In vitro response of essential oil extracted from Cassia tora against dermatophytes of infected soil

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Abstract: Fungal activity of the essential oil extracted from Cassia tora (Fabaceae) was studied against dermatophytic fungi like Microsporum gypseum. Study of dermatophytes in soil samples collected from Jaipur, was carried out but only 88% samples contained dermatophytic fungi. In screening of the essential oil of Cassia tora the diameter of inhibition zone was found to be 19 mm for Microsporum gypseum. Result show that a Minimum inhibitory concentration of essential oil of Cassia tora with reference to Microsporum gypseum was 0.64µl/ml. Since the plants appeared to have broad spectrum of activity against dermatophytes so it could be useful in antiseptic or disinfectant formulations.

Key words: Keratinophilic fungi, Essential oil, Disc diffusion method, Micro dilution method

Introduction

Skin diseases, especially ringworm, are an important problem in India (Destra, 1993). It is due to different species of keratinophilic fungi like Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis, Trichophyton simii, Colletotrichum spp., Geotrichum spp., Fusarium spp., Scopulariopsis spp. etc. Soil constitutes the main reservoir of fungi. Keratinophilic fungi are also reported from some common habitats viz. rice field soil, lake side soils, muddy soil and forest and farm yards. Two major techniques have been used for the qualitative and quantitative isolation of these fungi from soil surface- soil dilution plating and hair baiting techniques. In our research work we used hair baiting technique (Lewis et al., 1991). Ayurveda has a profound holistic approach towards skin care and only natural products are used for external application. There are a lot of plants (Essien and Akpan, 2004; Auna et al, 2005) which are used as antifungal agent for the treatment of ringworm infection. Cassia tora (Fabaceae) is a wild crop and grows in most parts of India as a weed. According to Ayurveda the leaves and seeds are acrid. (Ahmad et al., 1988) laxative, anti-periodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders (Chan and Peria, 2001). Cassia tora is a small annual herbs or under-shrub growing as common weed in Asian countries. It grows up to a height of about 30-90 cm.

The present study was carried out to investigate in vitro antifungal activity of essential oil of Cassia tora against dermatophytic fungi like Microsporum gypseum.

Material and Methods

Soil samples collection: Total 50 soil samples were collected from animal habitats of different areas of Jaipur. Sampling was carried out by using hair baiting method. Isolation of soil sample was carried out using hair- bait method. The basic principle of the technique is - use of natural keratin substrate as baits to recover these fungi from soil. Twenty five grams of soil was placed in a sterilized Petri plate. Bits of sterilized human hair (1-2 cm) were used in this method. The hair bits were scattered uniformly on wet soil. Sterilised distilled water was added to provide moisture to the soil each Petri plate was incubated at 35±2°C for 3 to 4 week in the culture room. Fungal growth if any was observed on the hair bits periodically when it was observed under a stereoscopic binocular microscope the growth was cultured on sabouraud’s dextrose agar. Culture was examined regularly during the maximum period of four weeks. Strains were identified by their morphological and physiological characteristics according to the procedure described by Forbes et al. (2002).

Extraction of Essential oil: Extraction of essential oil of Cassia tora was carried out by standard hydro distillation method using Clevenger’s apparatus. The hydrated product was dehydrated by passing through anhydrous sodium hydroxide and refrigerated at 4°C.

Screening of essential oil using disc diffusion method: Oil was screened for their antifungal activity by disc diffusion method (Rios et al., 1988) Fresh culture of fungi was used for inculums preparation. Using a sterile cotton swab, fungi culture was swabbed on the surface of sterile Sabouraud’s Dextrose Agar Plates. Filter paper discs of 6 mm diameter were prepared and sterilized. Sterilized filter paper discs were soaked in neat, undiluted concentration of Cassia tora oil. The oil saturated disc was placed on agar plate containing test organism. Similarly standard antibiotic discs of Ketoconazole and Clotrimazole were also placed over the Sabouraud’s Dextrose Agar plates as standard drugs for comparison of antifungal activity of oil. The plates were incubated at 35°C + 2°C for 24 hours. The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. The activity of oils was measured as activity index

Activity Index = Inhibition zone of sample / Inhibition zone of standard

Minimum inhibitory concentration of essential oil: Minimum inhibitory concentration is the lowest concentration at which there was no visible growth of the organism. The Micro dilution method- Provine and Hadley (2000) was followed to determine Minimum inhibitory concentration using semisolid agar media (Brain Heart Infusion Agar), aliquots of semisolid agar media (Bacto Agar; Difco Laboratories) at a pH of approximately 7.4 were poured into a 16- by 125-mm glass tubes and were autoclaved. A suspension was prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing and homogeneous suspension was used for inoculation. Different concentrations of Cassia tora oil was added in media containing test-tubes, afterwards a standard platinum loopful (~0.001 ml, Himedia, Flexiloop) of the inoculums suspension was inserted deep into each tube of medium containing a different concentration of oil, as well as a oil-free control, by a centered down-up motion to form a two dimensional inoculums. The tubes were then incubated at 30°C for 48-72 hours to determine the MIC.

