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IN VITRO ANTIFUNGAL ACTIVITY OF *Ixora brachita* 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 Presidensy 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 hydroalcholic 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 50^0C 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 1ml in sterile vials and stored at -20^0C 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 10^4-10^5 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 μgml^{-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

Figure 1. ***Ixora brachiata*** Roxb. flowerning twigs

Figure 2. Inhibitory effects of ethanolic extract of *Ixora brachiata* **Roxb. Root on growth of** *Trichophyton rubrum* **by Agar Dilution Method on Mycosel medium. MIC=125µgml^{-1}**

Figure 3. Inhibitory effects of ethanolic extract of *Ixora brachiata* **Roxb. Leaf on growth of** *T. mentagrophtes* **by Agar Dilution Method on Mycosel medium. MIC=250µgml**

in it. Inhibition of fungal growth was observed after seven days of incubation at 28-30ºC 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 Keteconazole (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.

		MIC *value ((µgml^{-1})				
Plant specie	Extract					
		T.m.[1]	T.r.[2]	M.g.[3]	M.c.[4]	E.f.[5]
Ixora brachita Root	EtOH	125	100	125	125	125
Ixora brachita Leaf	EtOH	250	250	250	250	250
Griseofulvin		<200	>200	30	150	50
Keteconazol		<200	>200	<200	50	150

***Values are the mean of three replicates.**
[1] *Trichophyton mentagrophytes* **TM[1].**
[2] *T. rubrum* **PTCC5143.**
[3] *M. gypseum* **PTCC5070.**
[4] *Microsporum canis* **PTCC5069.**
[5] *Epidermophyton floccosum* **EF[1].**

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

Organism	MIC & MFC *value ((µgml^{-1})				
(No. of isolates)	Range	50%	90%	MFC	
	[1]T.m. (3)	125-500	250	500	500
	[2]T.r. (2)	100-500	125	500	500
	[3]M.g. (4)	125-500	250	500	1000
	[4]M.c. (2)	125-500	250	500	1000
	[5]E.f. (3)	125-500	250	500	1000

***Values are the mean of three replicates.**
[1] *Trichophyton mentagrophytes* **TM[1].**
[2] *T. rubrum* **PTCC5143.**
[3] *M. gypseum* **PTCC5070.**
[4] *Microsporum canis* **PTCC5069.**
[5] *Epidermophyton floccosum* **EF[1].**

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

Organism	MIC & MFC* value ((µgml^{-1})				
(No. of isolated)	Range	50%	90%	MFC	
	[1]T.m. (3)	250-1000	500	1000	1000
	[2]T.r. (2)	250-500	250	500	1000
	[3]M.g. (4)	250-1000	500	1000	1000
	[4]M.c. (2)	250-500	250	500	1000
	[5]E.f. (3)	250-1000	500	1000	1000

***Values are the mean of three replicates.**
[1] *Trichophyton mentagrophytes* **TM[1].**
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Results and Discussion

To carry out the antifungal evaluation with agar dilution assays, extracts in concentrations of up to1000 μgml^{-1} were incorporated into the growth media according to Material and methods. Extracts with MIC values <1000 μgml^{-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 μgml^{-1} (MIC90 and MIC50 values=500 & 250μgml^{-1} respectively). The MFC values of these compounds were between 500-1000μgm^{-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 unsure the safety of the extract.

The discovery of a potent herbal remedy that is safe will be a big advancement in fugal 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.

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References

1. **Brass, C., Shainhouse, J.Z. and Steven, D.A.:** Variability of Agar Dilution Replicator Method of yeast Susceptibility Testing. *Antimicrob. Agents Chemother.* 15: 763 **(1979).**
2. **Cooke, T.** : The flora of the Presidency of Bombay, Botanical Survey of India. Vol. II : 224-231 **(1958)**.
3. **Domenico, B.** : Novel antifungal drugs. *Curr. Opin. Microbial*. 2: 509-15 **(1999)**.
4. **Evans, E.G.** : Tinea pedis: Clinical experience and efficacy of short treatment. *Dermatology*. 1:3-6 **(1997)**.
5. **Gupta, A.K. *et al.***: Prevalence and epidemiology of toenail onychomycosis in diabetic subjects: a multicenter survey. *Br. J. Dermato*. 139: 665-671 **(1998)**.
6. **Irobi, O.N. and Darambola, S.O.** : Antifungal activities of crude extract of *Mitracarpus villosus* (Rubiaceae). *J Ethnopharmacology*. 40: 137-140 **(1998)**.
7. **Levy, L.A.** : Epidemiology of onychomycosis in special risk populations. *J. Am. Podiatr.*

Med. Assoc. 87:546–550 **(1997).**

8. **Marco, F. *et al*.**: *In-vitro* activities of voriconazole (UK-109,496) and four other antifungal agents against 394 clinical isolates of Candida spp. *Antimicrob. Agent Chemother.* 42:161–163 **(1998).**
9. **Rebell, G. and Taplin, D.** : *Dermatophytes, Their Recognition and Identification*, University of Miami Press, Coral Gables, Florida **(1970)**.
10. **S. Shin and S. Lim.** : Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. *Journal of Applied Microbiology*. 97: 1289–1296 **(2004)**.
11. **Zacchino, S., Lo pez, S. *et al.* :** *In-vitro* evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes. *J. Nat. Prod*. 62:1353–1357 **(1999).**

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