



Trade Science Inc.

December 2010

ISSN : 0974 - 7427

Volume 4 Issue 2

# BioCHEMISTRY

An Indian Journal

Regular Paper

BCAIJ, 4(2), 2010 [122-125]

## Antidermatophytic activity of *Caesalpinia pulcherrima*

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Received: 10<sup>th</sup> June, 2010 ; Accepted: 20<sup>th</sup> June, 2010

### ABSTRACT

Increase in the use of antifungal agents for the treatment of infections, but the expanded use of antifungal agents has accelerated to commercial drugs. Antidermatophytic activities of the *Caesalpinia pulcherrima* plant aerial parts were tested against eleven fungi including pathogenic dermatophytes. *Caesalpinia pulcherrima* leaf extracts showed stronger and wider range of antidermatophytic activities at lowest concentration. The Minimum inhibitory Concentration and zone of Inhibition of the flower and stem extract of the plant shown *Trychophyton sp.* are resistant to these extracts.

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### KEYWORDS

Antidermatophytic;  
*Caesalpinia pulcherrima*;  
Flavonoids.

### INTRODUCTION

Dermatophytes are among the few fungi causing communicable disease, that is, diseases acquired from infected animals or birds or from the fomites they have engendered. The humid weather, over population and poor hygienic condition are conducive to the growth of dermatophytes<sup>[1]</sup>. Treatment failure, relapse and various side effects with available antifungal drugs emphasize the need or a novel, plant – based drug for the treatment of dermatophytes<sup>[2]</sup>. *T. rubrum* was the chief isolate from skin scales<sup>[3]</sup>. This is the commonest agent isolated from glabrous skin of the body, groin folds and the feet<sup>[4]</sup>. *T. mentagrophytes* was the second common isolate from the body site<sup>[5]</sup>. *E. floccosum* was isolated from two specimens obtained from the skin and this was the third common isolate from the glabrous skin<sup>[6]</sup>. Conventional antifungal agents such as

chlorhexidine and imidazole derivatives have limited uses in the pregnant and the young and can produce many adverse effects<sup>[7,8]</sup>.

*Caesalpinia pulcherrima*, an evergreen, low-branching and fast growing shrub. Leaves, flowers, bark and seeds are largely used in Indian medicine. Literature study reveals that *C. pulcherrima* bark contain terpenoids is considered as antibacterial and antifungal agent. In Indo china, the plant is used as a tonic, stimulant, and emmenagogue<sup>[9]</sup>. The leaves and flowers of this plant is considered as an antioxidant, cytotoxic agent<sup>[10]</sup>, analgesic<sup>[11]</sup>, antiulcer agent<sup>[12]</sup> and anti-inflammatory agent<sup>[13]</sup>. Flavonoid combinations or flavonoids with conventional antibiotics could be an effective alternative in the treatment of infections produced by microorganisms<sup>[14]</sup>. Some compounds of *C. pulcherrima* which possess antiviral activities against herpesviruses (HSV-1, HSV-2) and adenoviruses (ADV-3, ADV-8, ADV-11) may be de-

**TABLE 1 : In vitro susceptibilities of fungi to extracts of *C.pulcherrima* Quercetin, and Ketaconazole by broth microdilution method**

Fungi	Quercetin (mg ml <sup>-1</sup> )	CL* (mg ml <sup>-1</sup> )	CS** (mg ml <sup>-1</sup> )	CF*** (mg ml <sup>-1</sup> )	Ketaconazole (microgram ml <sup>-1</sup> )
<i>Aspergillus niger</i>	2.5	2.5	2.5	2.5	12.5
<i>Aspergillus flavus</i>	2.5	2.5	2.5	2.5	12.5
<i>Aspergillus fumigatus</i>	2.5	2.5	2.5	2.5	12.5
<i>Candida albicans</i>	5.0	5.0	5.0	5.0	12.5
<i>Candida utilis</i>	5.0	5.0	5.0	5.0	25.0
<i>Fusarium oxysporum</i>	2.5	5.0	5.0	5.0	25.0
<i>Fusarium solani</i>	5.0	5.0	5.0	5.0	12.5
<i>Microsporium gypseum</i>	5.0	5.0	5.0	10.0	25.0
<i>Trichophyton metagraphytes</i>	5.0	5.0	5.0	10.0	25.0
<i>Epidermophyton floccosum</i>	5.0	5.0	10.0	10.0	12.5
<i>Trichophyton rubrum</i>	5.0	5.0	10.0	10.0	25.0

