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Determination of antifungal potential of secondary metabolites from *Jatropha curcas* and *Abrus precatorius*

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Abstract
Antifungal potential of flavonoids and alkaloids from Jatropha curcas and Abrus precatorius was determined against the wilt fungus Fusarium oxysporum. Total 21 extracts were tested at three different concentrations (2.5, 5 and 10 mg/ml) for the mycelia and spore inhibition of the test fungus. All tested extracts were recorded to have significant antifungal potency at 10 mg/ml concentration as all inhibited mycelia and spore germination. Free flavonoids, alkaloids from seeds of *J. curcas,* bound flavonoids from stem and leaves of *A. precatorius* showed excellent inhibitory activity against the wilt fungi.

Keywords: *Jatropha curcas, Abrus precatorius,* flavonoids, alkaloids

Introduction

Plants and their products especially secondary metabolites have been proved untapped reservoir for the development of novel antimicrobial drugs. It is estimated that at least 12000 different secondary metabolites have been isolated from plants which constitute less than 10% of the total secondary metabolites (Schultes, 1978; Cowan, 1999) [11, 3]. One hundred and nineteen secondary metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% pharmacologically active plant derived compounds were discovered. It clearly indicates enormous potential of plants and it becomes extremely important in the present scenario where synthetic drugs are being extensively used against disease causing microorganisms, making them multi drug resistant. Therefore, it is always desirable to explore different plants for their hidden antimicrobial potential.

Fusarium oxysporum also known as panama diseases or agent green, notorious for causing fusarium wilt in economically important plants such as tomato, potato, *Musa* spp, sugar cane, cow pea, ginger etc. It destroys xylem of the host plant that leads to development of wilt symptoms such as leaf wilting, yellowing and later death of the diseased plant. It not only infects crop plants but also known for producing mycotoxins in stored grains. Moreover, it can also survive for a considerable long time in soil without a host. Therefore, management of the *Fusarium oxysporum* becomes very essential and indispensable for crop protection (Sharma *et al*., 2016, Hashem *et al*., 2010, Maheshwar *et al*., 2009, Larena *et al*., 2003) [6, 7]. Although several synthetic antifungal drugs are available for the prevention of disease but side effects of synthetic drugs are well known. Hence, exploration of new potential formulations of herbal origin are always welcome because of being less or no hazardous and toxic to environment. Sporulation or microconidial dissemination is considered as the one among the causes of wilt disease. Therefore, prevention of fungal dissemination can be managed by inhibition of sporulation. Mycelial inhibition becomes very important in the management of the fungus in terms of destruction of fungus at initial stages.

Present investigation is an effort to explore hidden antifungal potential of selected plants i.e. *Jatropha curcas* and *Abrus precatorius*against *Fusarium oxysporum.* Antifungal potential of various extracts from different parts of plants was investigated in terms of percent mycelial inhibition and percent spore inhibition.

Material and methods

Different parts of *Jatropha curcas* (root, stem, leaf and Seeds), *Abrus precatorius* (root, stem, leaf) were collected from different localities of Jaipur and its nearby areas. The selected plant parts were separately shade dried, finely powdered and subjected to preliminary detection of flavonoid and alkaloid by well established methods (Harborne 1984) [5].
Fine powder of plant parts was then subjected to extraction of flavonoids and alkaloids following the well-established methods. (Subramanian & Nagarajan, 1969 and Harborne, 1984, respectively) [5].
Plant pathogenic microorganism (*F. oxysporum* MTCC No. 7678) was procured from IMTECH, Chandigarh. Fungal culture was grown and maintained on 'Potato Dextrose Agar' medium. Antifungal activity of plant extracts was determined by two methods i.e. percent mycelial inhibition and percent spore inhibition methods.
Three different concentrations (2.5 mg/ml, 5 mg/ml, and 10 mg/ml) of plant extracts were taken for the study. Antifungal activity of the extracts on mycelial growth of pathogen was carried out by food poisoning technique (Tian *et al*., 2011) [13].
Potato dextrose agar medium (20 ml) was poured into sterilized petri plates containing 5 ml of extract solution of different concentrations. A fungal disc of 6 mm diameter was cut with cork borer from seven days old fungal culture and placed at the center of the each petri plate. Then plates were incubated at $28 \pm 2\ ^0$C till full growth. Extracts were dissolved in acetone and control plates containing solvent were also incubated. Study was done in triplicates. Percent inhibition of

the radial growth by different extracts at different concentrations was calculated by using the following well established formula (Albuquerque *et al*., 2011).

