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Original Article

EFFECT OF *AZADIRACHTA INDICA* (NEEM) ON THE GROWTH PATTERN OF DERMATOPHYTES

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Abstract

Purpose: To determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for the extracts of the leaves and seeds of the plant *Azadirachta indica* against various dermatophytes. **Methods:** Clinical isolates of dermatophytes(*Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum nanum*) were treated with extracts of leaves and seeds of the plant *Azadirachta indica* (neem) for antifungal activity by *in vitro* tube dilution technique. **Results:** The MIC of neem seed extracts was 31□g/mL for all the dermatophytes tested. The neem seed extract at 15□g/mL concentration (below MIC) was observed to be sufficient for distorting the growth pattern of the organisms tested. **Conclusions:** The changes in growth curve of the treated dermatophytes were found to be statistically significant with reference to the untreated fungi.

Key words : *Azadirachta indica, dermatophytes, growth pattern, MIC*

Dermatophytes are the major cause of superficial mycoses of man and remain a public health problem especially in tropical countries such as India.[1] The humid weather, over population and poor hygienic conditions are conducive to the growth of dermatophytes. Even though it responds to treatment with conventional antifungals, the disease has a tendency to recur at the same or at different sites.

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. *Azadirachta indica* (*Neem*) is a tree which has been used for a long time in agriculture and medicine. *Azadirachta indica* is an indigenous plant widely distributed in India. The medicinal properties of the plant Azadirchata indica were studied by several workers. The antipyretic effect,[2,3] antimalarial effect,[4,5] antitumour effect,[6] antiulcer effect,[7] antidiabetic effect,[8] antifertility effect,[9] effect on the central nervous system,[10,11] and cadiovascular effect[12] were some of the studies of the earlier workers. Antimicrobial properties of *Azadirchata indica* were studied by several authors. Rao *et al*[13] reported the antimicrobial activity of the seed oil against a variety of pathogens. The antifungal effects were reported of gedunin against polyporous wood rot,[14] of a leaf extract against *Alternaria alternata*[15] and of a mixture of sulphorous compounds from the steam distillate of fresh matured leaves against *Trichophyton mentagrophytes* in a concentration of 125□g/ml.[16] We planned the present study to find out the antidermatophyte activities of 'neem' leaves and seeds and their effect on the growth pattern on dermatophytes.

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Materials and methods

Plant extract preparation

The plant materials used in this study were collected from Annamalainagar, Tamilnadu. It was identified and authenticated by the Department of Botany, Annamalai University. Fresh leaves and ripened fruits were collected and dried in shade. The dried leaves were ground to powder and suspended in petroleum ether and kept in refrigerator overnight for removing all the fatty substances. After overnight incubation, the supernatant was discarded and the residue was dried at room temperature. The residue was further divided in to three parts and each part was suspended in ethanol, ethyl acetate and hexane respectively in sterile 250 mL conical flasks and kept at 4°C overnight. Each 100 gms of powdered leaf material were soaked in 250 mL of ethanol, ethyl acetate and Hexane.

After overnight incubation, the supernatant was filtered through Whatman No.1 filter paper and the filtrate was dried to evaporate the organic solvent at room temperature. The sedimented extract was weighed and dissolved in 5% dimethyl sulfoxide (DMSO).

In the case of seed, the seed coat was removed and 1 gm of seed was ground in 100 mL of 5% DMSO aseptically so as to achieve 1000 □g/mL concentration and the filtrate was used.

Fungal Inoculum preparation

Twelve strains of *Trichophyton rubrum* which were isolated from clinical cases of tinea cruris, tinea corporis and tinea unguium, 10 strains of *T.mentagrophytes* which were isolated from tinea pedis, tinea corporis and tinea capitis and one strain of *Microsporum nanum* isolated from tinea corporis were tested.

These organisms were grown on Sabouraud dextrose (SD) agar. The 21 day old culture was scraped with a sterile scalpel and macerated in 10mL sterile distilled water. The suspension was adjusted spectrophotometrically to an absorbance of 0.600 at 450nm. Each tube was inoculated with 20 □L of fungal suspension.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

MIC and MFC were determined according to the method described earlier.[17] MIC was determined by incorporating various concentrations of extracts (1000 □g/mL to 31 □g/ml) in SD broth. 20 □l of standard fungal inoculum was added to each tube and incubated at room temperature for 21 days. Suitable controls were also included. SD broth with 20□l of inoculum served as positive control. SD broth alone served as negative control. The tubes in duplicate for each agent were incubated at room temperature for 21 days.

The MIC was regarded as the lowest concentration of the extract that did not show any viable growth after 21 days of incubation (compared with control).

