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Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes

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ABSTRACT

Objective: To evaluate the *in vitro* antifungal activity of *Aegle marmelos* leaf extracts and fractions on the clinical isolates of dermatophytic fungi like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum*. **Methods:** The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts and fractions of the leaves of *Aegle marmelos* were measured using method of National Committee for Clinical Laboratory Standards (NCCLS). **Results:** *Aegle marmelos* 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 μ g/mL) against dermatophytic fungi studied. **Conclusions:** *Aegle marmelos* 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.

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[1]. 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[2].

Medicinal plants represent a rich source of antimicrobial agents[3–36]. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine[37]. 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[38].

Aegle marmelos (L.) Correa (*A. marmelos*), a tree species belonging to the family Rutaceae, is commonly called Vilvam (in Tamil), and often cultivated in temples for its leaves are used for pujas. The leaves, stem, bark and fruits of this plant have long been used in traditional medicine for its medicinal value. The leaves are widely used to treat diarrhoea, dysentery, skin and eye diseases[39–43]. They contain terpenoids which act as an antifungal agent[44]. After these facts were known, the present work was done to investigate the antifungal activity of the leaves of *A. marmelos* against the clinical isolates of dermatophytic fungi from patients attending the Department of Dermatology of Bharath Heavy Electrical Limited (BHEL) Hospital, Tiruchirappalli, India and Annal Gandhi Memorial Government Hospital, Tiruchirappalli, India.

2. Materials and methods

The plant material used in this study was collected from Tiruchirappalli, Tamil Nadu, India. It was identified and authenticated by the Botanist of Department of Plant Sciences, Bharathidasan University, Tiruchirappalli. Fresh leaves were collected and shade dried. The dried leaves were ground to powder and stored in an airtight container

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until further use. Known quantity of *A. marmelos* 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[45].

Known quantity of *A. marmelos* 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*), *Microsporium canis* (*M. canis*), *Microsporium 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[46]. MIC & MFC were determined after 21 days incubation at 35 °C. To know the phytoconstituents of *A. marmelos*, 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

The results revealed that the extracts and fractions of *A. marmelos* leaves inhibited the growth of clinical isolates of dermatophytic fungi. All six fractions showed MIC and MFC at 400 μ g/mL concentration against all the organisms tested. Methanol fraction, ethanol extract and water extract showed the MIC and MFC at 200 μ g/mL against *T. mentagrophytes*, *M. canis* and *E. floccosum* (Figure 1–5). Steroids and alkaloids were totally absent in *A. marmelos*. Trace amounts of triterpenoids, phenolic compounds, tannins and flavonoids were seen in the extracts and fractions.

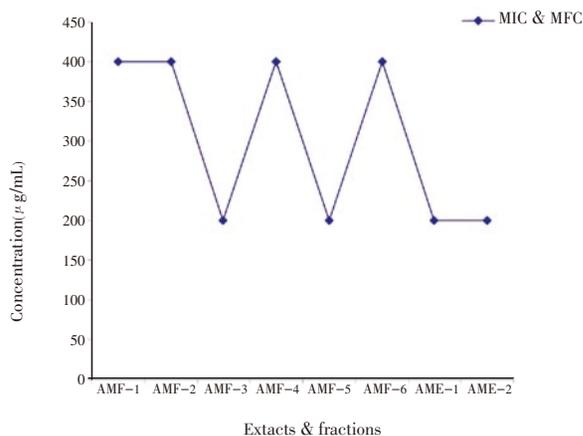


Figure 1. Effect of *A. marmelos* leaf extracts and fractions on *T.*

rubrum.

AMF-1–Hexane fraction; AMF-2–Benzene fraction; AMF-3–Chloroform fraction; AMF-4–Ethyl acetate fraction; AMF-5–Methanol fraction; AMF-6–Water fraction; AME-1–Crude ethyl alcohol extract; AME-2–Crude water extract.

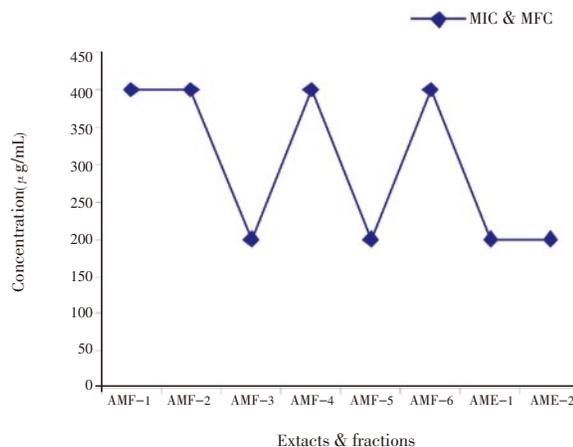


Figure 2. Effect of *A. marmelos* leaf extracts & fractions on *T. mentagrophytes*.

