

In Vitro Antimycotic Activity of Some Medicinal Plants Against Human Pathogenic Dermatophytes

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ABSTRACT

The water extract, methanol, free flavonoids and bound flavonoids of *Allium sativum*, *Cymbopogon martinii* and *Catharanthus roseus* were screened for their antimycotic activities using disc diffusion method. They were tested against dermatophytes, isolated from patients having tinea infection. The activities of the extracts were compared with the standard antibiotics. The bound flavonoid extracts as well as free flavonoid extracts showed prominent antidermatophytic activity against all the dermatophytes used in the study. From the zones of inhibition produced by the extracts, it was observed that *Candida albicans* was most resistant to water and methanolic extracts taken from all the medicinal plants used.

Key Words: Antimycotic activity, dermatophytes, inhibition zone, extracts, flavonoids, *Candida albicans*, antibiotics

INTRODUCTION

Plants have a long history of antibiotic usage for the cure of disease caused by antimicrobial, including antiviral, antibacterial and antifungal, agents. Much attention has been refocused on plant origin, antimicrobial and antidermatophytic agents after the discovery of Penicillin, that is considered to be one of the most important life-saving phytodrugs. Since then, many extracts from different plants have been tested for their antimicrobial activities but there are so many un-opened pages of plant life which require more careful investigation to reveal their hidden characteristics. Natural products are generally harmless or have minimum side effects as compared to synthetic drugs. In addition to the antimycotic drugs, the effect of certain indigenous plant extracts on the spore germination of dermatophytes *in vitro* has also been studied, but the work is fragmentary. Antifungal activities of some plants have been reported by various researchers throughout the world like Sharma 1988, Caceres *et al.*, 1993, Mehrabian *et al.*, 1995, Farombi 2003, Mahesh and Satish 2008, Tewari (1990), Rajendheran *et al.*, (1998), Nair *et al.*, (2005) and Prusti *et al.*, (2008).

The use of antimycotic drugs in controlling superficial mycoses has been in practice since long and has been recognised as an effective means of control of dermatophytoses caused by dermatophytes. Griseofulvin is still found to be the most effective and widely used antidermatophytic drug. This drug could not be accepted as a routine treatment for every case because it is

expensive and the duration of the treatment is long. Similarly, topical treatment with Tolnaftate is costly and ineffective against conidial infections of the skin (Wilmer and Wollina, 1998). Some of the newer agents including Clotrimazole, Miconazole, Fluconazole, Itraconazole and Ketoconazole (Imidazole group) are also very effective but they can cause side effects, which could be harmful to human health (Shahi *et al.*, 1999). In the present scenario, plants and their products have gained more importance as possible sources of alternative and effective drugs.

In India, references to the curative properties of some herbs (as available in the *Rigveda*) seem to be the earliest record of the use of plants for medicinal purposes. Singh *et al.*, (1988) reported the exhibition of activity of the essential oil of the leaves of *Eucalyptus rostrata* against four human pathogens, namely, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum gypseum* and *M. canis*. Iyer and Williamson (1991) investigated the efficacy of some plant extracts against *Trichophyton* species. Venugopal and Venugopal (1994) tested the antidermatophytic activity of allylamine derivatives. In 1994, Venugopal and Venugopal again reported antidermatophytic activity of aqueous and ethanolic extracts of neem leaves against 88 clinical isolates of dermatophytes. Tiwari and Dubey (1996) tested 14 essential oils against dermatophytes, namely, *Epidermophyton floccosum* and *Microsporum gypseum*.

