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Antifungal activity of *Polygonum minus* Huds. against plant pathogenic fungi

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Abstract

Methanol, chloroform and petroleum ether extracts of *Polygonum minus* Huds. were tested against the plant pathogenic fungi such as *Alternaria helianthi, Alternaria solani, Aspergillus flavus, Aspergillus niger, Colletotrichum musae, Fusarium oxysporum* fsp *lycopersici, Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii.* The methanol and chloroform extract of *P. minus* exhibited activity against *Alternaria solani*, *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* fsp *lycopersici.* Chloroform extract of *P. minus* inhibited these pathogens with the Minimum Inhibitory Concentration of 10mg/ml. Thin layer chromatographic studies revealed the presence of terpenoids in the chloroform extract of *P. minus.*

Keywords : antimicrobial, MIC, plant pathogens, *Polygonum minus*, terpenoids.

INTRODUCTION

Plants have provided man with all his needs in terms of shelter, clothing, food, flavours, fragrances and not the least, medicines. Much of the wealth of a country resides in its plant inheritance, whether the plants are endemic, naturalized or recent introductions (Mitchell and Ahmad, 2005). India has an extensive area of forest enriched with plant diversity. Several of these plants have been used as folklore medicines. In India, about 7300 plant species are used in traditional health care systems such as Ayurveda, Siddha, Unani and folk healing practices. The booming of traditional medicine industry results in an increasing demand on medicinal plant products. Nearly 90 % of the medicinal plants come from natural habitats. Since ancient times, plants have been an exemplary source of medicine.

In agriculture, the crop loss due to plant pathogens has become a 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 plant pathogens (Kubo *et al.*, 1981; Geyid *et al.*, 2005). During the past few years, there is a growing trend all over the globe to shift from synthetic to natural products including medicinal plants. It is high time now to consider the neglected and little known botanicals to cure the plant diseases, which create challenging problems in agriculture and pose real economic and environmental threats. This paper deals with the antifungal activity of *Polygonum minus* Huds. against the plant pathogenic fungi, viz., *Alternaria helianthi, Alternaria solani, Aspergillus flavus, Aspergillus niger, Colletotrichum musae, Fusarium oxysporum* fsp *lycopersici, Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii.*

MATERIALS AND METHODS

Plant sample and preparation of plant extracts

The plant sample (*Polygonum minus* Huds.) was collected from Aliyar forest, Tamil Nadu, India. Dried and powdered leaves of *P. minus* were extracted by percolation with 80% methanol (polar solvent), chloroform (medium polar solvent) and petroleum ether (non polar solvent) at the rate of 1:5 at room temperature for overnight (Geyid *et al.*, 2005). The extracts were then filtered with country filter paper and concentrated under vacuum in rotary evaporator to give (as a percentage of powdered plant materials) 6-11% gummy residue. All the extracts were kept in tightly stoppard bottle in a refrigerator until used for the anti-microbial testing.

Plant pathogens

The plant pathogens (bacteria and fungi) used in the study was obtained from the Department of Plant Pathology, TNAU, Coimbatore. The pathogens used for the study were maintained in respective slants under refrigerated condition (4°C). The cultures were frequently subcultures in fresh slants and stored for further study.

Agar well diffusion assay

The sterilized PDA medium was poured into the petriplates and allowed to solidify. Then each Petri plate

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was divided into four equal quarters using a marker pen. Using a sterile cork borer, wells of 6 mm in diameter were made in each quadrat of the plate containing the media. For each organism, 20µl of the prepared plant sample was loaded in each well using sterilized dropping pipette. Three replicates were maintained for each treatment. For each microorganism, the positive control (Ketoconazole) and the negative control (100% ethanol) (three replications each) were also loaded in a separate well. The plates were incubated for 3-4 days and the observations were recorded. The zone of inhibition (or halo like area) was measured. The Diameter of Inhibition Zone (DIZ) was measured and the mean D1Z was calculated (Iqbal *et al*., 1998).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was performed to test the antimicrobial activity of the chloroform extract of *Polygonum minus* and active bands using tube dilution method (Claeys *et al*., 1988). The MIC is the lowest concentration able to inhibit any visible microbial growth. This test was performed at four concentrations of the plant extract (10 mg ml^{-1}, 1 mg ml^{-1}, 0.1 mg ml^{-1}, 0.01 mg ml^{-1}).

Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal pathogens.

Mycelial discs (9 mm) of the pathogens (*A. solani, A. flavus, A. niger and F. oxysporum* f.sp. *lycopersici*) were inoculated into respective broth separately containing chloroform extract of *P. minus* (0.50, 1.0, 1.5, 2.0 and 2.5%). Conical flask without the extract was maintained as control. The treatments were replicated thrice and incubated for 21 days. The mycelium was harvested through filtration with Whatman No. 42 filter paper. The filter paper containing fungal mycelium was oven dried at 70°C for 24 h and the weight of the dried mycelium was determined (Singh *et al*., 1980).

