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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 inhibites 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].

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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

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*), *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[46]. MIC & MFC were determined after 21 days incubation at 35 ℃. 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 μg/mL concentration against all the organisms tested. Methanol fraction, ethanol extract and water extract showed the MIC and MFC at 200 μg/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.

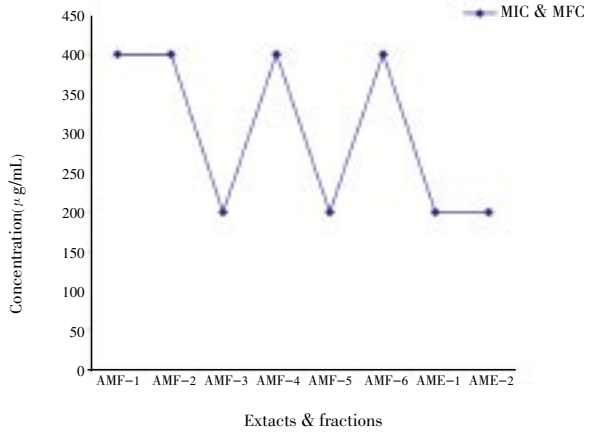

Figure 1. Effect of *A. marmelos* leaf extracts and fractions on *T. rubrum*.
AMF-1-Hexane fraction; AMF-2-Benzene fraction; AMF-3-Chloroform fraction; AMF-4-Ethyl acetate fraction; AMF-5-Methanol fraction; AMF-6-Water fraction; AME-1-Crude ethyl alcohol extract; AME-2-Crude water extract.

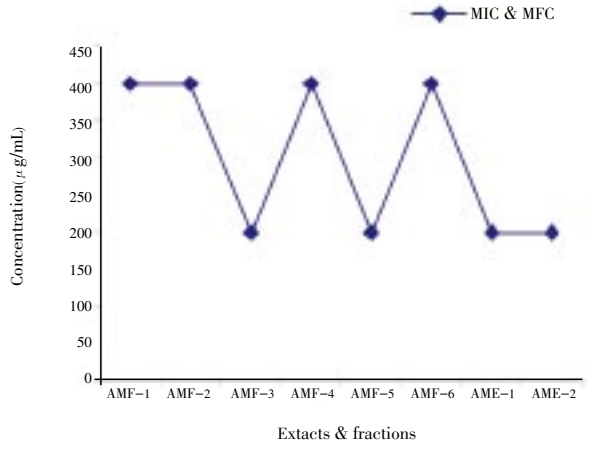

Figure 2. Effect of *A. marmelos* leaf extracts & fractions on *T. mentagrophytes*.

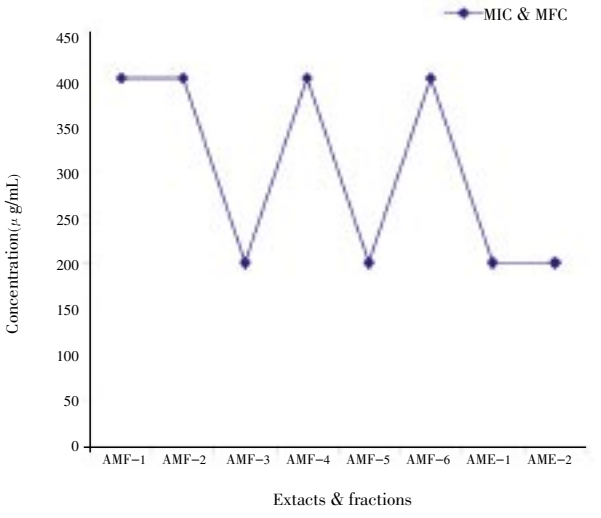

Figure 3. Effect of *A. marmelos* leaf extracts & fractions on *M. canis*.

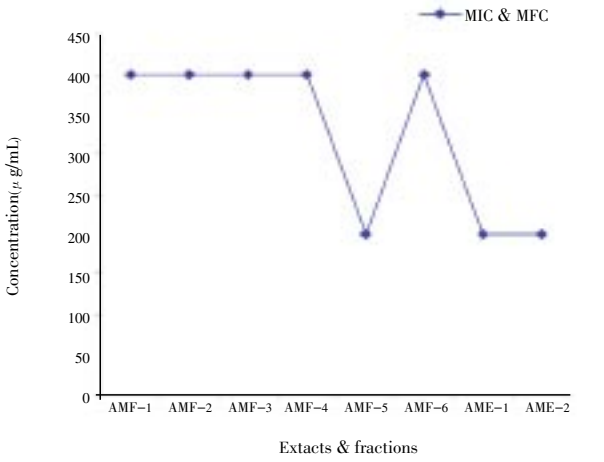

Figure 4. Effect of *A. marmelos* leaf extracts and fractions on *M. gypseum*.