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Antifungal activity of *Pterocarpus santalinus* an *in vitro* study

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ABSTRACT

In the present study leaves of *Pterocarpus santalinus* L.f (Fabaceae) were exhaustively extracted with different solvents like hexane, ethyl acetate and methanol in ascending order of polarity. All the three extract was subjected to antifungal screening and phytochemical analysis. The ethyl acetate extract showed significant MIC values against *Trichophyton rubrum* (62.5 µg/ml), *Trichophyton mentagrophytes* (500 µg/ml), *Trichophyton simmi* (125 µg/ml), *Epidermophyton floccosum* (500 µg/ml) and *Scropulariopsis sp* (500 µg/ml). Phytochemical screening revealed the presence of terpenoids, steroids, flavonoids, alkaloids, tannins and carbohydrates. The present study validates the traditional use of the medicinal plant and indicates that it can be effective potential candidate for the development of new antifungal drug to treat fungal infections.

Key words: antifungal, dermatophytes, *Pterocarpus santalinus*, minimum inhibitory concentration

INTRODUCTION

Microbial infections pose a health problem throughout the world with the alarming increase in the rate of infection by antibiotic resistant microorganism (Davies, 1994). The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern. In recent years, there has been renewed interest in the treatment against different diseases as herbal drugs are generally known to be non toxic (Rao, 2001). The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Perumalsamy *et al.,* 1999), and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi. *Pterocarpus santalinus* L.f (Red sanders) belongs to the family Fabaceae and is restricted to part of Andrapradesh. Traditionally it has been used in treatment of headache, skin diseases, fever, boils, and scorpion-sting and to improve sight (Chopra *et al.,* 1956). Previous chemical constituents revealed the presence of triterpene, isoflavone glucosides, savinin and calocedrin (Krishnaveni *et al.,* 2000). In this study we focused on the *in vitro* screening of antifungal activity of hexane, ethyl acetate and methanol extracts of *Pterocarpus santalinus* against dermatophytes and opportunistic pathogens.

MATERIAL AND METHODS

Plant collection and Extraction

Healthy, disease free mature leaves of *Pterocarpus santalinus* were collected from Andrapradesh, India a specimen was deposited at the department herbarium, St. Joseph's College, Trichy. Collected materials were washed thoroughly, shade dried in open air and grounded into powder. The powder was extracted by maceration in hexane (6.2 g) during 72 hr. Residuals were further extracted with ethyl acetate (46.2 g) and methanol (35.6 g) following the same procedure. The plant extracts were concentrated using rotary flash evaporator and preserved at 4^0C in air tight bottle until assay.

Fungal strains

The following fungi were used for experiments: *Trichophyton rubrum* MTCC 296, *T. mentagrophytes* 66/01, *T. simii* 110/02, *Epidermophyton floccosum* 73/01, *Scopulariopsis* sp. 101/01 *Aspergillus niger* MTCC 1344, *Botyritis cinerea, Curvularia lunata* 46/01, *Magnaporthe grisea* and *Candida albicans* MTCC 227.

Preparation of fungal spore

The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on Sabouraud Dextrose Broth (SDB) at 28°C for 48 h.

Antifungal assays

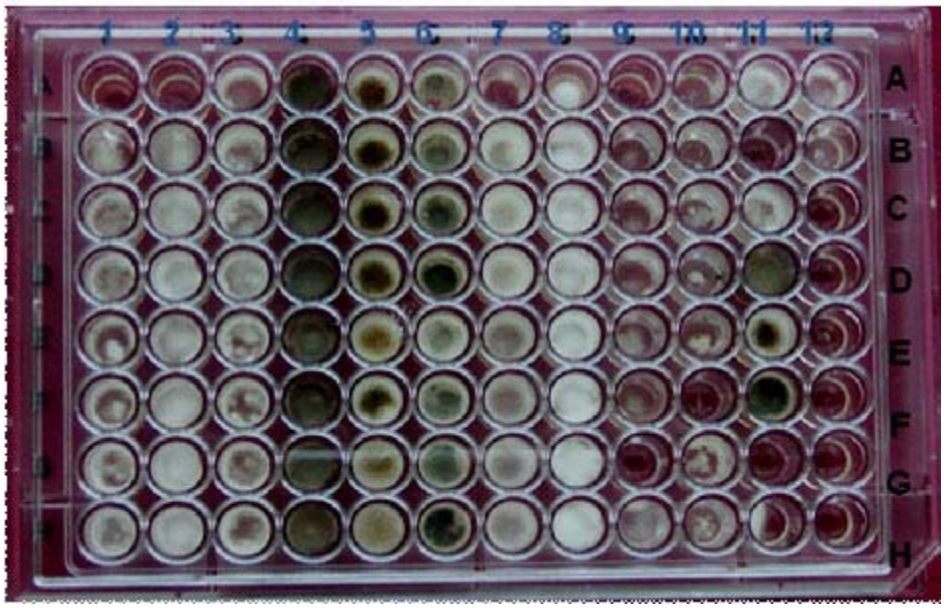
The antifungal activity was performed according to the standard reference method (NCCLS, 2002). The extracts were dissolved in 2% dimethyl sulfoxide (DMSO). The initial concentration of extract was 1mg/ml. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 µl of suspension containing 10^4 spore/ml of fungi. The antifungal agent Fluconazole was included in the assays as positive controls; the plates were incubated for 24h up to 9 days at 27°C for dermatophytes strains. MIC was defined as the lowest extract concentration, showing no visible fungal growth after incubation time.

