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Effect of antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse)

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ABSTRACT

The antifungal activity of five different medicinal plants namely Lawsonia inermis L, Mimosa pudica L, Phyllanthus niruri L., Tephrosia purpurea Pens., Vinca rosea L. were tested against plant pathogenic fungi Pythium debaryanum (causing damping off of disease) by agar well diffusion method. The plant leaves were extracted with various solvents like n-butanol, methanol, aqueous. Among the different plant tested, all the three solvents, the methanolic extracts of Lawsonia inermis showed maximum antifungal activity against Pythium debaryanum.

Key words: Antifungal, Damping off, *Pythium*, Medicinal plants.

INTRODUCTION

Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer (or) modern antibiotics that have been produced in the last three decades (Cohen, 1992; Nascimento *et al.*, 2000). Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (Shariff, 2001). Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Nascimento *et al.*, 2000; Rios and Recio, 2005). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in small quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal *et al.*,

2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin *et al.*, 1985).

The genus *Pythium* is a complex genus containing over 200 described species that occupy a variety of terrestrial and aquatic ecological habitats (Dick, 2001). Perhaps the most economically important members of this genus are plant pathogens (Hendrix and compbell, 1973), many of which have a broad host range and cause losses by both pre and post emergence damping off (Erwin and Ribeiro, 1996), as well as by reduction in plant growth and yield due to root rot (Plaats – Niterink, 1918).

Considering the vast potentiality of plant as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antifungal activity from *Lawsonia inermis, Mimosa pudica, Phyllanthus niruri, Tephrosia purpurea* and *Vinca rosea*.

MATERIALS AND METHODS

Plant Collection

The plants were collected from the non-irrigated cultivated lands in and around Thanjavur (Dt.), Tamilnadu. Medicinal plants species such as *Lawsonia inermis, Mimosa pudica, Phyllanthus niruri, Tephrosia purpurea, Vinca rosea* were taken for the antifungal study.

Sterilization of Plant Materials

The disease free and fresh plants were selected. About 2g of fresh and healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water (three times).

Preparation of plant extracts

Two grams of sterilized plant leaves were kept in the 10 ml organic solvents such as n-butanol, methanol and aqueous. Then they were ground well with the help of Mortar and Pestle. The plant materials were subjected to centrifugation, for 10-15 min (at 10000 rpm) again it was filtered through Whatman No. 1 filter paper. The supernatant was collected and made to known volume, by adding sterile n-butanol, methanol and aqueous stored for further antimicrobial screening purpose.

Microbial cultures and Growth conditions

The plant extracts were assayed for antifungal activity against the fungal strain *Pythium debaryanum* isolated from chilli field soil. This fungus was grown on PDA plate at 28°C and maintained with Periodic sub-culturing at 4°C.

Potato Dextrose Agar (PDA) Medium (pH 6.7)

Potato	-	250g
Dextrose	-	15g
Agar	-	18g
Distilled water	-	1000ml

The potato tubers were peeled off and weighed for about 250 g tubers were chopped into small pieces into the sterile conical flask. After boiling the supernatant were collected and dextrose (15g) with agar (18g) to dissolve the ingredients. The pH of the medium was adjusted to 6.5. Finally the medium was sterilized in pressure cooker for 20 min.

Screening for antifungal assay

Antifungal activity test

Antifungal activity was screened by agar well diffusion method (Perez *et al.,* 1990). The n-butanol, methanol and aqueous extracts of five different medicinal plants were tested against plant pathogen *Pythium debaryanum*. The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 200µl of each extracts were transferred into the separate wells. The plates were incubated at 27°C for 48 – 72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the well indicated the presence of antifungal activity. The zone of inhibition was recorded.

RESULTS AND DISCUSSION

Effect of antifungal activity of some medicinal plants against *P. debaryanum*

Antifungal activity of five medicinal plants extract was assayed by agar well diffusion method. The result revealed that the extract of five medicinal plants showed significant reduction in growth of *P. debaryanum.*

Among all the five plants extract the n-butanol and methanol extract of *Lawsonia inermis* exhibited maximum antifungal activity (15 and 20 mm) followed by *Phyllanthus niruri* (15 and 20 mm), *Tephrosia purpurea* (10 and 15 mm) *P. debaryanum*. The methanolic extract of *Mimosa pudica* (20 mm), *Vinca rosea* (10 mm) exhibited least activity against *P. debaryanum*. The results of antifungal effect of aqueous extract of all tested five plants showed no activity against *P. debaryanum*. (Table1)

The methanol leaf extracts of various medicinal plants showed significant antibacterial and antifungal activity against *Aspergillus flavus, Dreschlera turcica* and *Fusarium verticillioides* have been reported (Mahesh and Satish, 2008).

Methanolic extracts of *Tephrosia purpurea* showed excellent antibacterial and antiviral activity (Kokila *et al.,* 2010). Extracts from fruits of *Schisandra chinensis* separated into n-butanol and diethyl ether showed antagonistic effects on *Alternaria alata* (Kim *et al.,* 1996). Methanolic extracts of root and shoots of the herb *Heracleum candicans* wall (Apiaceae), showed antifungal effect against *Pythium* and *Aspergillus* species only. Aqueous and chloroform shoot extracts and aqueous root extract did not show any antifungal effect against the *Alternaria, Aspergillus, Fusarium, Penicillium, Phytophthora* and *Pythium* have also been reported (Mohinder Kaur *et al.,* 2006).

Table 1 Effect of Antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse)

S. No.	Medicinal Plants	Zone of Inhibition (mm)		
		n-butanol	Methanol	Aqueous
1.	*Lawsonia inermis*	15	25	-
2.	*Mimosa pudica*	-	20	-
3.	*Phyllanthus niruri*	15	20	-
4.	*Tephrosia purpurea*	10	15	-
5.	*Vinca rosea*	-	10	-

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