Results

A total of 50 samples were collected from different parts of Jaipur district. Only 44 out of 50 (88%) samples contained dermatophytic fungi. By using disc diffusion method, the diameter of inhibition zone is found to be 19 mm for Microsporum gypseum (Table 1). Result show
In vitro response of essential oil against dermatophytes

<table>
<thead>
<tr>
<th>Test strain</th>
<th>IZ of sample</th>
<th>IZ of standard, Ketoconazole</th>
<th>Al</th>
<th>IZ of standard, Clotrimazole</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsporum gypseum</td>
<td>19</td>
<td>35</td>
<td>19/35=0.54</td>
<td>40</td>
<td>19/40=0.475</td>
</tr>
</tbody>
</table>

Table 1: Antifungal activity of Cassia tora oil (neat) against fungi

Table 2: MIC of Cassia tora oil against Microsporum gypseum

<table>
<thead>
<tr>
<th>Different Concentrations of Cassia tora oil used in µl/ml</th>
<th>Growth visually inspected in different Contrations of oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>0.3</td>
<td>3</td>
</tr>
<tr>
<td>0.4</td>
<td>2</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Control without oil</td>
<td>100% growth</td>
</tr>
</tbody>
</table>

that a Minimum inhibitory concentration of essential oil of Cassia tora with reference to Microsporum gypseum is 0.6µl/ml (Table 2).

Discussion

Cassia tora Linn is a medicinal plant traditionally used as laxative, for the treatment of leprosy and various skin disorders. The phytochemical analysis of leaf showed the presence of polyphenols which confirms its antioxidant and antiproliferative activity (Abraham et al., 2009). Chemical component of Cassia tora are anthraquinones, chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides. Naphthophrone, nucrofuscun, nucrofusarin, rubrofusarin, entiobiocide. Toralactone, torachrysone. Roots contains 1,3,5-trihydroxy-6-7-dimethoxy-2-methylanth- roquinone and beta-sitosterol. While Seeds contains Naptho-alpha-pyrene-toralactune, chrysophanol, phycin, emodin, rubrofusarin, chryso.picric acid-9,anthrone, Emodin, tricoten-1-ol, stigmasterol, Beta-sitosterol-beta-D-glucoside, frieldin, palmitic, stearic, succinic and d-tartaric acids uridine, quer cinin and isoquer cetrin are isolated from leaves (Desta, 1993).

Antibacterial, anti platelet aggregation, hepatoprotective, cAMP phosphodiesterase inhibitory activity antifungal, antifeast, anti-inflammatory and antiestrogenic, Hypolipidemic, anti-mutagenic and antioxidant activities of Cassia tora has been evaluated (Karaman et al., 2003).

Ethanol and Aqueous extracts from the leaves of Cassia tora was investigated for their antibacterial activity. Their concentrations 0.15mg, 0.31mg ethanolic and aqueous extracts were studied in activity, which involved the determination of inhibition zone in mm. Both the extracts exhibited significant antibacterial activity. Ciprofloxacin used as standard reference (Sharma et al., 2010). The chloroform, ethanol and aqueous extract of leaf of Cassia tora Linn., showed antibacterial activity (0-5000mg ml⁻¹) against 38, 58 and 29 bacterial strains respectively out of 120 various bacterial strains and methanol extracts showed antifungal activity (0-64mg ml⁻¹) against 3 strains out of 4 strains. Five strains of Shigella dysenteriae, four strains of Staphylococcus aureus, and three strains of Escherichia coli, have shown sensitivity against in vitro treatment of the methanol extracts up to 2000 mg ml⁻¹ concentration. The minimum inhibitory concentration (MIC) values ranges from 2–64 mg ml⁻¹ for dermatophytes. Minimal Bactericidal Concentration (MBC) value lies in the range of 2000-2500 mg ml⁻¹ against Escherichia coli ATCC25938 and Shigella dysenteriae 1. Phytochemical study indicates that the leaf extract contains flavonoids, saponins, resins, phytosterol, alkaloids and carbohydrates. The traditional claim of leaves of Cassia tora as an antimicrobial property have been confirmed as the extracts displayed activity against some bacteria and fungi which cause skin infection (Das et al., 2010). Methanolic extract of Cassia tora L. had antifungal properties on Candida albicans, Candida lichinum, and Cryptococcus neoformans. (Rahman, 1996).

Ethanol extracts of Cassia tora seeds shows positive results for Candida albicans. Clear inhibition zones at 25 mg ml⁻¹ and 30 mg ml⁻¹ of Cassia tora seeds extracts was observed. The inhibition zone at 25 mg ml⁻¹ concentration of Cassia tora seeds extract is 8.8 mm and at 30 mg ml⁻¹ is 11.1 mm in diameter (Omar et al., 2002). In the present study with the essential oil of Cassia tora, by using disc diffusion method, the diameter of inhibition zone was found to be 19 mm for Microsporum gypseum. In our findings, by using micro dilution method, the Minimum inhibitory concentration (MIC) of the essential oil was studied. Results show that Minimum inhibitory concentrations of essential oil of Cassia tora with reference to Microsporum gypseum are 0.6µl/ml (Table 2).

The results demonstrate that antifungal activity of Cassia tora against dermatophytes correlates well with the claims of traditional uses for skin infections. Since the plants appeared to have broad spectrum of activity against dermatophytes. They could be useful in antibiotic or disinfectant formulations. However further studies are needed including in vivo investigations and toxicity evaluation.

References