Thin Layer Chromatography (TLC) for Detection of chemical groups

Over the pre-coated silica gel sheets, chloroform extract of plant *Polygonum minus* was run using the mobile phase 100% dichloromethane and after TLC separation the sheets were kept in room temperature for drying. The TLC sheets were sprayed with chemical reagents *viz*., folins reagent, vanillin sulphuric acid, phosphomolybdic acid, dragendroffs reagent and diazotized sulphanillic acid and the observations were made.

RESULTS AND DISCUSSION

The chloroform extract of *P. minus* exhibited comparably more activity against *Alternaria solani*, *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* fsp *lycopersici* than the methanol extract (Plate 1). The Diameter of Inhibition Zone (DIZ) produced by the chloroform extract was comparable/equal to that of the positive control – ketaconazole (Table 1).

Table 1. Antifungal activity of *P. minus* against plant pathogenic fungi

Organisms	Diameter of Inhibition Zone (cm)				
	ME	CE	PE	Negative control	Positive control
Alternaria helianthi	2.2	2.6	-	-	4.0
Alternaria solani	2.7	3.3	-	-	3.8
Aspergillus flavus	2.5	3.1	-	-	3.5
Aspergillus niger	3.0	3.2	-	-	3.6
Colletotrichum musae	1.1	1.0	-	-	2.9
Fusarium oxysporum fsp. *lycopersici*	3.1	3.4	-	-	3.5
Macrophomina phaseolina	-	-	-	-	2.9
Rhizoctonia solani	-	-	-	-	3.1
Sclerotium rolfsi	-	-	-	-	3.0

ME – Methanol Extract; CE – Chloroform Extract; PE – Petroleum ether Extract; Negative control-Ethanol; Positive control -Ketaconazole -10mg/ml

Polygonum cuspidatum (Polygonaceae) has traditionally been used in folk medicine to control oral diseases. The fraction (F1) of *Polygonum cuspidatum* showed bacteriostatic and bactericidal activity against mutants of streptococci in suspension, with a minimum inhibitory concentration (MIC) range of 31.3–250 µg/ml and minimum bactericidal concentration (MBC) range of 0.5–1 mg/ml (Song *et al.,* 2007). According to Kubo *et al.* (1981) roots of *P. cuspidatum* contain compounds called stilbenes, which are aromatic hydrocarbons having the general formula C14-H12. One stilbene called resveritrol has several physiological activities. Primarily, it inhibits the growth of several bacteria and fungi.

The results of minimum inhibitory concentration (MIC) (Table 2) studies revealed that the methanol and chloroform extract at a concentration of 10mg/ml checked the growth of these pathogens effectively. Further purification of this extract by various chromatographic techniques could provide lower MIC of this extract. Antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the crude extracts of *Polygonum cuspidatum* roots were assayed against five common food borne bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli*, and *Salmonella anatum*) by Shan *et al.* (2008) and found to exhibit potent antibacterial properties.

The mycelial growth of all the tested fungal pathogens were significantly reduced by 2.5 and 2.0 per cent chloroform extract of *P. minus* (Table 3). Two per cent

Table 2. Minimum inhibitory concentration for methanol, chloroform and petroleum leaf extracts of *Polygonum minus* against plant pathogens

Name of the fungi	Dilution 1 (10mg/ml)			Dilution 2 (1mg/ml)			Dilution 3 (0.1mg/ml)			Dilution 4 (0.01mg/ml)		
	ME	CE	PE	ME	CE	PE	ME	CE	PE	ME	CE	PE
Alternaria helianthi	NG	NG	G	G	G	G	G	G	G	G	G	G
Alternaria solani	NG	NG	G	G	G	G	G	G	G	G	G	G
Aspergillus flavus	NG	NG	G	G	G	G	G	G	G	G	G	G
Aspergillus niger	NG	NG	G	G	G	G	G	G	G	G	G	G
Colletotrichum musae	G	G	G	G	G	G	G	G	G	G	G	G
Fusarium oxysporum fsp. *Lycopersici*	NG	NG	G	G	G	G	G	G	G	G	G	G
Macrophomina phaseolina	G	G	G	G	G	G	G	G	G	G	G	G
Rhizoctonia solani	G	G	G	G	G	G	G	G	G	G	G	G
Sclerotium rolfsi	G	G	G	G	G	G	G	G	G	G	G	G
Solvent control		G			G			G			G	
Ketoconazole		NG			NG			G			G	
Cells		G			G			G			G	