The MFC was determined using the method of Rotimi *et al*.[18] The tubes which showed no visible growth after 21 days incubation were subcultured on extract free SDA plates and incubated at room temperature for 21 days. The MFC was regarded as the lowest concentration of the extract that prevented the growth of any fungal colony on the solid medium.

Growth Pattern

The growth pattern of the organisms (*T. mentagrophytes, T. rubrum, M. nanum)* at dilutions of 15□g/mL (below MIC) of 'neem' seed extract in SD agar were compared with the control. One millilitre of the standard suspension of the organism was inoculated on test and control plates. Both the plates were incubated at room temperature and observed for growth on every third day upto 30 days. Three sets of replicate test was done. A growth curve was plotted using time interval on X axis and extent of growth on (cm) Y axis. The results of the test were compared with that of the control. The statistical significance of the growth pattern of the test with reference to control was done by using two-way analysis of variance.

Results

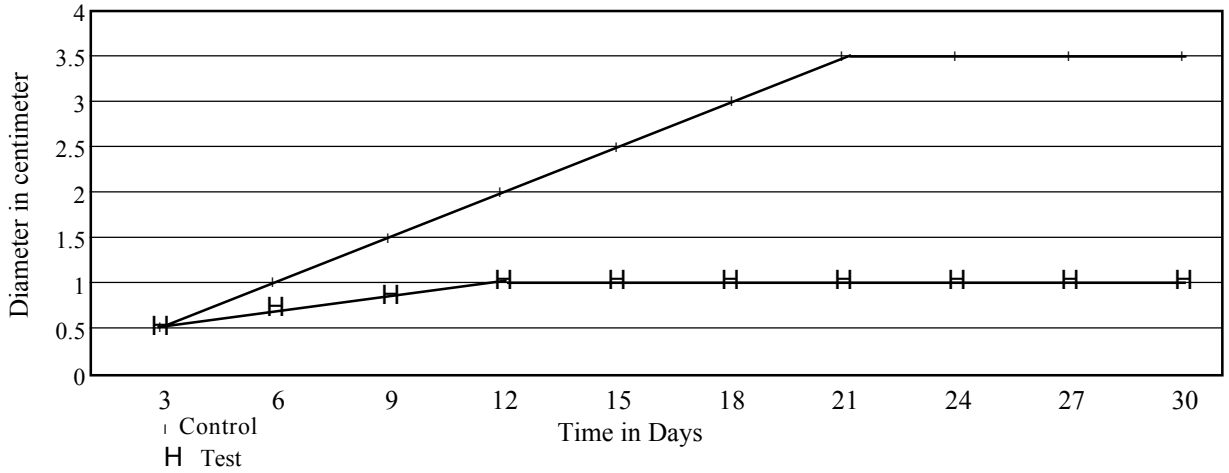
The ethanol extracts of 'neem' leaves showed MIC and MFC at 250 □g/mL concentration for all the strains of *T. rubrum* and *M. nanum* tested. MIC and MFC recorded for stains of *T. mentagrophytes* was 125 □g/mL. The ethyl acetate extract of 'neem' leaf showed MIC and MFC at 125 □g/mL for all the stains of *T. rubrum, T. mentagrophytes* and 250 □g/mL for *M. nanum*. Hexane extracts of 'neem' leaf showed MIC and MFC at 500 □g/mL for all the strains of *T. rubrum, T. mentagrophytes* and *M. nanum*. The 'neem' seed extract showed MIC and MFC at 31 □g/mL for all the dermatophytes tested (Table).

Table : *In vitro* susceptibility of various organic extracts of 'neem' leaf and seed

Organism tested	No. of isolates	Ethanol extract of 'neem' leaf		Ethyl acetate extract of 'neem' leaf		Extract of 'neem' leaf in hexane		'neem' seed extract	
		MIC100 □g/mL	MFC100	MIC100 □g/mL	MFC100	MIC100 □g/mL	MFC100	MIC100 □g/mL	MFC100
Trichophyton rubrum	12	250	250	125	125	500	500	31	31
Trichophyton mentagrophyte	10	125	125	125	125	500	500	31	31
Microsporum nanum	01	250	250	250	250	500	500	31	31

The growth pattern of *T. rubrum* treated with 15 □g/mL (below MIC) of 'neem' seed extract is shown in figure 1. The log phase was regular and continuous till the 18th day with 0.5cm growth in diameter on every third day in the control.

