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### Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum*

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#### ABSTRACT

The antifungal activity of eight different medicinal plants namely *Aloe vera*, *Ocimum sanctum*, *Cenetella asiatica*, *Piper betle*, *Calotropis gigantea*, *Vitex negundo*, *Ocimum basilicum* and *Azadirachta indica* were tested against plant pathogenic fungus (red rot disease causing agent) *Colletotrichum falcatum* by agar well –diffusion method. The plants leaves were extracted with various solvents like chloroform, ethanol and aqueous. Among the different plant tested, all the three solvents of the *Vitex negundo* showed maximum antifungal activity (25 mm) against the plant pathogen tested. Whereas the other plant extracts were showed moderate to minimum antifungal activity.

**Key Words:** Medicinal plants, Antifungal activity, *Colletotrichum falcatum*, Red rot disease.

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#### INTRODUCTION

Synthetic fungicides are currently used as primary means for the control of plant disease. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicide among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee *et al.*, 2007). In agriculture, the crop loss due to plant pathogens has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases. Crude extracts of some well known medicinal plants are used to control some of the plants pathogens (Kubo *et al.*, 1981).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs has been isolated from natural resources. Traditional medicine is an important source of potentially useful are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and large number of diverse types of plants grow in different parts of the country. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al.*, 1998). The effects of plant extracts on bacteria have been studied part of the world (Reddy *et al.*, 2001; Ateb and Erdoural, 2003). Much work has been done on ethno medicinal plants in india (Maheswari *et al.*, 1986; Negi *et al.*, 1993). Interest in a large number of traditional natural product has increased (Taylor *et al.*, 1996; Negi *et al.*, 1993). Medicinal plants were used as excellent antimicrobial agents because it posses a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of medical agents.

Red rot, caused by *C.falcatum* Went, is an important disease of sugarcane (interspecific hybrids of *saccharum-L*) which can cause severe losses in sucrose yield in many cane growing areas of the world. Sugarcane is a vegetatively propagated crop. Fall planting is needed to obtain adequate stalk maturity and sucrose yield the following growing reason. The planting season extends from august through early October. To ensure an adequate shoot population the following spring, whole stalks are planted at a rate of approximately 4 to 8 stalks running in the planting furrow. Keep the above facts in mind, the present study deals with the screening of antifungal activity of some medicinal plants against *C. falcatum* and extraction of the antimicrobial compounds by using various solvents.

## MATERIALS AND METHODS

### Plant collection

The plants were collected from the non-irrigated cultivated lands in and around Thanjavur (Dt), Tamil Nadu. Medicinal plants species such as *Aloe vera*, *Ocimum sanctum*, *Cenetella asiatica*, *Piper betle*, *Calotropis gigantea*, *Vitex negundo*, *Ocimum basilicum* and *Azadirachta indica* were collected from Sri Gowri Biotech Research Academy (SGBRA), Thanjavur, Tamilnadu for the study.

### Sterilization of Plant Materials

The disease free and fresh plants were selected. About 2g of fresh and health 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 10ml organic solvents such as methanol, ethanol 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 aqueous, methanol and ethanol stored for further antimicrobial screening purpose.

### Microbial Cultures and Growth Conditions

The plant extracts were assayed for antifungal activity against the fungal strain *C.falcatum* obtained from sugarcane breeding institute, Coimbatore. 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 250g tubers were chopped in to small pieces in to the sterile conical flask. After boiling the supernatant were collected and dextrose (15g) with agar (18g) to dissolve the ingredients. The medium was mentioned and adjusted to 6.5pH. Finally the medium was sterilized in pressure cooker for 20min.

### Screening for antifungal assay

#### Antifungal activity test

Antifungal activity was screened by agar well diffusion method (Perez *et al.*, 1990). The methanol, ethanol and aqueous extracts of eight different plants were tested against plant pathogen *C.falcatum*. The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spreaded 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 in to 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 calculated.

## RESULTS AND DISCUSSION

### Effect of antifungal activity of some medicinal plants against *Colletotrichum falcatum*

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

### Inhibition Spectrum of the Medicinal Plants against *Colletotrichum falcatum*

S. No.	Name of the Medicinal plants	Zone of inhibition (mm)		
		Chloroform	Ethanol	Aqueous
1.	<i>Aloe vera</i>	-	10	-
2.	<i>Azadirachta indica</i>	5	5	-
3.	<i>Calotropis gigantea</i>	23	24	-
4.	<i>Centella asiatica</i>	20	25	-
5.	<i>Ocimum basilicum</i>	15	20	-
6.	<i>Ocimum sanctum</i>	20	20	-
7.	<i>Piper betle</i>	19	25	-
8.	<i>Vitex negundo</i>	25	25	-

Among all the eight plants extract the chloroform and ethanol extract of *Vitex negundo* exhibited maximum antifungal activity (25 and 25 mm) compared with other plant extract. The ethanol extract of *Calotropis gigantea* (24 mm) and *Centella asiatica* (25 mm) showed prominent

antifungal activity against *C. falcatum*. The chloroform and ethanol extract of *Ocimum sanctum* (20 and 20 mm), *Piper betle* (19 and 25 mm) showed moderate activity against *C. falcatum*. The ethanol extracts of *Aloe vera* (10 mm) and *Azadirachta indica* (5 mm) exhibited least activity against *C. falcatum*. The results of antifungal effect of aqueous extract of all tested eight plants showed no activity against *C. falcatum*.

Evidently Mathur *et al.*, (2011) reported that hydro-alcohol extract of *Valeriana jatamansi*, *Coleus barbatus*, *Berberis aristata*, *Asparagus racemosus*, *Andrographis paniculata*, *Achyranthes aspera*, *Tinospora cordifolia*, *Plantago depressa* showed maximum antifungal activity against *aspergillus niger* and *candida albicans*. Similarly Sule *et al.*, (2010) evaluated the antifungal activity of *Senna alata linn*. Crude leaf extract exhibited moderate activity against *Microsporum canis*, *Trichophyton jirrucosum*, *Trichophyton mentagrophytes* and *Epidermophyton jlorrcosum*. Abera *et al.*, (2011) reported that the antifungal potential of aqueous and ethanol extracts of eight different plants species *invitro* and *invivo* against *Colletotrichum kahawae* in completely randomized design with three replication. The extracts were from *Hogenia abyssinica*, *Allium sativum*, *Phytolacca dodcandera*, *Croton macrostachyus*, *Maesa lanceolata*, *Eucalyptus globules*, *Eucalyptus citriodera* and *Lippia adoensis*. The study indicated that the inhibitory effect of the extracts depended on the type of plant species used, method of extraction and time of application of the extracts. *A.sativum* reduced radial growth of the pathogen in ethanol an aqueous extracts by 83 and 100%, respectively and *C. macrostchys* by 68 and 88%, respectively. In the present study revealed that the ethanol and chloroform extract of *Vitex negundo*, showed maximum activity. Therefore, this study suggests that *Vitex negundo* plant extract would be helpful in treating in sugarcane field (Red Rot disease Caused by *C.falcatum*). To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant disease the interest on plant extracts has been increased. This study provides new scientific information on antifungal activity of *Vitex negundo* against *C. falcatum*. The extracts should be tested against the disease under the disease field conditions.

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