

Biological activities of some medicinal plants against *Setosphaeria rostrata* causing seedling blight disease in sugarcane

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ABSTRACT

The antifungal properties of the leaf extracts of Pandanus odoratissimum, Cedrus deodara, Capparis Zeylanica, Mirabilis jalapa, Eicchornia crassipes, Gaedenia jasminoides, Spermacoce hispida, Nymphaea nouchali, Melothria heterophylla, Enicostemma littorale, against Setosphaeria rostrata isolated from sugarcane fields. The pathogen Setosphaeria rostrata commonly causing the seedling blight disease in sugar cane. The antifungal assay was determined by well diffusion method. Three different solvents such as methanol, ethanol and aqueous were used. Among the three solvents methanolic extracts of all the treated plant leaves showed maximum significant inhibitory activity followed by ethanolic extract. There was no activity found with aqueous extract of all the plants tested.

Key Words: Antifungal activity, Medicinal plants, *Setosphaeria rostrata*, Seedling blight diseases.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (mann et al., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. 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 by a very large number of researchers in different part of the world (Reddy et al., 2001; Ateb and Erdoural, 2003). Much work has been done on ethno medicinal plants in india (Maheshwari 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 possesses a variety of chemical constituents. In nature, recently much attention has been 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 sources of medical agents.

MATERIALS AND METHODS

Plant collection

The plants were collected from the non-irrigated cultivated lands in and around Thanjavur (Dt), Tamil Nadu. Medicinal plant species such as *Pandanus odoratissimus*, *Cedrus deodara*, *Capparis zeylanica*, *Mirabilis jalapa*, *Eichhornia crassipes*, *Gardenia jasminoides*, *Spermacoce hispida*, *Nymphaea noucholi*, *Melothria heterophylla*, and *Enicostemma littorale* were collected from Sri Gowri Biotech Research Academy (SGBRA), Thanjavur, Tamil Nadu for the 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 10ml organic solvents such as Methanol, Ethanol and aqueous. Then they were ground with the help of mortar and pestle. The plant material was 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 distilled water and stored for further antimicrobial screening purposes.

Microbial cultures and growth conditions

The plant extracts were assayed for antifungal activity against the fungal strain *Setosphaeria rostrata* (F2149) obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. 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) (g/l)

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

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

Screening for antimicrobial assay

Antifungal Activity Test

The methanolic extracts of ten different plant extracts were screened for antifungal activity by agar well diffusion method (Perez et al., 1990) with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of Fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molton. PDA medium was poured in to a petri dish by pour plate technique. After solidifaction, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of ten different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24-28 hours at 28^oc was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experimen was carried out in triplicates.

Minimum out inhibitory concentration (MIC) assay

Based on the preliminary screening methanol and Ethanol extracts revealed potent antimicrobial activity. The Minimum Inhibitory concentration (MIC) of the extracts were determined according to Elizabeth et al., (1999). A final concentration of 0.5% (v/v) Tween-20 (Sigma) was used to enhance crude extract solubility. A serious of two fold dilution of each extract, ranging from 0.2 to 100mg/ml was prepared. After sterilization, the medium was inoculated with 3 μ l aliquots of culture containing approximately 105 CFU/ml of each organism of 24 hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After the media solidified a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. Different plant crude extracts ranging from 0.2 to 100mg/ml were added to the wells of each petri dish and the control plates without extract. Inhibition of organism growth in the plates containing test crude extracts was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate. Similarly the MICs of methanolic extracts were determined against all other microorganisms. The results were given in (Table 1).

RESULTS AND DISCUSSION

Antifungal activity of ten botanical extracts was assayed and the effect of plant extracts on the growth of *S.rostrata* was observed. The data revealed that significant reduction in growth of *S.rostrata* was observed with extracts of ten medicinal plants and the extracts showed significant differences in their efficacy. Among all the ten plant methanolic extracts, 100% plants showed inhibition of mycelial growth of *S.rostrata* over control and three plants *Cedrus deodara*, *Spermacoce hispida*, *Melothria heterophylla*, showed exceptionally prominent activity.

Evidently Mathur *et al.*, (2011) reported that hydro-alcohol extract of *Valeriana jatamanasi*, *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 jlrrcosum*.

Table -1. Inhibition spectrum of the medical plants against *Setosphaeria rostrata*

Name of plant	Zone of Inhibition (Diameter in mm) <i>Setosphaeria rostrata</i> solvents		
	Methanol	Ethanol	Aqueous
1. <i>Pandanus odoratissimum</i>	11	0.5	-
2. <i>Cedrus deodara</i>	21	16	-
3. <i>Capparis zeylanica</i>	12.5	15	-
4. <i>Mirabilis jalapa</i>	10	10	-
5. <i>Eichhornia crassipes</i>	20	5	-
6. <i>Gardenia jasminoides</i>	15	-	-
7. <i>Spermacoce hispida</i>	21	5	-
8. <i>Nymphaea nouchali</i>	19	-	-
9. <i>Melothria heterophylla</i>	21	5	-
10. <i>Encostemma littorale</i>	15	10	-

The extract of plant *Cedrus deodara* showed maximum activity even at lower concentrations. There fore, this study suggests that methanolic extracts of screened plants would be helpful in treating diseases in plants (Caused by *S.rostrata*). The control plate representing DMSO did not exhibit inhibition on the tested fungi where as standard antifungal drug Bavistin have antifungal activity even at 5µg/well. In particular, the authors may recommend that the methanolic extract of *Cedrus deodara* to be used as potent biocide to treat diseases in plants caused by *S.rostrata* as it showed maximum activity even at lower concentrations nearly equal to the standard antifungal agent. It revealed that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. It also supports the earlier investigation (Banso and Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Extensive bioprocess parameter studies should be undertaken for the methanolic extract of *Cedrus deodara* as a strong antifungal agent against *S.rostrata* causing plant diseases.

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