Figure 1 : Growth pattern of *T.rubrum* treated with 'neem' seed extract (15mg/mL)



The maximum growth in diameter-recorded on 18th day was 3.5 cms. After the 18th day the growth was observed to become stationary. In contrast, distorted growth pattern was recorded for *T. rubrum* in the test plate. The log phase continued till the 12th day with a growth of 1cm diameter on 12th day after which the growth became stationary. The growth patterns of *T. mentagrophytes* and *M. nanum* on 'neem' seed extract and control plates are shown in figure 2 and 3 respectively.

Figure 2 : Growth pattern of *T.mentagrophyte* with 'neem' seed extract (15mg/mL)

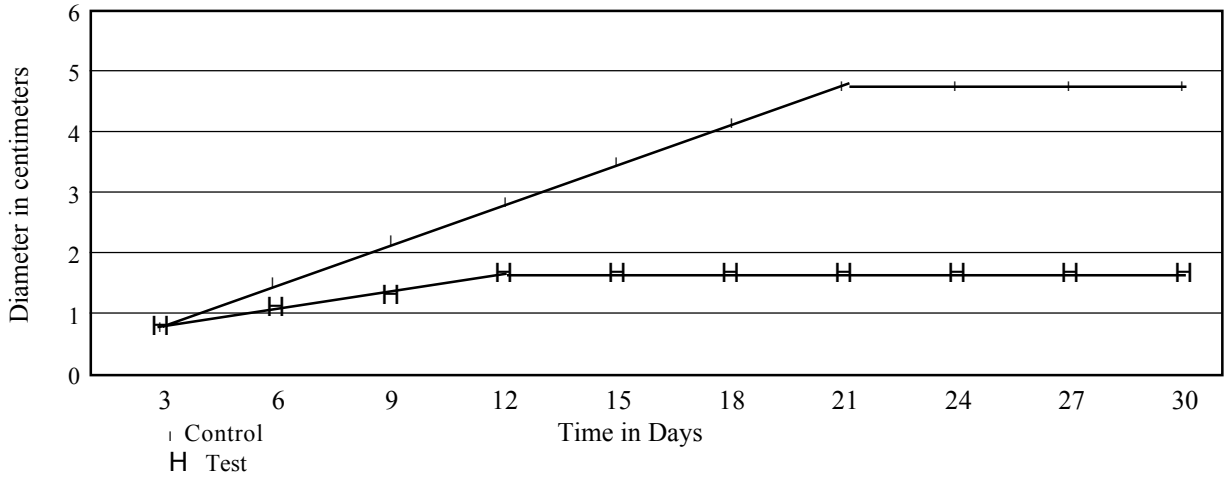
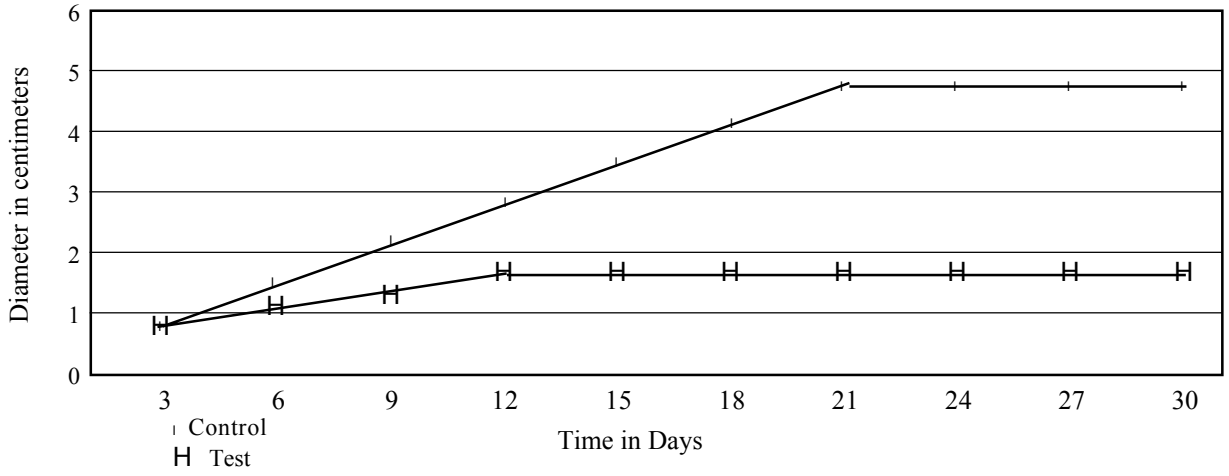


Figure 3 : Growth pattern of *M. nanum* with 'neem' seed extract (15mg/mL)



Statistical analysis showed that the growth pattern changes in the test were significantly different from the growth pattern in untreated culture.

