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ANTI DERMATOPHYTIC ACTIVITY OF *AZADIRACHTA INDICA* AND *ACALYPHA INDICA* LEAVES- AN *IN VITRO* STUDY

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ABSTRACT

This study was conducted to evaluate the effect of ethanolic, ethyl acetate and hexane extracts of Indian medicinal plants *Azadirachta indica* and *Acalypha indica* leaves against dermatophytes (60 isolates of *Trichophyton rubrum*, 22 isolates of *Trichophyton mentagrophytes,* 9 isolates of *Microsporum gypseum* and 9 isolates of *Trichophyton tonsurans)*. Of the three extracts used the ethanolic and ethyl acetate extracts were equally good in inhibiting all the isolates. The extracts of *Azadirachta indica* leaves showed antifungal activity with an MIC and MFC ranging from 125µg to 500µg. On the contrary, *Acalypa indica* leaf extracts showed less activity with a MIC ranging from 500µg to 1000µg against all the dermatophytes tested. The minimum activity was seen with hexane at a conc of 250µg/ml for *Azadirachta indica* and at a concentration of 500µg/ml for *Acalypha indica.*

KEY WORDS: **Antidermatophytic activity, *Azadirachta indica, Acalypha indica***

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INTRODUCTION

Azadirachta indica commonly called Neem is a evergreen popular tree found in India, Africa, America.[1] The leaves are known to have antiallergenic, antidermatic, antifeedent, antifungal, antiinflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, and spermicidal and other biological activities.[2] *Acalypha indica* (commonly called kuppaimeni) is a common annual herb found throughout the plains of India. The whole plant contains various saponins and alkaloids. It has been reported to be useful in treating pneumonia, asthma, rheumatism and has antibacterial and antifungal activity. [3] The dried leaves of *Acalpha indica* was made into a poultice to treat bed sores and wounds and to treat a variety of skin disorders. Several chemicals and biological investigations have been carried out on this plant. The leaves of both the plants have shown a varying degree of antidermatophytic effects against some of the human infectious fungal pathogens due to their different chemical compositions [4,5] Hence the present study was undertaken to evaluate the antidermatophytic activity of the solvent extracts of the leaves of *Azadirachta indica* and *Acalypha indica* against 100 isolates of dermatophytes by tube dilution technique.

MATERIALS AND METHODS

Preparation of plant extract [6]

The leaves of *Azadirachta indica* were dried at room temperature and then crushed into a coarse powder using a blender. Powdered leaves were suspended in petroleum ether and kept in refrigerator overnight to remove fatty substances. After incubation the supernatant fluid was discarded and the residue dried at room temperature. The residue was further divided into three parts and 25 grams each was suspended in 100 ml of ethanol, ethylacetate, and hexane respectively in a sterile conical flask and kept at 4 °C overnight. After incubation the supernatant was filtered through a whatmann filter paper no.1 and the filtrate was dried to evaporate the organic solvent at room temperature. The sedimented extract was weighed and dissolved in 5% dimethyl sulphoxide.

Fungal inoculum preparation [7]

One hundred clinical isolates of dermatophytes was used in the study which included 60 isolates of *Trichophyton rubrum* 22 isolates of *Trichophyton mentagrophytes,* 9 isolates of *Microsporum gypseum* and 9 isolates of *Trichophyton tonsurans*. The organisms were grown on Sabouraud's dextrose agar plates. The 21 day old culture was scrapped with a sterile scalpel and macerated in 10ml sterile distilled water. The ground fungal suspension was adjusted spectrophotometrically to an absorbance of 0.600 at 450nm.

Susceptibility test [7]

One ml of the plant extract was incorporated into one ml of Sabouraud's dextrose broth and was serially diluted so as to achieve concentrations ranging from 1000 µg/ml to 31.25 µg/ml respectively. 20 µl of fungal inoculum was added to each tube and incubated at room temperature for 21days. Suitable controls were included. Sabouraud's dextrose broth with 20 µl of inoculum served as positive control whereas, SD broth alone served as negative control. The whole setup in duplicate was incubated at room temperature for 21 days.

Minimum Inhibitory Concentration (MIC) determination[7]

MIC was determined by incorporating various concentrations of the extracts 1000 µg /ml to 31.25 µg /ml in SD broth. 20 µl of standard fungal inoculum was added to each tube and inoculated at room temperature for 21 days. The MIC was regarded as the lowest concentration of the extract that did not permit any visible growth after 21 days of inoculation when compared with control.

