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Full Length Research Paper

Antidermatophytic activities of *Ixora brachiata Roxb*

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The *in vitro* antifungal activity of *Ixora brachiata Roxb.* leaf and root extracts were evaluated against three different genera of dermatophytes viz. *Microsporum*, *Trichophyton* and *Epidermophyton* by Dilution Agar Method. The crude extracts of *Ixora brachiata* root and leaf showed *in-vitro* antifungal properties and completely prevented the growth of tested dermatophytic species with minimum inhibitory concentration (MIC) values of *I. brachiata* leaf (IBL) and *I. brachiata* root (IBR) between 5.0 - 10 and 2.5 - 10 mg ml^{-1} medium, respectively. Minimum fungicidal concentration (MFC) values of *I. brachiata* leaf (IBL) and *I. brachiata* root (IBR) was also similar to 5.0 and 2.5 mg ml^{-1} of the medium, respectively. Results of phytochemical screening indicated that the leaf and root of *I. brachiata* gave positive test for starch, saponins, reducing sugars, anthraquinones, glycosides, phenols and proteins while they gave negative test for alkaloids. IBL gave positive test for tannins while IBR gave positive test for flavonoids. Detection of coumarins and triterpenes in IBL suggested that these compounds might be responsible for the anti-dermatophytic activity in this study.

Key words: Antidermatophytic activity, *Ixora brachiata Roxb.*

INTRODUCTION

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 exhibit several side effects and have limited efficacy. Therefore, 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 (Irobi et al., 1993), because herbal remedies used in traditional folk medicine may help to overcome the growing problem of resistance to antifungal drugs and their relative toxicity. *Ixora brachaita* Roxb. belongs to the family *Rubiaceae*. It is a small tree 15 - 30 ft. high, found to be growing in high rainfall locality (Cooke, 1958).

Human infections particularly those involving the skin are increasing at an alarming rate, especially in tropical and subtropical developing countries, with dermatophytes as the most common pathogens (Fenner et al., 2005; Portillo, 2001; Mahesh et al., 2009). This increase is directly related to the growing population of immunocompromised individuals. Human mycoses are not always successfully treated, since the available many advance effect 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). According to Siddha literature, *I. brachaita* Roxb. have anti-inflamatory, aromatic and antipyretic properties. But the literature on antioxidant and antimicrobial properties are scanty. Since no review of literature are available on the antifungal effect of *I. brachiata*, thus, the present study was undertaken to evaluate the antifungal activity of ethanolic extracts of leaf and root against tested dermatophytic species.

MATERIALS AND METHODS

The leaves of *I. brachiata* were extracted in ethanol. To 10 g of each powdered material air dried was added 100 ml of 80% ethanol

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(drug/solvent ratio=1:10 w/v) for maceration, and then kept on a rotary shaker for 72 h at room temperature (Fenner et al., 2005). Following filtration of the suspension through a Buckner funnel and Whatman filter paper #1, the crude ethanol extracts were evaporated in oven at 45°C.

Dermatophyte isolates

Five dermatophytic species vis. *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, Microsporum gypseum* and *Epidermophyton floccosum* were used in the present study. They were collected from medical diagnosis laboratory, Ahwaz, Iran.

Preparation of fungal inoculum

For this, the suspension of conidia was prepared by using 0.85% sterile physiological saline which containing 0.05% Tween 80 (Sigma). The final suspension of conidia was counted with a hemocytometer cell counting chamber. The inoculum of conidia suspensions were obtained according to Shin and Lim (2004) and Wright et al. (1983) and adjusted to 10^5 conidia with colony-forming units ($CFUml^{-1}$).