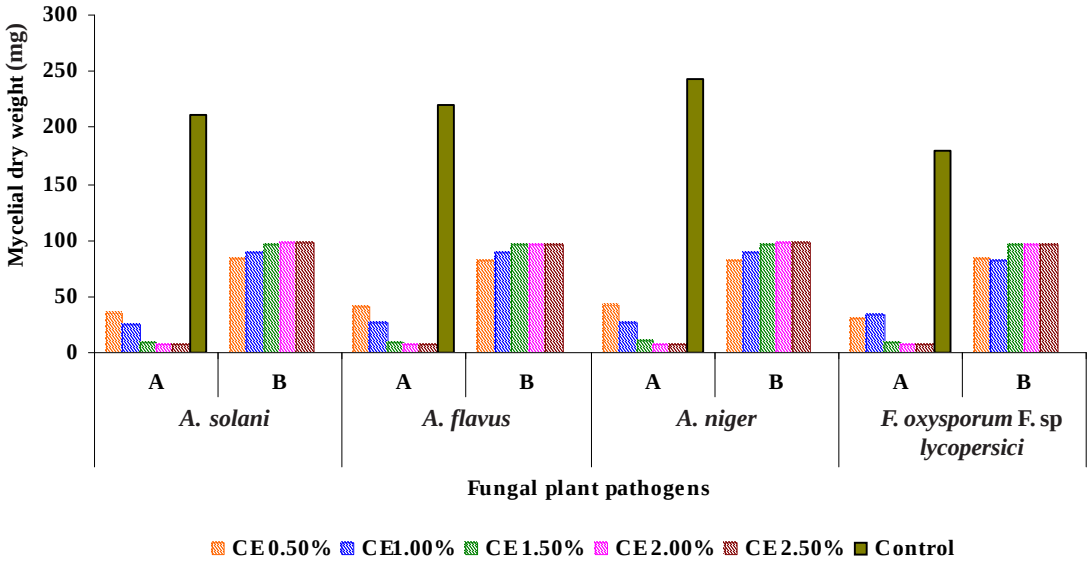
ME – Methanol Extract; CE – Chloroform Extract; PE – Petroleum ether Extract; NG – No growth; G – Growth

Table 3. Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal pathogens

Treatment	Mycelial dry weight (mg)							
	*A. solani**		*A. flavus**		*A. niger**		*F. oxysporum* f.sp. *lycopersici**	
	A	B	A	B	A	B	A	B
CE 0.50%	35.24	83.25	41.35	81.29	42.80	82.43	30.70	82.84
CE 1.00%	25.20	88.03	26.70	87.92	27.25	88.81	33.13	81.48
CE 1.50%	8.12	96.14	9.70	95.61	9.82	95.97	8.21	95.41
CE 2.00%	6.37	96.97	7.60	96.56	7.40	96.96	6.45	96.39
CE 2.50%	6.35	96.98	7.58	96.57	7.39	96.96	6.46	96.38
Control	210.50		221.00		243.60		178.90	

CE- Chloroform extract of *P. minus*
A- Mycelial dry weight (mg); B- Percent reduction over control
*Mean of four replications

Fig 1. Effect of chloroform extract of *P. minus* on mycelial dry weight of fungal pathogens

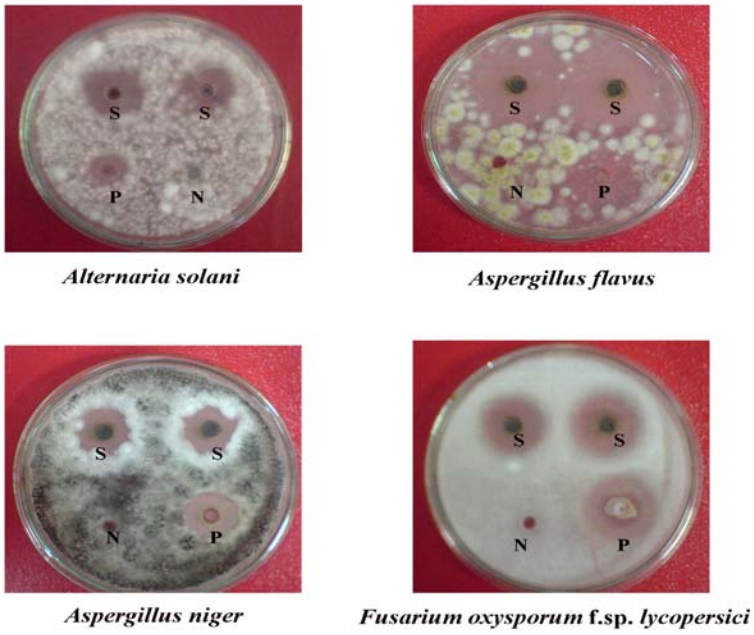


chloroform extract of *P. minus* recorded 96.97, 96.56, 96.96 and 96.39 per cent reduction in mycelial growth, whereas 2.5 per cent chloroform extract of *P. minus* recorded 96.98, 96.57, 96.96 and 96.38 per cent reduction in mycelial growth in *A. solani*, *A. flavus*, *A. niger* and *F. oxysporm* f.sp. *lycopersici*, respectively, when compared to the control (Fig. 1). All the treatments with various concentrations of chloroform extract of *P. minus* significantly reduced the mycelial growth of all the tested fungal pathogens more effectively when compared to the control.