\*MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* Leaves,

\*\*MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* stem,

\*\*\*MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* flowers

derived from the flavonoid of quercetin<sup>[15]</sup>.

The purpose of the investigation reported here is to provide a simple, reproducible and efficient method to control dermatophytic infection using *Caesalpinia pulcherrima* aerial parts.

## MATERIALS AND METHODS

### Plant material

*Caesalpinia pulcherrima* plant material was collected from Vivekanandha college campus, Tiruchengode and identified by the Department of Botany, Vivekanandha college of Arts and Sciences for women, Tiruchengode and placed in the Herbarium for future reference (Voucher No. RUBL- 19911). Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles. Quercetin (RM 6191) and Ketaconazole (RM 4322) and DMSO were purchased from Himedia Laboratories (Mumbai, India).

### Preparation of extract

The air-dried and powdered plant material leaves, flower, and stem (5 g of each part) was extracted with 95% of methanol, kept on a rotary shaker for 16 h. Thereafter it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and evaporated to dryness to give the crude dried extract. The extrac-

tive yield (%) of all the extracts is calculated.

### Microorganisms

A total of eleven fungal strains were used. Clinical isolates from vaginal swabs, blood, urine, onychomycosis and sputum: *Aspergillus niger*, *A.flavus*, *A.fumigatus*, *Candida albicans*, *C.utilis*, *Fusarium oxysporum*, *F.solani*, *Microsporium gypseum*, *Trichophyton metagraphytes*, *Epidermophyton floccosum*, and *Trichophyton rubrum*. strains were identified according to morphological and biochemical procedures. The strains were cultured in Sabouraud broth or agar and stored in glycerol at 80°C.

### Minimum inhibitory concentration

MICs were determined by broth dilution method as described earlier<sup>[16]</sup>. Duplicates of serial dilutions of broth and crude extract of *C.pulcherrima* were made. Sabouraud's broth and Potato Dextrose Broth were used for cultivation of yeast and fungus respectively. The MICs were determined against  $1 \times 10^9$  cells of each culture, as the lowest concentration of extract that reduced the growth of these microbes.

### Disc diffusion method

Yeast and fungal broth cultures aliquots were adjusted to ca.  $5 \times 10^4$  CFU ml<sup>-1</sup> were added to respective agar medium and spreaded uniformly. Sterile paper discs (8 mm, Whatmann filterpaper) were impregnated with 50 microlitre of 25% (v/v, 12.5mg) or 50% (v/v, 25mg) methanol extracts of plant leaves, stem and flowers and antifungal solution and placed on the culture plates after removing solvent by evaporation. The diameter of the zone of inhibition (mm) around the disk was measured after cultivation of at 24-28°C after 48hours. Quercetin and Ketaconazole were used as positive control and DMSO as Negative control. The values are the means of tests performed in triplicates.