Antifungal susceptibility testing

The antifungal activities of ethanol extracts were evaluated by the Agar Dilution Method (Fenner et al., 2005; Mitscher et al., 1972; Lucia et al. 2003). 1000 mg of the crude extract was dissolved in 1 ml of sterile DMSO served as stock solution (Fenner et al., 2005). For the assay, stock solutions of extracts were two-fold diluted with 0.85% sterile physiological saline to produce serial decreasing dilutions ranging from 0.078 - 20 mg ml^{-1}. 100 µl of the solution extracts and 50 µl of dermatophyte suspension of 10^5 $CFUml^{-1}$ were added to 5 ml Mycosel agar medium with 45°C and evenly fine mixed. The plates were incubated at 28 - 30°C up to 15 days for dermatophytic species. 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 leaf and root of the above extracts were further extracted with various polarities solvents like Diethyl ether, acetone, methanol and water. The antifungal activities of these extracts against *T. mentagrophytes* were tested by Disk Diffusion Method as described above.

Phytochemical study

In the present investigation the leaf and root of *I. brachiata* were evaluated qualitatively for the presence of saponins, reducing sugars, tannins, alkaloids, proteins, glycosides, anthraquinones and flavonoids. The ethyl acetate extracts of *I. brachiata* leaf and root were subjected to TLC by using precoated silica gel F_{254} plate. The mobile phase of Acetone/Ethyl acetate/Petroleum ether (0.5: 0.5:2.0) [AEP] was used for giving the best resolution of spots. High performance thin layer chromatography (HPTLC) fingerprint for same extract was obtained at UV- 254 nm and UV- 366 nm.

Statistical treatment of results

The analysis of data was performed with the SPSS program version 10. Analysis of variance tests were conducted using the general one-way ANOVA with post hoc comparison of mean values by LSD.

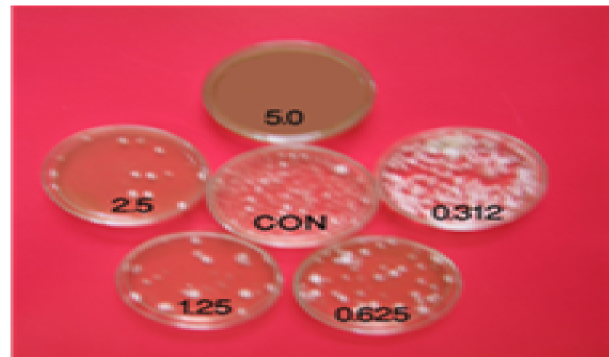

Figure 1a. Inhibitory effects of ethanolic extract of *I. brachiata* leaf on growth of *T. menta.* by Agar Dilution Method on Mycosel medium. Decreasing dilution ranging from 0.078 - 5.0 mg/ml medium. MIC = 5.0 mg/ml medium.

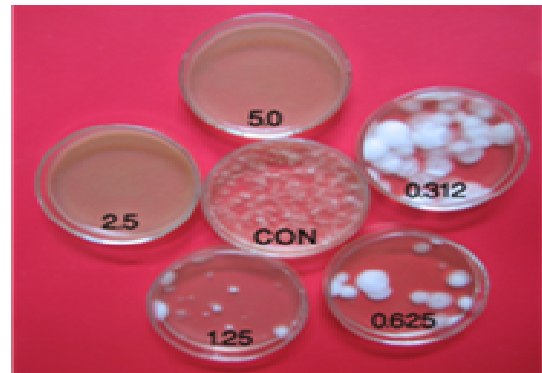

Figure 1b. Inhibitory effects of ethanolic extract of *I. brachiata* root on growth of *T. menta.* by Agar Dilution Method on Mycosel medium. Decreasing dilution ranging from 0.078 - 5.0 mg/ml medium. MIC = 2.50 mg/ml medium.

RESULTS

The ethanolic extracts of *I. brachiata* leaf and root completely prevented the growth of tested dermatophytic species with MIC values for IBL and IBR ranged between 5.0 - 10 and 2.5 - 10 mg ml^{-1} medium, respectively, MIC90 and MIC50 values equal 2.50 and 0.625 mg ml^{-1} medium for IBL and 1.250 and 0.312 for IBR (Figure 1a and 1b). MIC90s and MIC50s were similar in all tested clinical and standardized dermatophytic species of *T. mentagrophytes, M. canis, M. gypseum*, *T. rubrum* and *E. floccosum*. MFC values for IBL and IBR were also similar in all tested clinical and standardized dermatophytic species (5.0 and 2.5 mg ml^{-1} medium) respectively. The results are shown in Tables 1 and 2.

