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 ATCC26575 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).

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 Candidiasis 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

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

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 was 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 luteola* 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.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Candida albicans</th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida tropicalis</th>
<th>Cryptococcus luteolus</th>
<th>Cryptococcus neoformans</th>
<th>Trichosporon begelli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500*</td>
<td>250</td>
<td>125</td>
<td>500</td>
<td>250</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>Caesalpinia pulcherrima (L.) Swartz.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Cyperus rotundus L.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euphorbia tirucalli L.</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Holarrhena antidysenterica (Heyne. ex Roth.) A. DC.</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Mangifera indica L.</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Mesua ferra L.</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Saussurea lappa Costus.</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trapa natans L.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitex negundo L.</td>
<td>-</td>
<td>9</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole (10 mcg/disc)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphotericin B (100 units/disc)</td>
<td>12</td>
<td>17</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

*Concentration of plant extract (µg/disc).
Values are mean of three replicates.

Antifungal activity of the screened plants and amphotericin 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 families 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 antidysenterica and Trapa natans which showed little activity against C. tropicalis. The higher concentrations (500 and 250 µg/disc) of all the screened plants did not show any activity against C. albicans 1 and 2 while the lowest concentration i.e. 125 µg/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
plants showed some activity against this fungus. No concentration effect was observed. The methanol extract of *Holarrhena antidysenterica* 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 antidysenterica* 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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