was introduced to molten sabouraud dextrose agar and poured in the sterile Petri plates. Sterile filter paper discs (7.0 mm diameter) were impregnated with 500 µg/disc, 250 µg/disc and 125 µg/disc of the plant extracts dissolved in 100% DMSO (dimethylsulphoxide) and dried. The discs were placed on fungal-seeded plates and incubated at 28°C for 48 h. Discs impregnated with only 100% DMSO served as the negative control. As a positive control, fluconazole (10 mcg/disc) and amphoitericin B (100 units/disc) were used. Following an incubation period of 48 h, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth-inhibition. The whole experiment was performed three times to minimize error.

RESULTS AND DISCUSSION

Effect of three different concentrations (500, 250 and 125 µg/disc) of 9 plants belonging to different plants was tested against yeast and moulds. All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The antifungal activity of the screened plants against some strains of yeast is shown in Table 2a, and that against moulds are shown in Table 2b. The yeast species were more resistant when compared to moulds. Amongst *Candida* species, *C. glabrata* and *C. tropicalis* did not show any susceptibility except to the lowest concentration of *Holarrhena antidysentrica* and *Trapa natans* which showed little activity against *C. tropicalis*. The higher concentrations (500 and 250 ug/disc) of all the screened plants did not show any activity against *C. albicans* 1 and 2 while the lowest concentration i.e. 125 ug/disc showed some activity against these 2 strains. *C. luteolus* and *C. neoformans* also were resistant to the screened plant extracts except *Trapa natans* which showed slight activity. *T. begelli* was the most susceptible fungal strain. All the 3 concentrations of all the

Table 2b. Screening of some Indian medicinal plants for antifungal activity against some strains of moulds.

Plant species [Extractive yield (%)]	Inhibition Zone (mm)*											
	Aspergillus candidus			*Aspergillus flavus*			*Aspergillus niger*			*Mucor heimalis*		
	500	250	125	500	250	125	500	250	125	500	250	125
Caesalpinia pulcherrima (L.) Swartz. [10.27]	13	11	10	10	13	15	-	12	10	10	12	-
Cyperus rotundus L. [7.35]	13	12	-	-	-	12	-	14	11	-	-	12
Euphorbia tirucalli L. [8.10[	-	-	-	12	10	9	15	10	10	-	-	10
Holarrhena antidysenterica (Heyne. ex Roth.) A. DC. [15.60]	11	10	10	-	10	11	15	14	16	10	10	-
Mangifera indica L. [10.58]	12	12	-	-	-	15	12	15	13	12	-	-
Mesua ferra L. [7.29]	10	-	-	-	12	12	-	12	13	11	10	-
Saussurea lappa Costus. [11.56]	10	11	-	11	10	20	13	12	11	10	10	-
Trapa natans L. [2.49]	9	-	-	15	12	10	12	-	12	-	-	-
Vitex negundo L. [13.48]	10	11	-	-	10	11	10	10	15	10	-	-
Fluconazole (10 mcg/disc)	-			22			15			-		
Amphotericin B (100 units/disc)	20			18			19			16		

[a]Concentration of plant extract (µg /disk).
Values are mean of three replicates.

plants showed some activity against this fungus. No concentration effect was observed. The methanol extract of *Holarrhena antidysentrica* showed best antifungal activity against *T. begelli*. Poor activity was shown by *Cyperus rotundus* and *Trapa natans*. These two plants have been previously observed to possess good antibacterial activity (Parekh and Chanda, 2006a,b), but they turned out to be poor antifungal agents.

The methanolic extracts of all the screened plants showed good antifungal activity against the strains of moulds screened. The three *Aspergillus* spp. were more susceptible than *Mucor heimalis*. The lowest concentration of all the plants almost did not show any activity against *A. candidus* and *M. heimalis*, while the other two higher concentrations showed good antifungal activity. The lowest concentration of all the plants showed good antifungal activity against *A. flavus* while the highest concentration showed inhibitory effect against *A. flavus* and *A. niger*.

The effect of plant extracts was different with different fungal strains. The methanolic extract of *Caesalpinia pulcherrima* and *Cyperus rotundus* showed best antifungal activity against *A. candidus*. *Saussurea lappa* showed best antifungal activity against *A. flavus* followed by *Trapa natans* and *Mangifera indica*. The methanolic extract of *Holarrhena antidysentrica* showed best antifungal activity against *A. flavus* and *M. indica* was the best plant against *M. heimalis*. The overall results suggest that *A. flavus* is the most susceptible fungal strain and the most resistant was *C. glabrata*. The antifungal activity of the studied plant extracts was compared with standard antifungals; Fluconazole (10 mcg/disc) and Amphotericin B (100 units/disc). A similar study of screening natural plant extracts against different fungal pathogens was well recorded in literature (Ahmad et al., 2000; Rani and Murty, 2006).

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