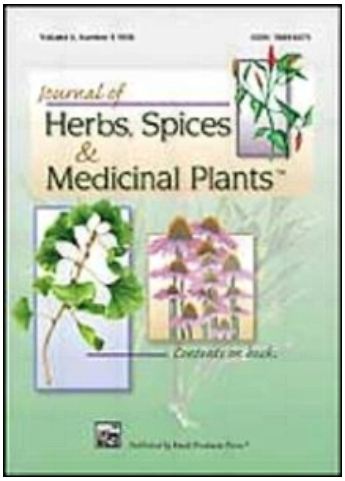

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Antifungal Activity of *Mucuna pruriens* Seed Extractives and L-dopa

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The seeds of Mucuna pruriens (L.) *D.C. (Leguminosae) were extracted first with hexane, then with ethyl acetate, and finally with methanol. The solvents were removed to get the respective extractives. These were assessed for the mycelial growth inhibition of three phytopathogenic fungi—Colletotrichum gloeosporioides, Colletotrichum capsici, and Fusarium solani—by the poisoned-food technique. Methanol extractive showed highest activity against all the three test organisms. L-dopa [3-(3,4-Dihydroxyphenyl)-L-alanine], which was qualitatively detected in the ethyl acetate and methanol extractives and quantitatively assessed in the seeds, showed antifungal activity against all the three test organisms, showing that it is one of the active principles in these extractives.*

KEYWORDS phytopathogenic fungi, plant fungicides

INTRODUCTION

Plants parts of *Mucuna pruriens* (Leguminosae) used for traditional medicine include seed, leaf and root (8). The seeds have been used for treatment of Parkinson's disease, edema, impotence, intestinal gas, and worms. In Indian Ayurvedic medicine, it is considered a diuretic, nerve tonic, and

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aphrodisiac. In Nigeria, it is used as oral prophylactics for snakebite, and studies demonstrated the protective effect of these seeds against snake venom poisoning (12). L-dopa, alkaloidal constituents, epoxy fatty acids, and many diverse phytochemicals have been isolated from the seeds such as a multiform glycoprotein that elicits anti–snake venom antibodies against *Echis carinatus* venom (1). *M. pruriens* contains the bioactive alkaloids mucunine, mucunadine, mucuadinine, pruriendine, and nicotine, besides β-sitosterol, glutathione, lecithin, oils, and venolic and gallic acids (10). Earlier investigators have reported antifungal activity of the crude extractives (2,3), but less attention was paid to compare the extractives obtained by solvents of varying polarities and to understand the chemical nature of the active principle(s). The objective of the present work was to compare the antifungal activities of extractives of the seeds of *M. pruriens* obtained by solvents of varying polarities and to ascertain whether L-dopa present in the plant is an antifungal active principle.

MATERIAL AND METHODS

Plant Material

Mucuna pruriens seeds were harvested from the experimental plot of Indian Institute of Horticultural Research, Hessaraghatta (IIHR), Bangalore, India in May 2009, were dried at 60°C, and were powdered. The powdered plant material was extracted in a Soxhlet apparatus first with hexane, then with ethyl acetate, and finally with methanol. The respective extractives were obtained by completely distilling out the solvents on a water bath and were tested for fungicidal activity. L-dopa (98.5%) was obtained from S.D. Fine Chemicals, Mumbai, India.

Estimation of Active Principle

For qualitative estimation, hexane, ethyl acetate, and methanol extractives (0.25 g each) and L-dopa (0.05 g) were spotted on silica gel TLC plates (0.5-mm thickness) and eluted with an n-propanol-ethyl acetate-water-acetic acid (20:19:10:1) mixture. The plate was sprayed with ninhydrin (0.1% in acetone) and warmed in the oven at 100°C for 30 min (6). L-dopa had an Rf value of 0.65.

Quantitative estimation of L-dopa was done by high-performance liquid chromatography (HPLC) (9) with the following column conditions: column-Spherisorb, 5 μm ODS2; analytical column, 4.6 × 250 mm; detector–U.V. detector set at 280 nm; time: 10 min; mobile phase– sodium dihydrogen orthophosphate, pH 2.8 (1.162% w/v); flow rate: 2 mL/min; retention time of L-DOP: 3.5 to 3.8 min.