## RESULTS AND DISCUSSION

Antidermatophytic activity of plant material was investigated on 11 fungal strains by MIC and disc diffusion method. Several herbal preparations that can enhance the body's immune status are extensively being used in the indigenous system of medicines. There is an upsurge in clinical usage of indigenous drugs as they are

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**TABLE 2 : Antidermatophytic activity of Quercetin and the plant extracts of *C.pulcherrima* estimated by disc diffusion method**

Fungi	Zone of Inhibition (mm)							
	Quercetin		CL*		CS**		CF***	
	12.5	25	12.5	25	12.5	25	12.5	25
<i>Aspergillus niger</i>	8.3±0.5	11.3±0.5	3.83±0.79	5.0±1.3	2.2±0.4	3.6±1.0	2.5±0.2	4.5±0.8
<i>Aspergillus flavus</i>	9.5±0.4	11.5±1.5	4.3±0.55	5.7±1.5	3.5±0.6	5.1±0.5	3.8±1.1	5.4±0.1
<i>Aspergillus fumigatus</i>	6.6±0.5	7.6±1.5	4.3±0.66	5.8±1.6	3.2±0.3	4.2±0.5	2.8±1.6	4.9±1.3
<i>Candida albicans</i>	7±1.0	9.6±0.5	4.8±0.5	5.5±0.9	4.3±1.0	5.7±0.2	3.6±0.6	6.0±1.8
<i>Candida utilis</i>	6.3±0.5	8.6±0.4	5.6±0.8	6.8±1.2	2.8±0.8	3.6±1.6	3.7±0.4	6.2±0.5
<i>Fusarium oxysporum</i>	7±0.0	8.6±0.5	5.5±1.0	5.7±0.8	3.0±0.4	4.2±0.8	5.2±0.2	6.5±0.7
<i>Fusarium solani</i>	6.3±0.5	10.6±1.5	3.48±0.7	4.6±1.5	4.2±0.6	5.1±0.4	3.6±1.5	4.5±0.6
<i>Microsporium gypseum</i>	5.3±0.0	6.3±0.5	6.1±0.8	6.7±0.9	3.1±0.5	4.1±0.7	5.4±1.1	5.8±0.2
<i>Trichophyton metagrophytes</i>	5.2±0.5	6.0±1.0	3.2±0.9	4.8±1.8	3±0.5	3.5±1.2	3.5±0.3	4.4±1.1
<i>Epidermophyton floccosum</i>	7.3±0.5	9.6±1.5	2.9±0.6	4.5±1.8	3.2±0.3	4.6±0.9	2.6±0.2	4.2±1.0
<i>Trichophyton rubrum</i>	7.6±0.7	12.3±1.5	4.1±0.6	5.0±1.2	3.6±1.0	4.6±1.0	3.5±1.3	4.6±0.7

Note: Values are mean ±SD n=3 in each group

\* MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* Leaves, \*\* MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* stem,

\*\*\* MICs (mg ml<sup>-1</sup>) of Methanolic extract of *C.pulcherrima* flowers

free from serious side effects<sup>[17]</sup>. TABLE 1 demonstrates the MIC ranges of the positive controls with reference and plant extracts to the yeasts and dermatophytes. The results of the present study indicates that *C.pulcherrima*. methanolic extract of leaves was found to be the most active against the all tested fungi and dermatophytes.

Alcohol extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possess antifungal properties<sup>[18]</sup>. *T.rubrum*, *T.metagrophytes* and *E.floccosum* with MIC values of 5mg/ml of methanolic extract of CL, while against *Aspergillus sp.*, the values were 2.5mg/ml. The results showed that the *Trycophyton* and *Epidermophyton sp.* are more resistant to flower extract of *C.pulcherrima*. Ketaconazole at a concentration of 12.5 microgram/ ml inhibited most of the fungi, but at 25 microgram/ ml concentration only it is inhibited dermatophytes. Quercetin at 5mg/ml values shown inhibition on the growth of dermatophytes.