The oil exhibited a broad antidermatophytic spectrum when tested against *Aspergillus flavus*, *A. niger*, *E. floccosum*, *M. audouinii*, *M. gypseum*, *M. nanum*, *Trichophyton rubrum* and *T. violaceum*. Lachoria *et al.*, (2000) obtained the essential oils of six plants and tested them against dermatophytes, namely, *Microsporum gypseum*, *Microsporum* sp., *Trichophyton rubrum* and *Epidermophyton floccosum*. The essential oil of *Lantana camara* showed higher activity against all dermatophytes as compared to other test oils. Alkofahi *et al.*, (1990) investigated the antimicrobial activity of 40 medicinal plants of Jordan. Taylor *et al.*, (1995) screened some selected medicinal plants of Nepal for antibacterial and antidermatophytic activity. Charchari *et al.*, (1996) extracted essential oil from the flowering and aerial parts of *Artemisia herba-alba* and *A. judaica* and both were found to be effective against *Candida* species and *Microsporum* species. Dutta *et al.*, (1998) tested the antifungal activity of ethanol (10 to 20 %) on *Trichophyton tonsurans*, *T. rubrum*, *Trichosporon beigelis*, *Microsporum fulvum*, *M. gypseum* and *Candida albicans*. Gopalakrishnan *et al.*, (2000) tested the effect of petroleum ether extract, chloroform and methanolic extract of dried leaves of *Acalypha indica* against some human pathogenic bacteria and *Candida albicans*. Kasiram *et al.*, (2000) reported *in vitro* antifungal activity of *Calendula officinalis* flower extracts against *Aspergillus niger*, *Rhizopus japonicum*, *Candida albicans*, and *C. tropicalis*. Bhadauria and Kumar (2004) screened medicinal plants for their antibacterial activity in Jaipur. Similarly in 2009 they were reported bioactive nature of *Solanum dulcamara* (collected from Mountabu) against dermatophytes (Kumar and Bhadauria 2009).

MATERIALS AND METHODS

Plant and Fungal Material

The present study deals with the screening of different extracts of plant parts (roots, stems, leaves, flowers and fruits) of three medicinal plants for antidermatophytic activity. The selected medicinal plants were: *Allium sativum* Linn. (Liliaceae), *Cymbopogon martinii* (Roxb) Wats. (Gramineae) and *Catharanthus roseus* (Linn.) G. Don. (Apocynaceae). The plant parts of *Cymbopogon martinii* were collected from Mount Abu region of Rajasthan. Four dermatophytic fungi selected for antidermatophytic activity were: *Trichophyton rubrum* (Castellani) Sabouraud, *Trichophyton mentagrophytes* (Robin) Blanchard, *Microsporum gypseum* (Bodin) Guiart and Grigorakis and *Candida albicans* Berkhout. All the four cultures were clinical isolates, isolated from

skin scrapings of patients suffering from ringworm disease, dermatomycoses. Skin samples were collected from the 'Skin Out Patients Department' of Sawai Man Singh Hospital, Jaipur. The fungal cultures of *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum gypseum* and *Candida albicans* were isolated and maintained on Sabouraud's Dextrose Agar medium (Peptone 10 gm, Dextrose 20 gm, Agar 20 gm in one liter distilled water and pH adjusted to 7.5). The cultures were allowed to grow at 28–37°C. The inoculum used for screening studies was prepared in sterile distilled water from freshly grown agar slant (fungal culture) before every antifungal assay.

Extraction Procedure

Plant parts were collected and washed in running tap water to remove dust. All parts (stems, leaves, roots, flowers and fruits) of the plants were shade-dried and powdered separately for extraction. Each of the dried and powdered samples was soxhlet extracted with water and 80 per cent methanol for 24 hours on water bath. For the extraction of free and bound flavonoids, the filtrate of 80 per cent methanol was subsequently extracted with petroleum ether, ether and ethyl acetate. Petroleum ether fraction was discarded because it was rich in fatty substances; ether fraction was used for free flavonoids whereas ethyl acetate fraction was used for bound flavonoids. Ethyl acetate fraction of each of the samples was hydrolysed further with 7% H₂SO₄ for two hours and was re-extracted with ethyl acetate. The fraction was washed with distilled water to neutrality and dried.

Screening for Antidermatophytic Activity

The filter paper disc method (Gould and Bowie, 1952) was used for screening the extracts for antifungal activity. Rinsed Petri plates were sterilized in an oven at 120°C for three hours. Each of the sterilized plates was half filled with Sabouraud's Dextrose Agar medium. The thickness of the agar medium was kept equal in all the petriplates. Standard size Whatman filter paper discs (6.0 mm in diameter) were sterilized in an oven at 140°C for one hour, saturated with the extract (0.04 ml) and air-dried at room temperature to remove any residual solvent that might interfere with the determination. The discs were then placed on the surface of sterilized Sabouraud's Dextrose Agar Medium that had been inoculated with the test fungi. Each of the plates was homogenized to ensure uniform distribution of the inoculum and air-dried to remove surface moisture. Petri plates containing the paper discs (6 mm) dipped in ethyl ether, ethyl acetate and 80 per cent methanol and water were then run as control.

