

IN VITRO ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS OF AZADIRACHTA INDICAS. M. RADHIKA¹, A. MICHAEL²¹Department of Microbiology, Karpagam University Coimbatore 641021, ²Department of Microbiology, PSG college of arts and science Coimbatore 641014, Tamil Nadu, India. Email: micro.radhika@yahoo.co.in

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ABSTRACT

Objective: The study was to evaluate the antidermatophytic activity of the ethanol, ethyl acetate and hexane extract of Neem (*Azadirachta indica*) leaves against dermatophytes isolated from patients with dermatophytosis.

Methods: Clinical samples from 200 patients were subjected to (KOH) examination and culture isolation. Causative agents were identified macroscopically and microscopically. The minimum inhibitory concentration (MIC) by broth dilution method and minimum fungicidal concentration (MFC) for the solvent extract of the leaves of the plant *Azadirachta indica* against the isolated dermatophytes was determined.

Results: Dermatophytes were isolated in 126/200 specimens. Of the three solvents used, ethanolic and ethyl acetate extract was found to be more active inhibiting 100% of the isolates at a concentration of 125µg/ml.

Conclusion: In conclusion ethanolic and ethyl acetate leaf extracts of *Azadirachta indica* plant can be used to treat infections with dermatophytic fungi.

Keywords: Dermatophytosis, Antidermatophytic activity, *Azadirachta indica*

INTRODUCTION

Dermatophytic infections are most prevalent in countries like India where they constitute a major public health problem. The reason for the increase of these infections is in the public's socio-economic status as well as poor hygiene and sanitary conditions. Different treatments are recommended to control dermatophytes and the pharmacological treatment include use of antifungal agents of which the side effects are many [1]. Plant continue to be a major source of medicine [2]. These plants can be exploited to find out effective alternative to synthetic drugs [3].

Recently the use of some natural products has emerged to inhibit these pathogens. Antimicrobials of plant origin are efficient in the treatment of infectious diseases with no side effects that are often associated with synthetic ones. The aim of the study was to identify the etiological agents of dermatophytosis and to investigate the antidermatophytic activity of different solvent extracts of the Indian medicinal plant *Azadirachta indica* commonly called as Neem. Neem has attracted worldwide prominence for its medicinal properties. Neem leaf extract have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antiulcer, antimalarial, antifungal, antibacterial, antioxidant, anticarcinogenic properties [4]. Hence, an *in vitro* study of anti dermatophytic properties of the leaves of the plant *Azadirachta indica* on dermatophytes has been attempted.

MATERIALS AND METHOD

Skin scales, hair, and nail samples from clinically suspected cases were subjected to mycological work up.

Microscopic examination

Direct microscopic examination was done in 10% potassium hydroxide (KOH) wet mount for the specimens of skin scales while 40% KOH was employed for hair and nail specimens [5].

Culture study

The KOH positive cases were subjected to culture study. The culture was performed in two different sets of antibiotic incorporated Sabouraud dextrose agar (SDA) media, one with chloramphenicol 50 mg/L to minimize bacterial contamination and the other with cycloheximide 500 mg/L, in addition to chloramphenicol to reduce contamination with saprophytic fungi [5]. The culture tubes were incubated at 30°C for 21 days and the growth was observed.

The mycological identification was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and pigment production on the reverse. The microscopic examination of fungal growth was observed with lactophenol cotton blue stain. Nature of mycelium and conidia formation (macro and micro conidia) helped to differentiate various genera and species.

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 and twenty six clinical isolates of dermatophytes was used in the study which included 36 isolates of *Trichophyton mentagrophytes*, 72 isolates of *Trichophyton rubrum*, 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 21 days. 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: Isolation of dermatophytes in relation to site involved**

Dermatophytes	Skin	Scalp/scalp hair	Nail
Trichophyton rubrum	60	-	12
Trichophyton mentagrophytes	24	-	12
Microsporum gypseum	9	-	-
Trichophyton tonsurans	-	9	-

Table 2: In vitro susceptibility testing of various organic extracts of Azadirachta indica leaves

Organism tested	No of strains	Ethanol extract		Ethyl Acetate Extract		Hexane Extract	
		µg/ml	MFC ₁₀₀	µg/ml	MFC ₁₀₀	µg/ml	MFC ₁₀₀
<i>Trichophyton rubrum</i>	72	125	125	125	125	500	500
<i>Trichophyton mentagrophytes</i>	36	125	125	125	125	500	500
<i>Microsporum gypseum</i>	9	125	125	125	125	500	500
<i>Trichophyton tonsurans</i>	9	125	125	125	125	500	500

From 200 samples, 93 isolates were obtained from skin scales, 9 from scalp/scalp hair and 24 from nail samples. The dermatophyte isolated were *T. rubrum* from 72 samples, *T. mentagrophytes* from 36 samples and *T. tonsurans* from 9 samples (table 1).

Results obtained during assay with organic extracts from *Azadirachta indica* leaves showed their inhibitory effect at all used concentrations against the 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 (table 2). Of the three solvents used ethanol and ethyl acetate were equally good and inhibited the isolates at a concentration of 125 µg/ml. Hexane inhibited the isolates at a concentration of 500µg/ml.

Among 200 patients with dermatophytosis, isolates were obtained from 126 (63%) patients. The isolation rate in the present study seemed to be higher when compared to various other studies where it ranged from 45.3-52.2% [9]. The number of isolates from skin scales was higher when compared to scalp/hair and the isolates were least from the nail samples.

Trichophyton rubrum was the main etiological agent from the skin scales (60/93) in the study similar to other reports [10]. *Trichophyton mentagrophytes* was the second common isolates 24/93 as observed in other studies [11]. *Microsporum gypseum* was isolated from 9 samples and was the third common isolate from the skin. *Trichophyton tonsurans* was the only species from the scalp/scalphair (9/9). This was not isolated from skin scales and nail samples. The isolates from the nail samples were *T. rubrum* 12/24 and *T. mentagrophytes* 12/24 and these are the common agents infecting the nail [10].

DISCUSSION

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 [12]. 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.

The leaf extract of *Azadirachta indica* at different concentrations significantly suppressed the growth of the pathogenic fungi. The extracts of *Azadirachta indica* from bark and leaf, inhibited both spore germination and mycelial growth of *Epidermophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophyte* [13]. Leaf extracts of neem were found to have a potent antidermatophytic activity against *T. rubrum*, *T. violaceum*, *M. nanum* and *E. floccosum* [14]. The higher rate of inhibition of neem extract

may be due to the presence of active ingredients like triterpenes or the limnoids such as meliantriol, azadirachtin, desactylimpin, quercetin, sitosterol, nimbin, nimbinin, nimbidin, nimbosterol and margisine [13].

CONCLUSION

From the present study it has become clear that the leaf extracts of *Azadirachta indica* has great potential to strongly inhibit the members of the *Trichophyton* and *Microsporum* species causing superficial infection of the skin. Therefore the results are of a significant value and confirms the therapeutic potency of such plant used in traditional medicine. It forms a good basis for further phytochemical and pharmacological investigation.

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