Minimum Fungicidal Concentration (MFC) determination. [8]

The dilution of extract which showed no visible growth after 21 days of incubation was subcultured onto extract free SDA plates with an inoculum size of 1 ml. The MFC was regarded as lowest concentration that prevented the growth of any fungal colony in the solid medium.

RESULTS

Table 1

In vitro susceptibility testing of various organic extracts of Azadirachta indica leaves

Organism tested	No of strains	Ethanol extract µg/ml		Ethyl Acetate Extract µg/ml		Hexane Extract µg/ml	
		MIC_{100}	MFC_{100}	MIC_{100}	MFC_{100}	MIC_{100}	MFC_{100}
Trichophyton rubrum	60	125	125	125	125	500	500
Trichophyton mentagrophytes	22	125	125	125	125	500	500
Microsporum gypseum	9	125	125	125	125	500	500
Trichophyton tonsurans	9	125	125	125	125	500	500

Table 2

In vitro susceptibility testing of various organic extracts of Acalypha indica leaves

Organism tested	No of strains	Ethanol extract µg/ml		Ethyl Acetate Extract µg/ml		Hexane Extract µg/ml	
		MIC_{100}	MFC_{100}	MIC_{100}	MFC_{100}	MIC_{100}	MFC_{100}
Trichophyton rubrum	60	250	250	250	250	1000	1000
Trichophyton mentagrophytes rubrum	22	250	250	250	250	1000	1000
Microsporum gypseum	9	250	250	250	250	1000	1000
Trichophyton tonsurans	9	250	250	250	250	1000	1000

Results obtained during assay with organic extracts from *Azadirachta indica* leaves showed their inhibitory effect against all the clinical isolates. All concentrations of the solvent extracts effectively suppressed the growth of these fungi and this effect was found to increase with concentration 125µg/ml to 500µg/ml for *Azadirachta indica*. Table 1 The leaf extracts of *Acalypha indica* at different concentrations suppressed the growth of tested fungi. Of the three extracts used, the ethanolic and ethylacetate extract were the most effective against all the species tested with a MIC and MFC OF 250 µg/ml. Higher concentrations of hexane extracts 1000µg/ml was required to treat all tested species when compared to ethanol and ethylacetate extracts. Table 2 The extracts of *Azadirachta indica* leaves showed

antifungal activity against *T.rubrum, T.mentagrophytes, T.tonsurans, M.gypseum* with an MIC and MFC ranging from 125µg to 500µg. On the contrary, *Acalypa indica* leaf extracts showed less activity with a MIC ranging from 250µg to 1000µg against all the isolated dermatophytes. Ethanolic extract of *Azadirachta indica* showed maximum antifungal activity against all the isolated dermatophytes. This credit to ethanol extraction is because ethanol is an organic solvent and will dissolve organic compounds better than aqueous extract and also liberate the active components required for antifungal activity.

DISCUSSION

In the present investigation, leaves of two plants *Azadirachta indica* and *Acalypha indica* belonging to two different families were collected and bioactive compounds were extracted with organic solvents and their antifungal activities were detected against dermatophytes. Three organic solvents such as ethyl alcohol, ethyl acetate and hexane were used to extract the active constituents from the leaves. Of the three extracts used, ethanolic and ethylacetate extract were the most effective against all the species tested. This data is in close agreement with previous reports using neem. [9] This could be related to the presence of bioactive metabolites present in *Azadirachta indica* which are not soluble in hexane but are soluble in ethanol and ethylacetate. [10] Leaf extracts of neem were found to have a potent antidermatophytic activity against *T.rubrum, T. violaceum, M.nanum* and *E.floccosum* [11] The higher rate of inhibition of neem extract may be due to their more amount of bioactive compounds.[2] Researchers explained this activity by the active ingredients like triterpenes or the limnoids such as azadirachtin, sitosterol, nimbin, nimbidin, nimbosterol and margisine. [13] The less significant inhibition in the extract of *Acalypa indica* may be due to the absence of lignin and alkaloids in the extracts which act as an effective antimicrobial agent. [3]

CONCLUSION

The findings of the present study showed that ethanol and ethylacetate was the best solution for extracting the effective antifungal substances from the medicinal plants *Azadirachta indica* and *Acalypha indica* than hexane. This could be related to the presence of bioactive metabolites present in *Azadirachta indica* and *Acalypha indica* which are not soluble in hexane but are soluble in ethanol and ethylacetate. Further studies may be carried out to purify these components to develop effective measure against fungal infections.

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