Different standard discs (Griseofulvin/Nystatin/Ketoconazole) for different fungi were used for determining the activity index of each extract. Before incubation, all the test and control petriplates were kept at 5°C for one hour to allow the diffusion of the substance from the disc into the agar medium plate. Plates were incubated at 37 °C for 48–72 hours, 24 hours in the case of *Candida albicans*, after which the zone of inhibition was measured. All the experiments were done in five replicates and the activity index was calculated for each of them using following formula:

$$AI = \frac{\text{Inhibition Zone of sample}}{\text{Inhibition Zone of standard}}$$

Reference Antibiotic Discs

The antibiotics known to be effective in their established doses against their respective test micro-organisms were used as reference compounds for comparison of the antifungal activity of the test samples. Griseofulvin was used for *Trichophyton rubrum* and *Microsporum gypseum*. Solutions of Griseofulvin (1000 µg/ml) and Ketoconazole (1000 µg/ml) for antifungal activity were prepared and impregnated in the filter paper disc. Nystatin (prepared disc) was used against *Candida albicans*.

RESULTS

Table 1-3 show the antidermatophytic activity of different water, methanolic and flavonoid extracts of three medicinal plants. The bound flavonoid extracts, as well as free flavonoid extracts, showed prominent antidermatophytic activity against all the dermatophytes used in the study. From the zones of inhibition produced by the extracts, it was observed that *Candida albicans* was the most resistant strain to water and methanolic extracts of all the medicinal plants used.

Antidermatophytic Activity of Standard Antibiotics Used

Antibiotics known to be effective against each of the test micro-organisms in their established doses were used as reference for comparison with the antidermatophytic activity of the test samples (Fig 1). These were Griseofulvin, Ketoconazole and Nystatin. Griseofulvin was used as a standard for *Trichophyton rubrum* and *Microsporum gypseum*. However, it showed negative response against *Candida albicans* and *T. mentagrophytes*. Inhibition zone of Griseofulvin (1000 µg/ml) was 25 mm for *T. rubrum* and 28 mm for *M. gypseum*. *T. mentagrophytes* and *C. albicans* were resistant to Griseofulvin at as high as 5000 µg/ml concentration. Ketoconazole was used as a standard for *T. mentagrophytes* and Nystatin was used for *C. albicans*. The inhibition zone of Ketoconazole was 35mm against

T. mentagrophytes and inhibition zone of Nystatin was 20mm against *Candida albicans*.

Antidermatophytic Activity of Different Extracts of Medicinal Plants

Allium sativum: Only bulb extracts were used for the antidermatophytic activity and free flavonoids showed the best results against all the four pathogenic fungi (Table 1 and Figure 1). Water extract inhibited the growth of *T. rubrum* only. Excellent efficacy of free flavonoids was recorded against *M. gypseum* and *T. mentagrophytes*, where inhibition zones were 35 and 28 mm showing results larger or nearly comparable to standards. Activity index of *M. gypseum* was 1.25 and for *T. mentagrophytes* it was 0.8. Inhibition zone against *T. rubrum* was 12 mm (AI = 0.48) and for *C. albicans* inhibition zone was 10 mm (AI = 0.5). Bound flavonoids showed a negative report against *T. mentagrophytes* and *C. albicans*.

Cymbopogon martini: Leaves, stems, flowers and roots were used for the present investigation. Data enumerated in Table 3E indicate that the leaves as well as the flowers exhibited prominent antifungal activity against all the pathogenic fungi used in the study.

Leaves: Free flavonoids showed negative activity against *Candida albicans*. For *T. rubrum* and *M. gypseum* inhibition zones were of 25 (AI = 1.0) and 28 mm (AI = 1.0) which was just equal to that of the standard. Inhibition zone of 20 mm and activity index of 0.57 was recorded for *T. mentagrophytes*. Bound flavonoids showed activity against all the four dermatophytes. The highest activity was measured in the case of *M. gypseum* (AI = 1.35) where the inhibition zone was 38 mm.