Discussion

The MIC and MFC of the 'neem' seed extract were similar, which shows that MIC is sufficient for measuring fungicidal activity. Use of plant extracts in the treatment regimen of various diseases are gaining importance as antimicrobial, antibacterial,antiviral and antifungal activities of many plants are reported. Antimicrobial properties of plant extracts are now recognized by several workers.[19,20] The present study showed that 'neem' seed extract has high antidermatophytic properties. 'Neem' seed extract at a concentration of 15 g/mL (below MIC) was observed to distort the growth pattern of the organisms tested. This finding supports the use of 'neem' oil in the treatment of various skin infections by alternative systems of medicine.

References

1. Venugopal PV, Venugopal TV. Antimycotic susceptibility testing of dermatophytes. *Indian J Med Microbiol* 1993;**11(2)**:151-154.
2. Okpanyi SN, Ezeukwk GC. Anti-inflammatory and antipyretic activities of *Azadirachta indica*. *Planta Medica* 1981;**41**:34-49.
3. Khattak SG, Gilani SN, Ikram M. Antipyretic studies on some indigenous Pakistani Medicinal Plants. *J Ethno Pharmacol* 1985;**14**:45-51.
4. Tella A. The effects of *Azadirachta indica* in acute *Plasmodium berghei* malaria. *Nigerian Medical Journal* 1977;**7**:258-263.
5. Rochankij S, Thebraranonth.Y, Yenjal C, Yuthavong. Nimbolide a constituent of *Azadirachta indica* inhibits *Plasmodium falciparum* in culture. *South East Asian J Trop Medi Public Health* 1985;**16**:66-72.
6. Fujiwara T, Takeka TO, Gihara Y, Shimizu M, Nomura T, Tomuta Y. Studies on the structure of Polysaccharides from the bark of Medica *Azardichta*. *Chemical Pharmaceu Bull* 1982;**30**:4025-4030.
7. Pillai NR, Santhakumari G. Toxicity studies on nimbidin, a potential antiulcer drug. 1984 b;**50**:146-148.
8. Shukla R, Singh S, Bhandari CR. Preliminary clinical trial on antidiabetic actions of *Azadirachta indica*. *Medicine and Surgery* 1973;**13**:11-12.
9. Sinha KC, Riar SS, Tiwary RS, Dhawan AK, Bhadhan J, Thomas P, Kain AK, Jain RK. Neem oil as a vaginal contraceptive. *Indian J Med Research* 1984;**79**:131-136.
10. Pillai NR, Shanthakumari G. Effect of nimbidin as acute and chronic gastro duodenal ulcer models in experimental animals. *Planta Medica* 1984;**50**:143-146.
11. Singh PP, Junnarkar AY, Reddi GS, Singh KV. *Azadirachta indica* neuro-psychopharmacological and antimicrobial studies. *Fitoterapia* 1987;**58**:233-238.
12. Thompson EB, Anderson CC. Cardiovascular effects of *Azadirachta indica* extract. *J Pharmaceu Sci* 1978;**67**:1476-1478.
13. Rao DVK, Singh K, Chopra P, Chabra PC, Ramanujalu G. *In vitro* antibactericidal activity of neem oil. *Indian J Med Research* 1986;**84**:314-316.
14. Sundarasivarao B, Nazma, Madhusudhanarao J. Antifungal activity of gedunin. *Curr Sci* 1977;**46**:714-716.
15. Bhomick BN, Chooudhary BK. Antifungal activity of leaf extracts of medicine plants on *Alternaria alternata*. *Indian Botanical Reporter* 1982;**1**:164-165.
16. Pant N, Garg HS, Madhusudhanan KP, Bhakuni DS. Sulpharous compounds from *Azadirachta indica* leaves. *Fitoterapia* 1986;**57**:302-304 .
17. Irobi ON, Daramola SO. Antifungal activities of crude extracts of *Mitra carpus villosus* (Rubiaceae). *J Ethno Pharmacol* 1993;**40**:137-140.
18. Rotimi VO, Lanhon BE, Bartlet JS, Mosadomi HA. Activities of Nigerian chewing sticks extracts against *Bacteriodes gingivalis* and *Bacteriodes melaninogenicus*. *Antimicrob agents chemother* 1988;**32**:598-600.
19. Dixit SN, Srivastava HS, Tripathi RD. Lawsone, the antifungal antibiotic from the leaves of *Lawsonia inermis* and some aspects of its mode of action. *Indian phytopathol* 1980;**31**:131-133.
20. Natarajan MR, Lalithakumar D. Leaf extracts of *Lawsonia inermis* as antifungal agent. *Curr Sci* 1987; **56**:1021-1022.