Fungi Tested

Colletotrichum gloeosporioides ITCC 4573 was obtained from the Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi, India, and *Colletotrichum capsici* and *Fusarium solani* isolated in Division of Plant Pathology at IIHR, and maintained on a potato-dextrose-agar medium.

Studied Activity

Antifungal activity of hexane, ethyl acetate, and methanol extractives and that of L-dopa were studied by observing the mycelial growth inhibition of *Colletotrichum sp.* and *Fusarium solani* by a poisoned-food technique; surfactant Tween-80 was added at a 0.3% to the media in both the control and the treated samples. Phenol was used as a standard. The percent mycelial growth inhibition was calculated by the formula $(C - T)/C \times 100$, where C is the mycelial diameter of the control and T is the mycelial diameter of the treated samples (4,5).

RESULTS AND DISCUSSION

Methanol extractive showed highest antifungal activity against the mycelial growth of *Colletotrichum sp.* and *Fusarium solani* (Table 1). Hexane extractive showed higher activity against the mycelial growth of *C. gloeosporioides* and *F. solani* than ethyl acetate extractive. In the case of *C. capsici,* the ethyl acetate extractive showed higher activity compared to hexane extractive. However, L-dopa (Figure 1) showed fungicidal activity against all the three phytopathogenic fungi tested.

Co-chromatography of the extractives with an authentic sample of L-dopa and detection with ninhydrin showed the presence of L-dopa in ethyl acetate and methanol extractives (Rf value: 0.65) but not in hexane extractive. The spot was more conspicuous in methanol extractive than in ethyl

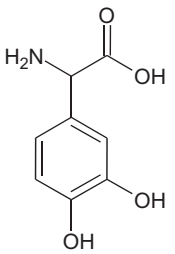

FIGURE 1 Structure of L-dopa.

TABLE 1 Antifungal Activity of *Mucuna pruriens* Seed Extractives and L-dopa against the Mycelial Growth of *Colletotrichum sp.* and *Fusarium solani*

Test organisms	Extractives/compound	Concentration (%)	Mycelial growth inhibition (%)
[1]*Colletotrichum gloeospoioides*	Hexane	0.2	3.8 ± 0.1
		0.5	7.4 ± 0.2
	Ethyl acetate	0.2	1.9 ± 0.1
		0.5	6.3 ± 0.2
	Methanol	0.2	14.1 ± 0.0
		0.5	20.8 ± 0.1
	L-dopa	0.05	15.5 ± 0.2
		0.1	51.4 ± 0.3
	Phenol (Standard)	0.025	55.6 ± 0.2
		0.05	78.3 ± 0.3
[2]*Colletotrichum capsici*	Hexane	0.2	10.7 ± 0.3
		0.5	17.7 ± 0.4
	Ethyl acetate	0.2	10.8 ± 0.0
		0.5	18.9 ± 0.5
	Methanol	0.2	17.1 ± 0.4
		0.5	29.0 ± 0.1
	L-dopa	0.05	29.7 ± 0.2
	Phenol (Standard)	0.025	22.6 ± 0.1
		0.05	51.1 ± 0.1
[1]*Fusarium solani*	Hexane	0.2	10.4 ± 0.0
		0.5	16.2 ± 0.4
	Ethyl acetate	0.2	8.8 ± 0.1
		0.5	12.1 ± 0.2
	Methanol	0.2	13.0 ± 0.3
		0.5	20.5 ± 0.2
	L-dopa	0.05	33.2 ± 0.2
	Phenol (Standard)	0.025	21.3 ± 0.1
		0.05	60.9 ± 0.1

NI = no inhibition.
[1]Observations were taken after an incubation of 8 days at 27 ± 2°C.
[2]Observations were taken after an incubation of 5 days at 27 ± 2°C.
Notes: Data are mean of two replications.
Concentrations of the extractives expressed as a percentage of the compounds in PDA (w/v).

acetate extractive. Quantitative estimation by HPLC revealed that the seeds contain 4.75% of L-dopa. Other workers also showed the L-dopa content in *Mucuna sp.* at a range of 3% to 7% (7,11).

The results clearly show that L-dopa is one of the antifungal compounds that contribute to the antifungal activity of polar extractives of *M. pruriens.* However, the absence of L-dopa in hexane extractive and in its antifungal activity clearly shows that there are compounds other than L-dopa that contribute to its activity.

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