Stems: Free flavonoids showed good activity against *T. rubrum*, *T. mentagrophytes* and *M. gypseum* but were not active against *C. albicans*. Bound flavonoids reported the best activity against all test dermatophytes, except *C. albicans*. Bound flavonoids produced inhibition zone of 30 mm and activity index of 1.07 for *M. gypseum*. Inhibition zones of 20 and 22 mm were produced for *T. mentagrophytes* and *T. rubrum* and activity indices were 0.57 and 0.88, respectively.

Flowers: Water extract showed good activity against *M. gypseum* (AI = 0.71), while the zone of inhibition was 20 mm. Bound flavonoids showed good response against all the four fungal cultures. The maximum size of inhibition zones was produced for *M. gypseum*, that is, 30 mm and minimum size of zone (11 mm) was produced for *C. albicans*.

Roots: Water extract showed good efficacy against the four dermatophytic fungi. Free flavonoids showed

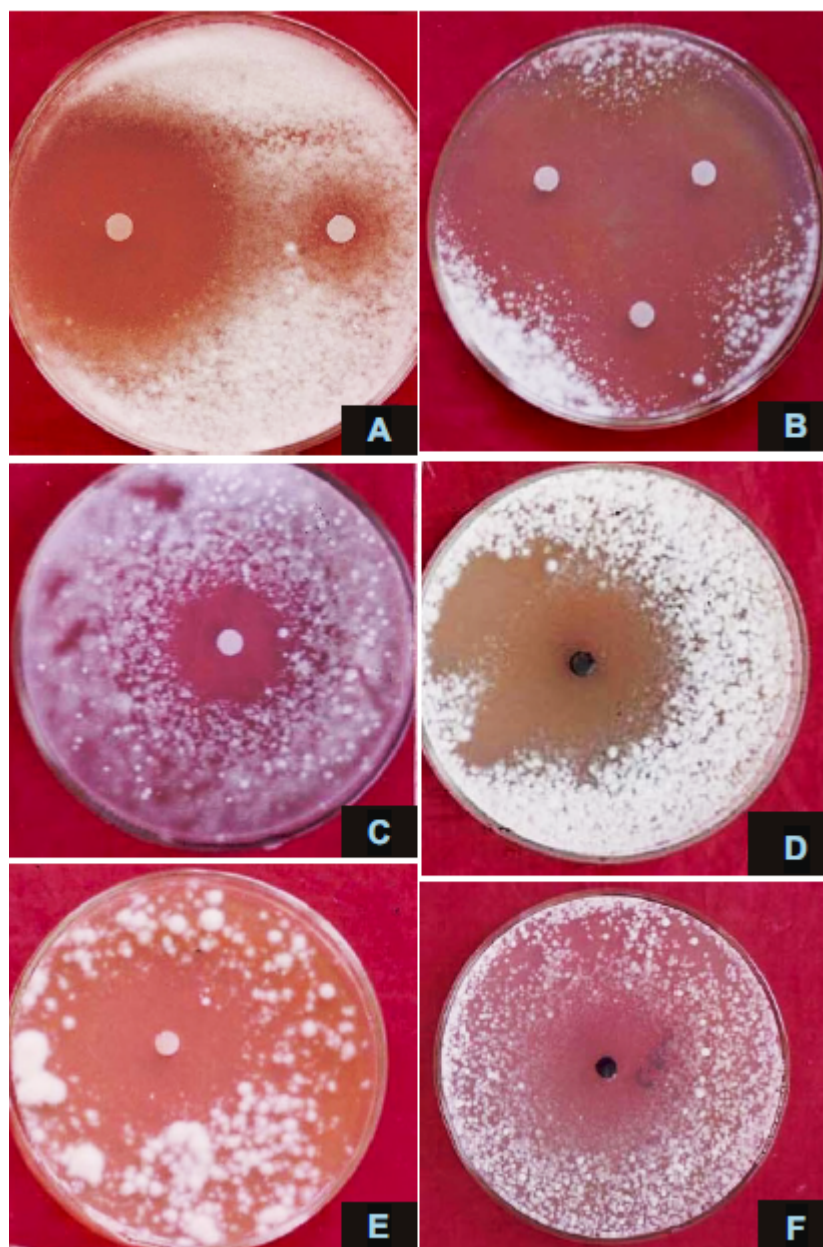


Fig. 1: Antidermatophytic Activity of Different Extracts of Medicinal Plants. A Petridish showing activity of Ketoconazole and Griseofulvin against *Trichophyton mentagrophytes*. B Activity of Free flavonoids extracted from *Allium sativum* against *M. gypseum*. C Activity of free flavonoids from *Cymbopogon martinii* against *Trichophyton rubrum*. D Activity of Bound flavonoids extracted from *Cymbopogon martinii* leaves against *M. gypseum*. E Activity of Bound flavonoids extracted from *Catharanthus roseus* against *Microsporum gypseum*. F Activity of bound flavonoids extracted from *Catharanthus roseus* leaves against *T. rubrum*.

negative efficacy against *T. mentagrophytes* and *C. albicans*. Free flavonoids were more effective for *M. gypseum* as compared to Griseofulvin. The maximum efficacy was shown against *M. gypseum* (AI = 1.07) where the zone of inhibition was 30 mm.

Catharanthus roseus: In the present study, leaves were most active for antidermatophytic activity (Table 3 and Fig. 1.)

Leaves: Bound flavonoids showed the best activity against all the four dermatophytes used in the present study. Inhibition zones of 40, 42 and 46 mm were recorded for *T. rubrum*, *T. mentagrophytes* and *M. gypseum*, respectively. All zones of inhibition were greater than reference antibiotics.

