IN VITRO ANTIFUNGAL ACTIVITY OF *Ixora brachiata* ROXB AGAINST DERMATOPHTES

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**Abstract:** Mycotic infections are probably the most common cause of skin disease in developing countries of tropical regions. Dermatophytosis is the most frequent superficial fungal infection in tropical and subtropical countries. The drugs used against dermatophytosis and exhibit several side effects and have limited efficacy. So that there is a distinct need for the discovery of new safer and more effective antifungal agents. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world. Because herbal remedies used in traditional folk medicine may help to overcome the growing problem of resistance to antifungal drugs and their relative toxicity. In this study, the *in vitro* antifungal activity of *Ixora brachiata* Roxb., leaves and root extract were evaluated against three different genera of dermatophytes viz. *Microsporum*, *Trichophyton* and *Epidermophyton* by Agar Dilution Method. *Ixora brachiata* belongs to family Rubiaceae. It is small tree 15-30 ft. high, found to be growing in high rainfall locality. The extracts inhibited the growth of the dermatophytes tested at different concentrations. The most biologically active was the ethanolic extract from the root which inhibited 14 isolates (100%) at a concentration of 125μgml⁻¹. The MFC values of these compounds were between 500-1000μgml⁻¹.

**Keywords:** Dermatophytes, *Ixora brachiata* Roxb., Antifungal activity, Herbal drugs.

**Introduction**

*Ixora brachiata* Roxb. belongs to family Rubiaceae. It is small tree 15-30 ft. high, found to be growing in high rainfall locality (Cooke, 1958) Figure 1. Human infections, particularly those involving the skin, is increasing at an alarming rate, especially in tropical and subtropical developing countries, with dermatophytes that the most common pathogens.

This increase is directly related to the growing population of immunocompromised individuals. Human mycoses are not always successfully treated, since the available, antifungal drugs are ineffective, produce many adverse effects, show recurrence, or lead to the development of resistance. It is therefore, essential to research for more effective and less toxic new antifungal agents (Zacchino et al. 1999). The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world (Irobi et al. 1993).

Review of literature revealed that no work has been carried out on the antifungal effects of *Ixora brachita*. Thus, the present study has been undertaken to evaluate the antifungal activity of ethanolic extract leaves and root against 14 strains of dermatophyte is describe.
Materials and Methods

(1) Plant material

Ixora brachiata was collected from Dapoli district Pune, Maharashtra state (India) in June 2004 and Oct. 2005. Efforts were made to collect this plant in flowering and fruiting for the correct Botanical identification. It was identified with the help flora of Presidency of Bombay (Cooke, 1958). The healthy and disease free leaves were separated and dried in shade so as to avoid decomposition of chemical constituents. These were powdered in grinder and stored in clean and dry airtight containers for further studies.

(2) Preparation of plant extract

Ixora brachiata leaves and root hydroalcoholic extracts prepared with 10 g (DW) of air dried powder to 100 ml organic solvent, ethanol 80% (drug/solvent ratio 1:10 w/v) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 3×24h by maceration at room temperature. Following filtration of the suspension through a Buckner funnel and Whatman filter paper #1, the crude ethanol extract was evaporated at 50°C to near dried. Then 1 gm dryness extract dissolved in 1 ml dimethyl sulfoxide (DMSO). Final concentration of each extract adjusted to 1000 mg ml⁻¹, then divided to volumes 1 ml in sterile vials and stored at -20°C for further studies.

(3) Dermatophyte isolates

For the antifungal evaluation, 3 strains were obtained from the Persian type culture collection (PTCC) Tehran, Iran. Tricophyton rubrum PTCC5143, Microsporum canis PTCC5069, M. gypseum PTCC5070 and 11 strains were from clinical isolates provided by Medical diagnosis laboratory, Ahwaz, Iran and identified by standard procedure (Rebell and Taplin, 1970). Pathogenic strains isolated from patients were Microsporum canis=1, M. gypseum=3, Trichophyton rubrum=1 and T. mentagrophytes=3, Epidermophyton floccosum=3. The samples were transferred to Sabouraud culture medium containing cyclohexamide and chloramphenicol agar (Difco, Detroit MI) slants and subcultured every 15 days to prevent pleomorphic transformations.

(4) Preparation of fungal inoculum

A standardized inoculum was prepared by counting the microconidia microscopically. Sterile normal saline (0.85%) solution containing 0.05% Tween 80 was added to the slant tube culture gently with a rod glass to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile centrifuges tube and the volume was adjusted to 5 ml with sterile normal saline. The final suspension of conidia was adjusted to 10⁴-10⁵ Cells/spores with colony forming units (CFUml⁻¹) on a hemocytometer cell counting chamber (Shin and Lim, 2004).

(5) Antifungal susceptibility testing

The fungistatic activities of different extracts were evaluated via the Agar Dilution Method (Brass et al. 1979). 1000mg of the crude extract was dissolved in 1 ml of sterile DMSO. It was served as stock solution. For the assay, stock solutions of extracts were two-fold diluted with sterile normal saline (0.85%) solution to produce serial decreasing dilutions ranging from 3.9-1000 μgm⁻¹. 5 ml of Sabouraud culture medium containing cyclohexamide and chloramphenicol agar (SCC) was added into Petri dishes (55 mm) and then cooled to 45°C.