### Disc diffusion method

Secondary compounds that would normally be extracted in the polar extracts included alkaloids, flavonoids and some phenols while non-polar secondary compounds may include tannins, terpenes, and quinines. The Zone of Inhibition in different fungal strains against *C.pulcherrima* extracts is shown in TABLE 2. Among

the various strains maximum zone of inhibition (6.8 mm) was recorded in *C. utilis* strain against *C.pulcherrima* leaf extracts. Minimum zone of inhibition (2.2 mm) was observed in *Aspergillus niger* strain by *C.pulcherrima* stem extracts. Methanolic extract of *C.pulcherrima* flower extracts shows significantly lowest rate of sensitivity against various fungal strains.

Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity. These plant compounds have different structures and actions when compared with conventional fungicides used to control the microbial growth and survival<sup>[19]</sup>. The growth of *T.rubrum*, and *E.floccosum*, was inhibited considerably particularly at the high dose (25 microgram/ml).

### CONCLUSION

Antimicrobial resistance is a major cause of significant morbidity and mortality globally. Ethnomedicine provides avenues for identification of compounds with antimicrobial properties and potential new antibiotics. The result of this study suggests that *C. pulcherrima* can be potential source of chemotherapeutic agents that can be used for the treatment of diseases. The present study has clearly demonstrated that the medicinal knowledge held by the traditional Indian drugs is relatively measurable in a laboratory-based assay.

**REFERENCES**

- [1] V.Natarajan, P.V.Venugopal, T.Menon; Indian journal of Medical Microbiology, **21**, 98 (2003).
- [2] S.Ranganathan, T.Menon, S.Arulmozhi Balajee, S.Mahendra Raja; Ind.J.Dermatol., **41**, 113 (1996).
- [3] J.T.Lim, H.C.Chua, C.L.Goh; Aust.J.Dermatol., **33**, 159 (1992).
- [4] A.Kamalam, A.S.Thambiah; Mykosen, **24**, 431 (1981).
- [5] M.M.Hunda, N.Chakraborty, J.N.Bordoloi; Indian J.Dermatol.Venereol.Leprol., **61**, 329 (1995).
- [6] P.Kannan, C.Janaki, G.S.Selvi; Indian Journal of Medical Microbiology, **24**, 212 (2006).
- [7] C.Curtis; In Practice, 244 (1998).
- [8] C.Curtis; In Practice, 48 (1999).
- [9] A.K.M.Nasimul Islam, M.Abbas Ali, Abu Sayeed, S.M.Abdus Salam, Anwarul Islam, M.Rhuman, G.R.M.Astaq Mohal Khan, S.Khatun; Asian Journal of Plant Sciences, **2**, 1162 (2003).
- [10] C.R.Pawar, R.E.Mutha, A.D.Landge, R.B.Jadhav, S.J.Surana; Ind. J.Biochem & Biophy., **46**, 198 (2009).
- [11] G.S.Chakraborty, S.Rohan, Babdujar, C.R.Pardesi; Journal of Pharmacy Research, **2**, 1199 (2009).
- [12] A.Kumar, V.Nirmala; Ind.J.Pharm.Sci., **66**, 676 (2004).
- [13] Y.K.Rao, S.H.Fang, Y.M. Tzeng; J.Ethnopharmacol., **100**, 249 (2005).
- [14] M.A.Alvarez, N.B.Debattisa, N.B.Pappano; Folia Microbiol., **53**, 23 (2008).
- [15] L.C.Chiang, W.Chiang, M.C.Liu, C.C.Lin; J.Antimicrobiol.Chemotherapy, **52**, 194 (2003).
- [16] K.M.Elizabeth; Journal of Human Ecology, **12**, 875 (2001).
- [17] J.Madan, A.K.Sharma, N.Inamdar, H.S.Rao, R.Singh; Int.J.Green Pharmacy, 154 (2008).
- [18] A.D.Webster, Pierre Taschereau, Ren'e J.Belland, Crystal Sand, Robert P.Rennie; J.Ethnopharmacol., **115**, 140 (2005).
- [19] K.Girija, P.Udaya Chandrika, K.Lakshman, N.Pruthvi; Int.J.Res.Pharm.Sci., **1**, 199 (2010).