Stems: Free flavonoids were not found effective for any of the dermatophytes tested, but bound flavonoids showed good efficacy against all the four test fungi. Bound flavonoids were found to be strongly active against *T. rubrum* and *M. gypseum*, where inhibition zones of 25 and 28 mm equalled the zone of Griseofulvin (standard); activity index for both the fungi was 1.0.

Flowers: *M. gypseum* was the only fungi which were sensitive to water, methanolic and free flavonoid extracts of flowers. The rest of the three fungi were resistant to all these extracts. The maximum activity recorded for *M. gypseum* was 1.0 and inhibition zone of 28 mm. Inhibition zones of 15 and 22 mm were recorded against *T. rubrum* and *T. mentagrophytes*, where activity indices were 0.6 and 0.62, respectively.

Pods: Free flavonoids showed negative efficacy against *T. mentagrophytes* and *C. albicans*. Inhibition zones of 10 and 15 mm were recorded for *T. rubrum* and *M. gypseum*, respectively. Bound flavonoids showed negative response against *C. albicans*. The maximum inhibition zone was 25 mm for *M. gypseum*, followed by *T. mentagrophytes* (20 mm) and *T. rubrum* (14 mm). Activity indices of *M. gypseum*, *T. mentagrophytes* and *T. rubrum* were 0.89, 0.57 and 0.56, respectively.

DISCUSSION

A review of literature indicates that garlic was used as a folk medicine all over the world from ancient times. Mehrabian *et al.*, (1995) investigated the antimicrobial effect of *Allium sativum* against mouth flora. Shivpuri *et al.*, (1997) tested fungitoxic properties of ethanol extract of 10 plant species. Of these, *Allium cepa* and *A. sativum* showed good efficacy against five pathogenic fungi, namely, *Alternaria brassicola*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia*

sclerotiorum. In the present study, free and bound flavonoids showed good efficacy against all the pathogenic fungi used. Inhibition zones of 35 and 30 mm were produced by free and bound flavonoids against *Microsporum gypseum*, whereas inhibition zone of standard Griseofulvin was smaller (28 mm) than flavonoid extracts of *Allium sativum*.

Lachoria *et al.*, (2000) tested essential oils of six plants for their antimicrobial activity against some dermatophytes. Of these plants, *Cymbopogon citratus* was tested against four dermatophytes, namely, *Microsporum sp.*, *M. gypseum*, *Trichophyton rubrum* and *Epidermophyton floccosum*.