The non-solidified SCC media was added with 100 μl of the serial dilutions extract and 50μl the inoculum removed after seven days from old culture of fungi and mixed thoroughly
Antifungal activity of *Ixora brachita* Roxb. against dermatophites

In it. Inhibition of fungal growth was observed after seven days of incubation at 28-30°C for dermatophyte strains in a moist, dark, and at a time according to the control of fungus growth up to 15 days for dermatophyte strains. The antifungal agents Ketconazole (Janssen pharmaceutical) and Griseofulvin (Sigma) were used as positive controls. Drug free solution (only with appropriate amount of DMSO) was also used as a blank control for verification of fungal growth. The minimal inhibition concentration (MIC) value was defined as the lowest extract concentration, and MFC minimal fungicidal concentration showing no visible fungal growth after incubation time. MIC50 and MIC90 values are the lowest extract concentration at which 50% and 90% of the clinical isolates inhibited (Marco et al. 1998). The results are depicted in Table 1,2,3,4 and Figure 2 and 3.
Table 1. *In vitro* evaluation of antifungal activity of ethanolic extracts of *Ixora brachiata* Root and Leaf against dermatophytes.

<table>
<thead>
<tr>
<th>Plant specie</th>
<th>Extract</th>
<th>T.m.</th>
<th>T.r.</th>
<th>M.g.</th>
<th>M.c.</th>
<th>E.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ixora brachiata</em> Root</td>
<td>EtOH</td>
<td>125</td>
<td>100</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td><em>Ixora brachiata</em> Leaf</td>
<td>EtOH</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td></td>
<td>&lt;200</td>
<td>&gt;200</td>
<td>30</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>Keteconazol</td>
<td></td>
<td>&lt;200</td>
<td>&gt;200</td>
<td>&lt;200</td>
<td>50</td>
<td>150</td>
</tr>
</tbody>
</table>

*Values are the mean of three replicates.

1 *Trichophyton mentagrophytes* TM1.
2 *T. rubrum* PTCC5143.

Table 2. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Ethanolic extracts of *Ixora brachiata* Roxb. Root against dermatophytes.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC &amp; MFC *value (µgml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(No. of isolates)</td>
<td>Range</td>
</tr>
<tr>
<td>1 T.m. (3)</td>
<td>125-500</td>
</tr>
<tr>
<td>2 T.r. (2)</td>
<td>100-500</td>
</tr>
<tr>
<td>3 M.g. (4)</td>
<td>125-500</td>
</tr>
<tr>
<td>4 M.c. (2)</td>
<td>125-500</td>
</tr>
<tr>
<td>5 E.f. (3)</td>
<td>125-500</td>
</tr>
</tbody>
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*Values are the mean of three replicates.

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2 *T. rubrum* PTCC5143.

Table 3. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Ethanolic extracts of *Ixora brachiata* Roxb. Leaf against dermatophytes.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC &amp; MFC* value (µgml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(No. of isolated)</td>
<td>Range</td>
</tr>
<tr>
<td>1 T.m. (3)</td>
<td>250-1000</td>
</tr>
<tr>
<td>2 T.r. (2)</td>
<td>250-500</td>
</tr>
<tr>
<td>3 M.g. (4)</td>
<td>250-1000</td>
</tr>
<tr>
<td>4 M.c. (2)</td>
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<td>5 E.f. (3)</td>
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</table>

*Values are the mean of three replicates.

1 *Trichophyton mentagrophytes* TM1.
2 *T. rubrum* PTCC5143.
Results and Discussion
To carry out the antifungal evaluation with agar dilution assays, extracts in concentrations of up to 1000 μg ml⁻¹ were incorporated into the growth media according to Material and methods. Extracts with MIC values <1000 μg ml⁻¹ were considered active.

The ethanolic extract of *Ixora brachiata* was strongly active against dermatophytes causative fungus of many superficial infections. We tested it against several strains of *E. floccosum* that produces arthroconidia, which survive for a longer time than other dermatophytes, therefore constituting an environmental source of contagion, sometimes leading to recurrent outbreaks of dermatophytosis in individuals and in institution (Domenico et al.1999) and *T. rubrum* and *T. mentagrophytes*, which are the main cause of athlete’s foot and onichomycoses in human beings.

Athlete’s foot is the most prevalent superficial infection in the developed world (Evans, 1997) and onichomycoses affects 2–13% of the population worldwide and up to 30% of groups at high risk such, as elderly and diabetic people (Levy, 1997 and Gupta et al. 1998). The ethanolic extract of *Ixora brachiata* root and leaves, inhibited all the species of dermatophyte genus tested, with MIC values between 125 and 500 μg ml⁻¹ (MIC90 and MIC50 values=500 & 250μgml⁻¹ respectively). The MFC values of these compounds were between 500-1000μgml⁻¹.

Conclusion
Based on the results of this study, we can consider ethanolic extract of *Ixora brachiata* as a new source for developing local antifungal agents. However, further studies are needed to determine the efficacy of active chemical constituent of this plant extract. Toxicological studies on the extract must also be performed to ensure the safety of the extract.

The discovery of a potent herbal remedy that is safe will be a big advancement in fungal infection therapies. It is vital for systemic fungal infections that are usually in immunocompromised patients as toxicities induced by commercial antifungal drugs are often observed in these patients due to the high dosage and prolonged therapy.

Acknowledgements
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References