Results on phytochemical screening indicated that the leaf and root of *I. brachiata* gave positive test for starch,

Table 1. MICs (mg ml $^{-1}$) of the extracts.

Name of plant	Plant part used	MIC value (mg ml $^{-1}$)[a]				
Ixora brachiata Roxb		M.C.[b]	M.g.[c]	E.f.[d]	T.r.[e]	T.m.[f]
	Leaf	5.00	5.00	5.00	5.00	5.00
	Root	2.50	2.50	2.50	2.50	2.50
[g]Griseofulvin		12.5	100	25	50	100
[h]Keteconazole		25	6.25	0.78	25	6.25

Abbreviations: [a]*Microsporum canis* PTCC5069. [b]*Microsporum gypseum* PTCC5070. [c]*Epidermophyton floccosunm EF-3.* [d]*Trichophyton rubrum TR-1.* [e]*Trichophyton mentagrophytes* PTCC5054. IBL (*Ixora brachiata* leaf), IBR (*Ixora brachiata* root).
MIC values are given as mean values (mg ml $^{-1}$) from the triplicate experiments.
MIC values griseofulvin and keteconazole (µg ml $^{-1}$) as positive control.

Table 2. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of IBL, IBR, Griseofulvin (GRS) and Keteconazole (KTZ) against dermatophytes by Agar Dilution Method

Dermatophytes No. of strains	Antifungal Compounds	MIC and MFC				
		Range	50%	90%	MFC	Geometric mean MIC
T. mentagrophytes (3) *T. menta.* PTCC5054	KTZ	0.78 - 6.25	1.56	6.25	12.5	3.52
	GRS	12.5 - 100	25	100	200	56.25
	IBL	0.312 - 2.500	0.625	2.500	5.000	1.46
	IBR	0.156 - 1.250	0.312	1.250	2.500	0.703
M. gypseum (3) *M. gypseum* PTCC5070	KTZ	0. 78 - 6.25	1.56	6.25	12.5	3.52
	GRS	12.5 - 100	25	100	200	56.25
	IBL	0.312 - 2.500	0.625	2.500	5.000	1.46
	IBR	0.156 - 1.250	0.312	1.250	2.500	0.703
M. canis (3) *M. canis* PTCC5069	KTZ	1.56 - 12.50	3.12	12.5	25	7.03
	GRS	3.12 - 25.00	6.25	25	50	14.06
	IBL	00.312 - 2.500	0.625	2.500	5.000	1.46
	IBR	0.156 - 1.250	0.312	1.250	2.500	0.703
T. rubrum (2)	KTZ	3.12 - 25.00	6.25	25	50	14.06
	GRS	6.25 - 50.00	12.5	50	100	28.13
	IBL	0.312 - 2.500	0.625	2.500	5.000	1.46
	IBR	0.156 - 1.250	0.312	1.250	2.500	0.703
E. floccosum (3)	KTZ	0.39 - 0.78	0.39	0.78	1.56	0.585
	GRS	3.12 - 25.00	6.25	25	50	14.06
	IBL	0.312 - 2.500	0.625	2.500	5.000	1.46
	IBR	0.156 - 1.250	0.312	1.250	2.500	0.703

Abbreviations: MIC values are reported as µgml $^{-1}$for KTZ (Keteconazole), GRS (Greseofulvin) and mg ml $^{-1}$ for IBL (*Ixora brachiata* leaf), IBR (*Ixora brachiata* root) extracts. *T. Trichophyton, M. Microsporum, E. Epidermophyton.* 50 and 90%, MICs at which 50 and 90% of the isolates in the test panel respectively are inhibited. Values are given as mean from the triplicate experiments.