A review of literature indicates that much work has been done on essential oils of different species of *Cymbopogon* but no work has been reported for antidermatophytic activity of water, methanolic extracts and flavonoids of *Cymbopogon martinii*. In the present study, leaves and flowers showed prominent activity against all the dermatophytes used. Bound flavonoids of leaves showed inhibition zones of 28 and 38 mm for *T. mentagrophytes* and *M. gypseum*, which were larger or nearly equal to that of Ketoconazole (35 mm) and Griseofulvin (28 mm), respectively. Free flavonoids of leaves also showed good efficacy against *T. rubrum* (IZ = 22 mm) and *M. gypseum* (28 mm) (Table 3E).

Singh and Singh (1997) investigated methanolic extracts of leaves of *Catharanthus roseus* in different concentrations of 5 and 10 per cent for antifungal activity against dermatophytes and related keratinophilic fungi, as *Microsporum gypseum*, *Trichophyton simii* and *Malbranchea gypsea* or *Chrysosporium tropicum*. *C. tropicum* was most resistant to the extracts of *C. roseus*. Rai and Upadhyay (1998) tested medicinal plants of Chhindwara district in Madhya Pradesh for antidermatophytic activity against *Trichophyton mentagrophytes*, a causal agent of Tinea pedis. Extracts of leaves and stems of *Catharanthus roseus* showed good efficacy against *T. mentagrophytes*. Stem of *C. roseus* showed highest antifungal activity (77.72 %) of water extract, whereas water extract of leaves reported 40.12 per cent antifungal activity.

In the present work, leaf extracts of *Catharanthus roseus* were most active against all the dermatophytes used. *Candida albicans* was susceptible to only the extracts of bound flavonoids of leaves and stems, where the inhibition zones were 17 and 10 mm, respectively. Water and methanolic extracts of all plant parts were least effective against all fungi. According to previous workers (Uniyal *et al.*, 2006),

Table 1: Antidermatophytic Activity of Bulb Extracts of *Allium sativum*

Test Material		Test Fungi							
		<i>Trichophyton rubrum</i>		<i>Trichophyton mentagrophytes</i>		<i>Microsporum gypseum</i>		<i>Candida albicans</i>	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
a.	Water extract	22	0.88	-ve	-	-ve	-	-ve	-
b.	80% Methanolic extract	12	0.48	-ve	-	15	0.53	-ve	-
c.	Free flavonoids	12	0.48	28	0.8	35	1.25	10	0.5
d.	Bound flavonoids	13	0.52	-ve	-	30	1.07	-ve	-

IZ = Inhibition Zone including diameter (6mm) of Filter paper disc; AI = Activity Index

$$AI = \frac{\text{Inhibition Zone of sample}}{\text{Inhibition Zone of standard}}$$

Inhibition zone of standard (Griseofulvin) against <i>T. rubrum</i>	=	25mm
Inhibition zone of standard (Griseofulvin) against <i>T. mentagrophytes</i>	=	Nil
Inhibition zone of standard (Griseofulvin) against <i>M. gypseum</i>	=	28mm
Inhibition zone of standard (Griseofulvin) against <i>C. albicans</i>	=	nil
Inhibition zone of standard (Nystatin) against <i>C. albicans</i>	=	20mm
Inhibition zone of standard (Ketoconazole) against <i>T. mentagrophytes</i>	=	35mm

Table 2: Antidermatophytic Activity of Different Extracts of *Cymbopogon martinii*