Phytochemical Screening

Preliminary phytochemical screening was carried out by using standard procedures (Edeoga *et al.,* 2005).

RESULTS AND DISCUSSION

Results of antifungal activity are summarized in Table-1. From this evaluation we found that ethyl acetate extract inhibited large



A1 to H1	-	*Trichophyton mentagrophytes*	A2 to H2	-	*Epidermophyton floccosum*
A3 to H3	-	*Trichophyton simii*	A4 to H4	-	*Curvularia lunata*
A5 to H5	-	*Aspergillus niger*	A6 to H6	-	*Botrytis cinerea*
A7 to H7	-	*Trichophyton rubrum*	A8 to H8	-	*Magnaporthe grisea*
A9 to H9	-	*Scropulariopsis sp*	A10 to H10	-	*Candida albicans*.

11A to 11H, 12A to 12B - Control (culture and Broth only)

12C to 12 H - Blank

Fig. 1: Antifungal activity of *Pterocarpus santalinus*

Table 1: Minimum Inhibitory Concentration (µg/ml) of ethyl acetate extracts of *Pterocarpus santalinus*

	Extract	T.m	E.f	T.s	C.l	A.n	B.c	Tr 296	Mg sp.	Scro	C.a
Flu		<12.5	<12.5	<12.5	<12.5	<12.5	NT	<12.5	NT	<12.5	<12.5
Pterocarpus	He	-	500	-	-	-	-	-	1000	-	-
santalinus	Ea	500	500	125	1000	-	-	62.5	1000	500	-
Linn (Leaf)	Me	-	500	-	-	-	-	-	1000	-	-

He – Hexane; Ea – Ethyl acetate; Me – Methanol, Flu – Flucanozole (Standard), NT – Not test

T.m - *Trichophyton mentagrophytes;* E.f – *Epidermophyton floccosum*; T.s - *T. simii; C. l – Curvularia lunata;* A.n - *Aspergillus niger;* B.c – *Botrytis cinerea; T.r – rubrum 296;* Mg. – *Magnaporthe grisea*; Scro – *Scropulariopsis sp*; *C.a- Candida albicans*.

Table 2: Preliminary phytochemical analysis of *Pterocarpus santalinus*

Extracts	Terpeonids	Steroids	Flavonoids	Alkaloids	Carbohydrates	Tannins	Saponin
Hexane	+	+	+	+	+	-	-
Ethyl acetate	+	+	+	+	+	+	-
Methanol	+	+	-	-	+	-	-

+ Positive, - negative

number of fungal growth. Hexane extracts also nearly showed the same level of inhibition against fungal growth. Methanol extract is highly in-active. Ethyl acetate extract of *P. santalinus* leaves showed activity against tested fungi namely (Fig. 1), *T. rubrum* 296 (62.5 µg/ml), *T. mentagrophytes* (500 µg/ml), *T. simii* (125 µg/ml), *E. floccosum* (500 µg/ml), *C. lunata* (1000 µg/ml) M. *grisea* (1000 µg/ml) and *Scopulariopsis sp* (500 µg/ml). The phytochemical analysis of ethyl acetate extract had showed the presence of flavonoids, terpenoids, steroids, alkaloids, tannins and Carbohydrates (Table 2)**.** In the present study *T. mentagrophytes*, *Epidermophyton floccosum*, *Trichophyton rubrum, Trichophyton simii, Magnaporthe grisea* were found to be the most sensitive fungal strains. *T. mentagrophytes*, *T. simii* and *T. rubrum* is pathogenic fungi; the dermatophytes have the ability to invade keratinized tissues of animals and humans and cause a disease. *Trichophyton rubrum* is the main agent isolated in superficial mycosis, corresponding to almost 60% of all clinical cases in Brazil (Esquenazi *et al.,* 2004). The basis of varying degree of sensitivity of test organisms of fungi may be due to the intrinsic tolerance of microorganisms, the nature and combinations of phytocompounds present in the crude extract. In previous findings flavonoids were found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extra cellular and soluble protein and to complex with bacterial cell wall: more lipophilic flavonoids may also disrupt microbial membrane (Tsuchiya *et al.,* 1996).

CONCLUSION

In conclusion all these findings raised some interesting exceptions about antifungal activity of this plant extract. Identification and elucidation of the active constituent in this plant may provide useful lead to the development of new and effective drugs.

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