The results of thin layer chromatographic (TLC) studies indicated the presence of terpenoids in the chloroform extract of *P. minus*. A total of 11 bands were observed, of which 7 bands were visible (Plate 2) and 4 bands were invisible and thus identified by using spray reagents (Folin-Ciocalteau reagent and 20 per cent sodium carbonate solution, vanillin sulphuric acid, diazotized sulphanillic acid, dragendroffs reagent and phosphomolybdic acid). The four invisible bands appeared as pink and purple colour, upon spraying with vanillin sulphuric acid indicating the presence of terpenoid group of compounds in the extract (Plate 3). Earlier Katsuhiro *et al.* (2005) examined the constituents of *Polygonum multiflorum* collected from various habitats using thin-layer chromatography (TLC) and detected. 2, 3, 5, 4'-tetrahydroxystilbene-2-O-β-D-glucoside with phosphomolybdic acid reagent in all the *Polygonum multiflorum* samples.

A more detailed study in the purification and identification of antimicrobial compound in the

Plate 1. Antimicrobial activity of chloroform extract of *P. minus* against plant pathogens by agar well diffusion assay



Alternaria solani | *Aspergillus flavus*

Aspergillus niger | *Fusarium oxysporum* f.sp. *lycopersici*

S - Sample (Chloroform extract of *P. minus*)
P - Positive control (Ketoconazole)
N - Negative control (100% Ethanol)

Plate 2. Thin layer chromatography for chloroform extract of *P. minus* - Visible observation

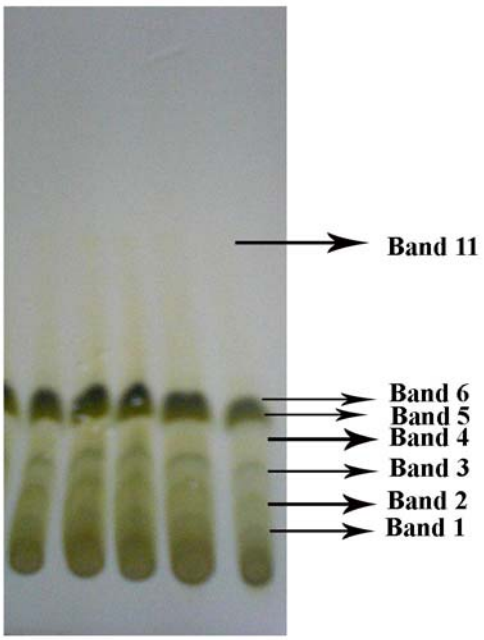
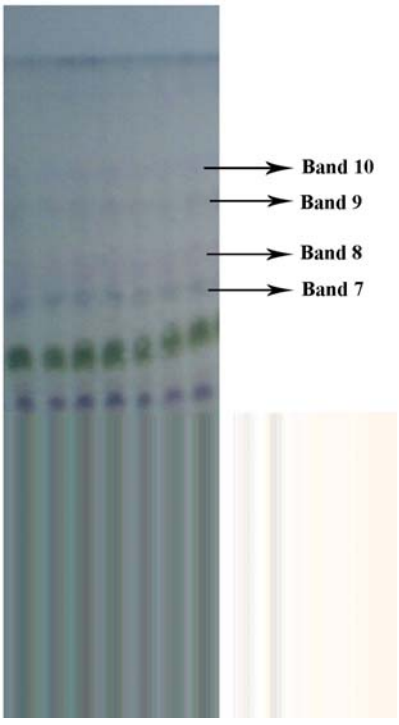


Plate 3. Thin layer chromatography for chloroform extract of *P. minus* - Bands observed using vanillin sulphuric acid



chloroform extract of *P. minus* could yield a novel antifungal compound against these pathogens. Structure elucidation of the respective antimicrobial principle could lead to its artificial synthesis and this may be used as an alternative to the commercial fungicides. Since these antimicrobial compounds are of plant origin they can be easily biodegraded, less toxic and does not create resistance in pathogens. Furthermore, use of this kind of compounds in plant disease control may save our environment from residual toxicity of the fungicides.

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