Test Material		Test Fungi							
		<i>Trichophyton rubrum</i>		<i>Trichophyton mentagrophytes</i>		<i>Microsporum gypseum</i>		<i>Candida albicans</i>	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
[A]	Leaves								
	a. Water extract	-ve	-	-ve	-	12	0.42	-ve	-
	b. 80% Methanolic extract	9	0.36	-ve	-	10	0.35	-ve	-
	c. Free flavonoids	25	1.0	20	0.57	28	1.00	-ve	-
	d. Bound flavonoids	15	0.6	28	0.8	38	1.35	10	0.5
[B]	Stem								
	a. Water extract	-ve	-	-ve	-	10	0.35	-ve	-
	b. 80% Methanolic extract	-ve	-	-ve	-	-ve	-	-ve	-
	c. Free flavonoids	20	0.8	18	0.51	20	0.71	-ve	-
	d. Bound flavonoids	22	0.88	20	0.57	30	1.07	-ve	-
[C]	Flowers								
	a. Water extract	9	0.36	-ve	-	20	0.71	-ve	-
	b. 80% Methanolic extract	10	0.4	-ve	-	30	1.07	-ve	-
	c. Free flavonoids	25	1.0	-ve	-	28	1.0	9	0.45
	d. Bound flavonoids	18	0.72	15	0.42	30	1.07	11	0.55
[D] Roots									
	a. Water extract	20	0.8	12	0.34	14	0.5	12	0.6
	b. 80% Methanolic extract	-ve	-	-ve	-	25	0.89	-ve	-
	c. Free flavonoids	10	0.4	-ve	-	30	1.07	-ve	-
	d. Bound flavonoids	12	0.48	13	37	30	1.07	-ve	-

IZ = Inhibition Zone including diameter (6mm) of Filter paper disc; AI = Activity Index

Inhibition zone of standard (Griseofulvin) against *T. rubrum* = 25mm; Inhibition zone of standard (Griseofulvin) against *T. mentagrophytes* = Nil; Inhibition zone of standard (Griseofulvin) against *M. gypseum* = 28mm; Inhibition zone of standard (Griseofulvin) against *C. albicans* = Nil; Inhibition zone of standard (Nystatin) against *C. albicans* = 20mm; and Inhibition zone of standard (Ketoconazole) against *T. mentagrophytes* = 35mm.

Table 3: Antidermatophytic Activity of Different Extracts of *Catharanthus roseus*

Test Material		Test Fungi							
		<i>Trichophyton rubrum</i>		<i>Trichophyton mentagrophytes</i>		<i>Microsporum gypseum</i>		<i>Candida albicans</i>	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
[A] Leaves									
a.	Water extract	-ve	-	15	0.42	-ve	-	-ve	-
b.	80% Methanolic extract	-ve	-	-ve	-	10	0.35	-ve	-
c.	Free flavonoids	13	0.52	12	0.34	12	0.42	-ve	-
d.	Bound flavonoids	40	1.6	42	1.2	46	1.64	17	0.85
[B] Stem									
a.	Water extract	-ve	-	-ve	-	15	0.53	-ve	-
b.	80% Methanolic extract	-ve	-	-ve	-	10	0.35	-ve	-
c.	Free flavonoids	-ve	-	-ve	-	-ve	-	-ve	-
d.	Bound flavonoids	25	1.0	10	0.28	28	1.00	10	0.5
[C] Flowers									
a.	Water extract	-ve	-	-ve	-	10	0.35	-ve	-
b.	80% Methanolic extract	-ve	-	-ve	-	15	0.53	-ve	-
c.	Free flavonoids	-ve	-	-ve	-	22	0.78	-ve	-
d.	Bound flavonoids	15	0.6	22	0.62	28	1.00	-ve	-
[D] Pods									
a.	Water extract	-ve	-	-	-	11	0.39	-ve	-
b.	80% Methanolic extract	-ve	-	-	-	12	0.42	-ve	-
c.	Free flavonoids	10	0.4	0.4	-	15	0.53	-ve	-
d.	Bound flavonoids	14	0.56	0.56	0.57	25	0.89	-ve	-

Inhibition zone of standard (Griseofulvin) against *T. rubrum* = 25mm; Inhibition zone of standard (Griseofulvin) against *T. mentagrophytes* = Nil; Inhibition zone of standard (Griseofulvin) against *M. gypseum* = 28mm; Inhibition zone of standard (Griseofulvin) against *C. albicans* = Nil; Inhibition zone of standard (Nystatin) against *C. albicans* = 20mm and Inhibition zone of standard (Ketoconazole) against *T. mentagrophytes* = 35mm

water and methanolic extracts of *Catharanthus roseus* showed good antimicrobial activity but none of the earlier workers reported activity of flavonoids for dermatophytes. Among dermatophytic fungi, much work has been done on *Candida albicans* by previous workers. They reported *C. albicans* as very resistant fungi. The present study is also in agreement with their works.

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