saponins, reducing sugars, anthraquinones, glycosides, phenols and proteins while they gave negative test for alkaloids. IBL gave positive test for tannins while IBR gave positive test for flavonoids (Table 3). In the present investigation, the organic solvents of acetone, methanol and water with different polarity did not show any efficacy for extraction from *I. brachiata* leaf and root but only Di-ethyl ether with semi-polarity had high affection with inhibition zone of 15 and 25 mm diameter against *T. mentagrophytes* for IBL and IBR, respectively. Results on High Performance Thin Layer chromatography (HPTLC) indicated that the ethyl acetate extract of *I. brachiata* leaf (Wagner, 1984) contains coumarin that it was observed in UV-254 nm with 8 peaks and in UV-366 nm with 9 peaks (Figure 2 a and 2b). The ethyl acetate extract of *I. brachiata* root contains triterpene and it was observed a

Table 3. Phytochemical screening of *Ixora brachiata* leaf and root extracts.

Name of the test carried out	Reagents used	*Ixora brachiata*	
		Leaf	**Root**
	(A.) Water Extract		
Starch	I2-KI	+ve	+ve
Tannins	Acidic $FeCl_3$	+ve	-ve
Saponins	H_2SO_4 + Acetic unhydride	+ve	+ve
Proteins	Million's test	+ve	+ve
Anthraquinones	Benzene + 10%NH_4OH	+ve	+ve
Reducing sugars	Benedict's	+ve	+ve
	(B.) Alcoholic Extracts		
Alkaloids	Mayer's	-ve	-ve
	Wagner's	-ve	-ve
	Dragendorff's	-ve	-ve
Flavonoids	HCl + Mg turnings	-ve	+ve
Glycosides	Benzene + hot ethanol	+ve	+ve

+ve: Present; -ve: Absent.

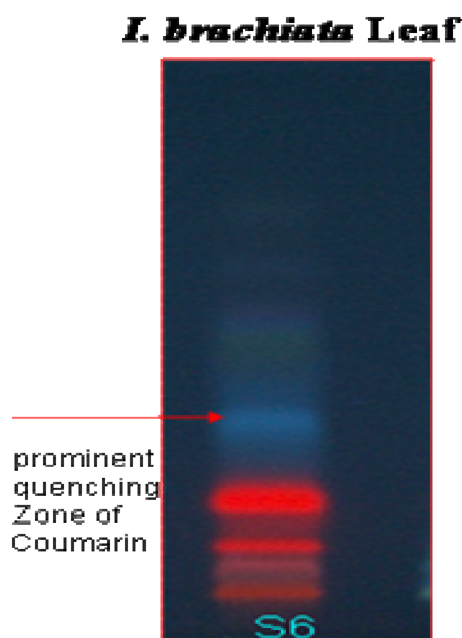

Figure 2a. Image in 366 nm after derivation with vanillin sulphuric acid.

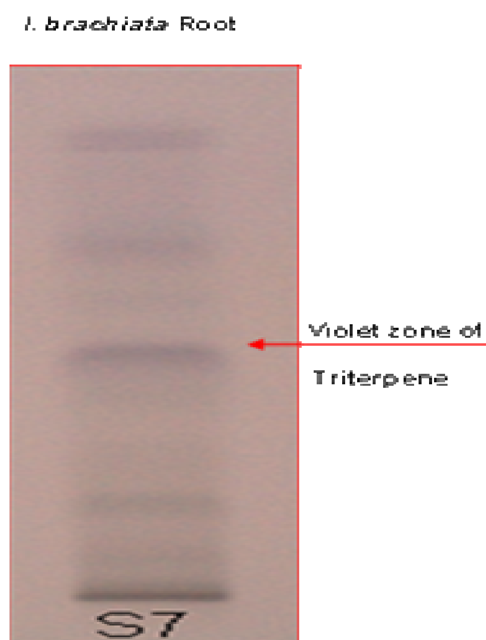

Figure 2b. Image in visible after derivation with vanillin sulphuric acid.

a violet zone in the visible region after derivation from Anisaldehyde – Sulphuric Acid (Figure 2a and 2b) with 10 peaks at UV-254 nm and 12 peaks at UV-366 nm.

