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Original Research Article

Antifungal Activity of Ethanolic Leaf Extract of *Limonia acidissima* against Dermatophytes

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Abstract: Antifungal activity of ethanolic leaf extract of *Limonia acidissima* was studied against three common dermatophytic fungai like *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum* by disc diffusion method and zone of inhibition was determined. Ketoconazle was used as reference control. The ethanolic leaf extract of *Limonia acidissima* exhibited antifungal activity against all the three dermatophytic fungai and the effects were comparable with the reference control Ketoconazole.
Keywords: *Limonia acidissima*, Antifungal activity, Dermatophytes, *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum*

INTRODUCTION

Fungal dermatological conditions are caused by a group of fungi called dermatophytes. They cause infections in almost all parts of the body. The most common cause of skin infections are dermatophytes and opportunistic fungi. Dermatophytoes are not life threatening but they affect the quality of life of the patients as they can cause depression, lack of self-confidence and isolation in case of deep lesions. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 -1600 B.C. and is supposed to be the oldest repository of human knowledge [1]. Plants produce a diverse range of bioactive molecules, making them rich source for the therapeutic efficacy. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found to have medicinal properties. *Limonia acidissima* L. Swingle Syn. *Feronia elephantum* Correa, *Schinus Limonia* L. (Rutaceae), is a tropical plant species, indigenous to India and locally known as elephant apple. All the parts of *Limonia* are prescribed in indigenous system of medicine for the treatment of various ailments. Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs, cures cough, hiccup and good for asthma, consumption, tumours, opthalmia and leucorrhoea [2]. Unripe fruit is astringent while seeds are used in heart diseases. The fruits are used as a substitute for bael in diarrhea and dysentery [3]. The bark and leaves are used for vitiated conditions of vata and pitta [4]. Leaves are astringent and carminative, good for vomiting, indigestions, hiccup and dysentery. The leaves have hepatoprotective activity [5]. The gum is demulcent and constipating, and is useful in diarrhoea, dysentery, gastropathy, haemorrhoids and diabetes [6]. *Limonia acidissima* plant parts showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, fats steroids, saponins, glycosides, gum, mucilage and fixed oils [7]. The present study was conducted to evaluate the antifunagal activity of ethanolic leaf extract of *Limonia acidissima* against some common dermatophytic fungai.

MATERIALS AND METHODS

Plant Material

The leaves of *Limonia acidissima*, Linn. were collected from Ammapattai, Bhavani Taluk, Tamilnadu the outskirts of Bhavani, Erode District, Tamilnadu in the month of July 2014. The Plant was identified and authentified as *Limonia acidissima*, Linn. by Botanist Dr. Saravana Babu, Department of Botany, Chikkaiah Naicker College, Erode. The voucher specimen was kept in the laboratory (Specimen No: CNC/ERD/01/30/15 for future reference.

Preparation of Extract

The leaves were washed with water and dried in sunlight for one hour and then it was dried under shade. By the help of grinder the dried leaves were powered to get coarse. Dried course powders of the leaves were extracted with ethanol (70%) and by using Soxhlet apparatus. The extracts were then concentrated, dried and stored in desiccators. Obtained dark green ethanolic extract were used for the pharmacological study.

Dermatophytic Fungi for Antifungal Study

Trichophyton mentagrophytes, Microsporum canis and *Epidermophyton floccosum* were the three different clinical isolates of dermatophytic fungai taken for the study. The pure culture of the pathogenic fungal strain of *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum* were obtained from Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry.

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. These adjusted suspensions approximately corresponded to 0.5 to 2.5 X 10^3 cells/ml and were used as inoculum for antifungal susceptibility testing [8].

Antifungal assay

Antifungal activity tests were performed by using the disk diffusion agar method of Bauer *et al*., 1966, [9]. Test plates were prepared with 20 ml of sterile SDA. The standardized fungal suspension was applied on the solidified culture medium by using sterile cotton swabs and allowed to dry for 5 min. A sterile paper disk (Whatman AA disk, 6 mm) was impregnated with 10 µl of a stock solution (50 mg/ml) from crude extract. The disks were aseptically transferred on the inoculated agar plates and incubated for 48 h to 7 days, depending of the tested fungi. Antifungal activity was determined by measuring clear zones of inhibition around the test crude extract discs. The clear zones indicated the fungicidal effect while fungi static effect referred to the unclear zone of inhibition. Ketoconazole (250 µg) disks were used as a standard reference or positive controls, and the solvent or empty disks were used as negative controls. All assays were performed in triplicate.

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's '*t*' test. P values < 0.05 were considered as significant.

RESULTS

The results of antifungal activity of ethanolic leaf extract of *Limonia acidissima* was studied against three common dermatophytes and the results were shown on table 1. The zone of inhibition of *Limonia acidissima* against *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum* were 32.42±1.43, 27.56±0.95 and 28.62±1.37 respectively. The zone of inhibition of reference control Ketoconazole against *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum* were 45.62±2.24, 39.86±1.43and 32.61±1.88 respectively. Both the *Limonia acidissima* and Ketoconazole showed significant (P<0.001) increase in zone of inhibition against the tested dermatophytes. The ethanolic leaf extract of *Limonia acidissima* showed similar antifungal effect as that of standard Ketoconazole.

Table-1: Antifungal Activity of ethanolic leaf extract of *Limonia acidissima.*

S.No	Organisms	Zone of Inhibition (mm)		
		Vehicle	Ethanolic Extract of *Limonia acidissima*	Ketoconazole
1	*Trichophyton mentagrophytes*	7.62±0.52	32.42±1.43***	45.62±2.24***
2	*Microsporum canis*	5.32±0.17	27.56±0.95***	39.86±1.43***
3	*Epidermophyton floccosum*	5.79±0.24	28.62±1.37***	32.61±1.88***

Values are in mean ± SEM (n=6),
*P<0.05, **P<0.01, ***P<0.001 Vs Vehicle Control

CONCLUSION

Limonia acidissima leaf extract was evaluated for its antifungal activity against three common dermatophytes. From the results, it was concluded that, ethanolic leaf extract of *Limonia acidissima* exhibited antifungal activity against *Trichophyton mentagrophytes, Microsporum canis* and *Epidermophyton floccosum.*

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