ANTIFUNGAL ACTIVITY OF BURSERA PENCILLATA LEAF EXTRACT ON DERMATOPHYTES

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ABSTRACT
To evaluate the in vitro antifungal activity of *Bursera pencillata* leaf extracts and fractions on the clinical isolates of dermatophytic fungi like Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, Microsporum gypseum and Epidermophyton floccosum. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts and fractions of the leaves of *Bursera pencillata* were measured using method of National Committee for Clinical Laboratory Standards (NCCLS). *Bursera pencillata* leaf extracts and fractions were found to have fungicidal activity against various clinical isolates of dermatophytic fungi. The MIC and MFC was found to be high in water and ethyl alcohol extracts and methanol fractions (200 g/mL) against dermatophytic fungi studied.

*Bursera pencillata* leaf extracts significantly inhibits the growth of all dermatophytic fungi studied. If this activity is confirmed by in vivo studies and if the compound is isolated and identified, it could be a remedy for dermatophytosis

KEYWORDS: *Bursera penicillata*, Antimicrobial activity, Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC).

1. INTRODUCTION
Mycotic infections are the most common cause of skin infection in tropical developing countries. The incidence of dermatophytosis raised dramatically in the past one decade. Humid weather, over population and poor hygiene are the ideal conditions for the growth of dermatophytes. These dermatophytes invade skin, hair and nail and cause dermatophytosis. Though these dermatophytes respond to treatment with conventional antifungal agents, the
disease had a tendency to reoccur in the same area or other ones. Medicinal plants represent a rich source of antimicrobial agents. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. Treatment based on Indian medicinal plants is becoming increasingly popular among patients with dermatophytes and physicians are also looking for alternative treatments because the present–day cures have side effects.

*Bursera* is an aromatic essential oil plant introduced into India by two private enterprising Scotsman - P. J. Anderson and G. N. Hamphries in 1912 at Thatgunni estate near Bangalore, Karnataka State. Forest department has started it's cultivation since 1958. It grows well on lateristic red soils and prefers arid tropical climate with temperature variation between 18°C and 35°C and rainfall between 450mm and 650mm annually. Being a deciduous species tree remains leafless from November to March and new flush starts during April-May with simultaneous flowering. Trees start bearing 3 to 4 years after planting. The oil is distilled by usual steam distillation of air dried husks which yield 10 to 14% of oil. About 25kg of oil can be expected from one hectare plantation. Rosita Arvigo (*Bursera simaruba*) reports that the bark is a common topical remedy in Belize for skin affections like skin sores, measles, sunburn, insect bites and rashes. A bark decoction is also taken internally for urinary tract infections, pain, colds, flu, sun stroke, fevers and to purify the blood.

2. MATERIALS AND METHODS

The plant material used in this study was collected from Nallamalla forest, Telangan District, India. It was identified and authenticated by the Botanist of Department of Botany, Osmania University. Fresh leaves were collected and shade dried. The dried leaves were ground to powder and stored in an airtight container until further use. Known quantity of *Bursera pentillata* leaf powder was subjected to cold extraction with water and 100% ethyl alcohol separately and the aqueous extracts were collected. The extracts were dried in a vacuum desiccator and were stored in a sterile container for further use. Known quantity of *Bursera pentillata* coarse powder was also successively extracted with various organic solvents like hexane, benzene, chloroform, ethyl acetate, methanol and water. Different fractions collected were filtered and evaporated to dryness in a vacuum concentrator. Coding was given to various extracts and fractions and was stored till use. The dried extracts and fractions were weighed and dissolved in 5% dimethyl sulfoxide (DMSO) and were used for further analysis.
Trichophyton mentagrophytes (T. mentagrophytes), Trichophyton rubrum (T. rubrum), Microsporum canis (M. canis), Microsporum gypseum (M. gypseum) and Epidermophyton floccosum (E. floccosum) were the five different clinical isolates of dermatophytic fungi taken for this study.

The selected isolates were grown on sabouraud dextrose agar (SDA). Twenty–one–day old culture of dermatophytic fungi was scraped with a sterile scalpel and macerated with sterile distilled water. The suspension was adjusted spectrophotometrically to an absorbance of 0.600 at 450 nm. By this way the fungal inoculum was prepared. For further study known quantity of this inoculum was used. Susceptibility testing was performed by the reference broth micro dilution method. MIC & MFC were determined after 21 days incubation at 35⁰C. To know the phytoconstituents of *Bursera pencillata*, the extracts were subjected to the analysis of macromolecules and secondary metabolites by using thin layer chromatography and high performance thin layer chromatography.

### 3. RESULTS AND DISCUSSION

The results revealed that the extracts and fractions of leaves inhibited the growth of clinical isolates of dermatophytic fungi. All six fractions showed MIC and MFC at 400μg/mL concentration against all the organisms tested. Methanol fraction, ethanol extract and water extract showed the MIC and MFC at 200μg/mL against T. mentagrophytes, M. canis and E. floccosum (Figure 1–5). Steroids and alkaloids were totally absent in *Bursera pencillata*. Trace amounts of triterpenoids, phenolic compounds, tannins and flavonoids were seen in the extracts and fractions. They showed in vitro antimicrobial activity against Propionibacterium acnes, Staphylococcus aureus, Escherichia coli, Candida albicans, T. mentagrophytes and T. rubrum. Angela Malheivos et al, reported that Drimys brasiliensis could be of use for developing new antifungal agents for treating dermatomycosis produced by E. floccosum.
4. CONCLUSION

Occurrence of fungal diseases is a serious problem of the present world. This is because of the development of antifungal drug resistance of the pathogens and side effects exhibited by the drugs used for fungal diseases. Hence there is a great demand for safer, alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of skin diseases including mycotic infection is an age old practice in many parts of the world. Plants contain a spectrum of secondary metabolites such as triterpenoids, phenols, flavanoids, quinines, tannins and their glycosides, alkaloids and their essential oils. The importance of these substances as antimicrobial agents against pathogens has been emphasized by several workers.

A wide spectrum of antifungal activity of ethanol and water extracts and methanol and chloroform fractions of Bursera penicillata leaves against 5 different dermatophytic fungi was observed in this study. In an earlier study Rana et al verified that the essential oil of this plant inhibited the growth of dermatophytes and Fusarium species at a concentration of 500 µg/mL. Souza et al[48] showed that a crude extract of Hyptis ovalifolia had activity against the same organisms at a concentration that ranges from 500 µg/mL to 1000 µg/mL. The ethanol extract of Azadiracta indica leaves showed MIC and MFC at 250µg/mL concentration against T. rubrum and Microsporum nanum. Crude methanol extract of the plant Piper solmsianum exhibited antifungal activity against M. canis, M. gypseum, T. mentagrophytes, E. floccosum and T. rubrum. Bagy et al demonstrated an in vitro antifungal activity of some natural compounds like onion oil, aloe sap and garlic extracts. All of these showed less antifungal activity against T. rubrum. Cassia alata leaf extract showed antimicrobial activity against dermatophytic fungi T. mentagrophytes, T. rubrum, M. canis and M. gypseum. It also
showed susceptibility towards the dermatophytic fungi than the non dermatophytic fungi like Fusarium solani, Aspergillus niger, Cladosporium wenneckii and Penicillium sp. Rai and Acharya reported that the essential oil of both the species of the genus, Tagetes erecta and Tagetes patula can be utilized topically on dermatophytic infections. It showed maximum inhibition against T. mentagrophytes and Fusarium oxysporum. Monica Bedi et al, reported that Allium sativum (garlic) and Scontains ajoene, showed antifungal activity.

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