DISCUSSION AND CONCLUSION

These results indicated that the ethanolic extract of *I. brachiata* possesses antifungal properties not only against standardized strains of clinically important dermatophytes but also against their clinical phytochemical studies on other species of *Ixora* such as *Ixora coccinea* which consists of anthocyanins in flowers, oleic and linoleic acids in root oil and octadecadienoic acid from root bark (Chopra et al., 1956) and saponins and tannins (Grainge et al., 1988). In previous reports it is seen that screening of ether extract of *I. coccinea* gave positive test

for alkaloids, flavonoids, sapogenins, sterols, terpenes and that of methanol extract for alkaloids, phenols and sterols (Annapurna and Raghavan, 2003). These results are similar to those results obtained in the present invest-tigation from phytochemical screening of *I. brachiata* whereas it gave negative test for alkaloids.

Finally after detection and confirmation of triterpenes and coumarin by HPTLC in *I. brachiata*, it was suggested that these compounds might be responsible for the anti-dermatophytic activity in this plant which gave a positive test results by *in-vitro* isolates as suggested by Cowan (1999).

Based on the results of this study, we can consider ethanolic extract of I. 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.

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REFERENCES

Annapurna J, Amarnath PVS, Amar Kumar D, Ramakrishna SV, Raghavan KV (2003). Antimicrobial activity of *Ixora coccinea* leaf. Fitoterapia. 74: 291–293.

Chopra RN, Nayar SL, Chopra IC (1956). Glossary of Indian medicinal plants. Council of Scientific and Industrial Research India (CSIR).

Publication and information directorate New Delhi-110012.: 57, 102, 139, 198 and 256.

Cooke T (1958). Flora of the Presidency of Bombay, Botanical Survey of India.Vol. I, II & III.

Dymock D, Warden CJH, Hooper D (1810). Pharmacographia Indica.(III): 74-75.

Fenner M, Sortinob SM, Ratesa R, Agnola S, Zacchino B (2005). Antifungal activity of some Brazilian *Hypericum* species. Phytomedicine 12: 236-240.

Lucia KHS, Cecilia MAO, Pedro HF, Juldasio GOJ, Ary Henrique SJ, Orinalda de Fatima LF, Maria do Rosario RS (2003). Antimicrobial Activity of *Hyptis ovalifolia* Towards Dermatophytes. Mem Inst Oswaldo Cruz, Rio de Janeiro. 98 (7): 963-965.

Grainge M, Ahmed S (1988). Handbook of plants with pest control properties. New York: Wiley.

Irobi ON, Darambola SO (1993). Antifungal activities of crude extract of *Mitracarpus villosus* (Rubiaceae). J. Ethnopharmacol. 40: 137-140.

Marco F, Pfaller MA, Messer S, Jones RN (1998). 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.

Mitscher L, Leu R, Bathala M, Beal J (1972). Antimicrobial agents from higher plants. Introduction, Rationale Methodology. 35: 157–166.

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001). Antifungal activity Paraguayan plant usrd in traditional medicine. J. Ethnopharmacol. 76: 93-98.

Rebell G, Taplin D (1970). Dermatophytes, Their Recognition and Identification, University of Miami Press, Coral Gables, Florida.

Shin S, Lim S (2004). Antifungal effects of herbal essential oils alone and in combination with ketoconazole against Trichophyton spp. J. Appl. Microbiol. 97: 1289–1296.

Wagner H, Bladt S, Zgainski EM (1984). Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edition, Springer- Verlag, New York: 305-327.

Wrigh L, Scott E, Gorman S (1983). The sensitive of mycelium, arthrospores and microconidia of *Trichophyton mentagrophytes* to imidazoles determined by *in-vitro* tests. J. Antimicrob. Chemother. 12: 317-323.

Zacchino S, Lo Pez S (1999). *In-vitro* evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes. J. Nat. Prod. 62: 1353-1357.