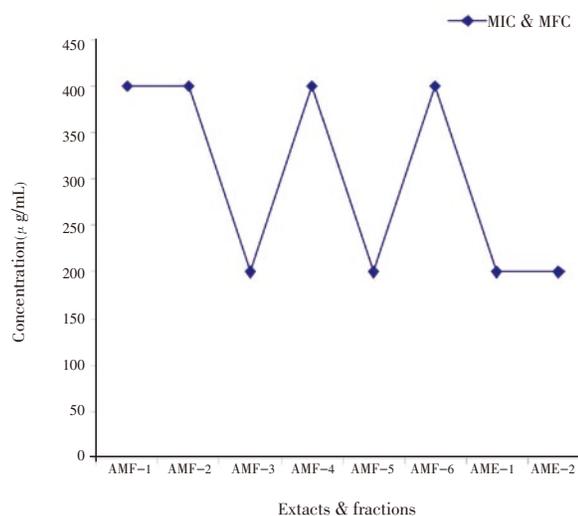


Figure 3. Effect of *A. marmelos* leaf extracts & fractions on *M. canis*.

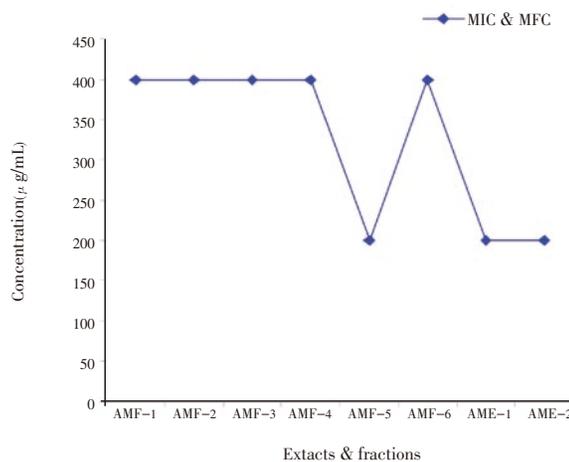


Figure 4. Effect of *A. marmelos* leaf extracts and fractions on *M. gypseum*.

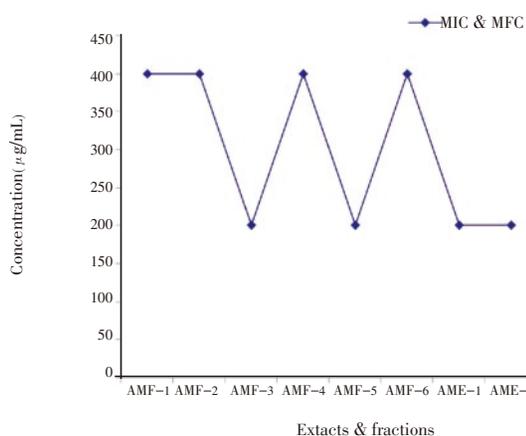


Figure 5. Effect of *A. marmelos* leaf extracts and fractions on *E. floccosum*.

4. Discussion

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[47]. 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[1].

A wide spectrum of antifungal activity of ethanol and water extracts and methanol and chloroform fractions of *A. marmelos* leaves against 5 different dermatophytic fungi was observed in this study. In an earlier study Rana *et al*[44] 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 1 000 µg/mL.

The ethanol extract of *Azadiracta indica* leaves showed MIC and MFC at 250 µg/mL concentration against *T. rubrum* and *Microsporum nanum*[2]. Crude methanol extract of the plant *Piper solmsianum* exhibited antifungal activity against *M. canis*, *M. gypseum*, *T. mentagrophytes*, *E. floccosum* and *T. rubrum*[49].

Bagy *et al*[50] 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 werneckii* and *Penicillium sp.*[51].

Rai and Acharya[52] 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*[53], reported that *Allium sativum* (garlic) and *Scontains ajoene*, showed antifungal activity. They have also reported that tea tree

oil has been widely used topically for the treatment of bacterial and fungal infections. They showed *in vitro* antimicrobial activity against *Propionibacterium acnes*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *T. mentagrophytes* and *T. rubrum*. Angela Malheiros *et al*[54], reported that *Drimys brasiliensis* could be of use for developing new antifungal agents for treating dermatomycosis produced by *E. floccosum*.

From the present study it was concluded that the crude extracts made of water and ethanol and methanol fractions of the plant *A. marmelos* has potent antifungal activity against the clinical isolates of dermatophytes. Leaves of *A. marmelos* yield an essential oil, 4 alkaloids besides aegelenine and aegeline; also condensed tannins, phlobotannins, flavan-3-oil, leucoanthocyanins anthocyanins, flavonoid glycosides, skimmianine, β-sitosterol, rutin and marmesinin. These compounds stated either alone or in combination may be the reason for its antifungal activity. Thin layer and high performance thin layer chromatography also confirmed the presence of these compounds. It is evident that the antidermatophytic activity of these plants may be due to secondary metabolites present in it. Further work on this study may help to design a new drug against dermatophytosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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