% mycelial inhibition=

$$\frac{\text{Mycelial inhibition in control- mycelial inhibition in extract}}{\text{Mycelial inhibition in control}} \times 100$$

Spore germination inhibition was determined by spore counting method (Milosevick *et al.,* 2007) [9]. Spore suspension was prepared from 15 days old culture of the fungus in sterile distilled water. Fungal suspension (100 µl) was added to 100 µl extracts of different plants of three concentrations (2.5 mg/ml, 5.0 mg/ml, 10 mg/ml) prepared in acetone. Each was incubated at 25±2° C for 24 h. Control vials contained acetone in place of plant extracts. After incubation, content of the vials were stained with cotton blue and mounted in lactophenol. Study was done in triplicates. Spores were observed under microscope for their germination status. Percentage inhibition was calculated, using established formula (Tiwari *et al*., 2003; 2004) [14, 15].

%Spore inhibition=

$$\frac{\text{Spore inhibition in control - Spore inhibition in extract}}{\text{Spore inhibition in control}} \times 100$$

Results and Discussion

Total 21 extracts were tested from the different parts of selected plants. Three concentrations of each extract i.e. 2.5 mg/ml, 5 mg/ml and 10 mg/ml were taken for the experiment. Most of the extracts showed significant antifungal potency at different concentrations. Hundred percent mycelial inhibition by the free flavonoids from stem, seed and alkaloid extract from seed, leaf of *J.curcas* was recorded at 10 mg/ml concentration. Free flavonoids from stem and leaf, bound flavonoids from stem of *A.precatorius* showed 100 % mycelial inhibition. These extracts were also showed remarkable potency at 2.5 and 5 mg/ml concentration as well. Among 21 extracts only 7 were found not to be active at all tested three concentrations, remaining 14 extracts were found to have significant potential at all three concentrations.

All the extracts were recorded to inhibit spore germination at all concentrations except ethyl acetate extract of leaf of *J. curcas* that was found to be inactive at 2.5 and 5 mg/ml concentration. Alkaloid extract from seeds of *J. curcas* was found to be the most potent extract where 100% inhibition of spore germination was recorded at two concentrations (5 and 10 mg/ml). It also showed 80.50 % inhibition at 2.5 mg/ml. Ethyl acetate extracts of *A. precatorius* from stem and leaf were found to inhibit spore germination 100 % at 10.mg/ml. Flavonoids (free and bound) from the leaf of *A. precatorius* exhibited remarkable inhibition at all three tested concentrations. Alkaloids from this part of the plant were too showed significant potency against the fungi. Free flavonoids and alkaloids from seeds of *J. curcas* were observed to have great antifungal potential against the wilt fungi.

In the present investigation almost all the tested extracts were found to be active against spore germination of the test fungi. Promising results were noted at 5 and 10 mg/ml concentration for most of the extracts although inhibition was also recorded at 2.5 mg/ml concentration. Worldwide efforts are being done to protect economically important crops form pests and pathogens. Although, investigations are being carried out at national and international level to explore new antimicrobials from selected plants against various pathogens (Yasmin *et al*., 2015, Garaniya and bapodara, 2014, Adamu *et al*., 2013, Rampadarath *et al*., 2016) [16, 1, 10] but systematic studies to find active principle is still lacking. Present study is an effort towards this direction.

Table 1: Inhibition of mycelial growth of *Fusarium oxysporum* by different plant extracts.

Plant	Part	Extract	%Inhibition of mycelial growth		
			2.5 mg/ml	5 mg/ml	10 mg/ml
Jatropha curcas	Root	E_1	30.00	42.20	55.20
		E_2	15.50	35.20	48.20
		A	45.30	55.00	65.20
	Stem	E_1	65.50	90.10	100
		E_2	-	-	15.00
		A	-	-	35.00
	Leaf	E_1	15.40	25.20	45.00
		E_2	-	30.30	45.50
		A	57.00	89.70	100
	Seeds	E_1	68.50	95.00	100
		E_2	-	34.10	50.00
		A	71.00	87.30	100
Abrus precatorius	Root	E_1	35.20	57.00	70.30
		E_2	-	27.20	35.50
		A	-	25.00	39.80
	Stem	E_1	65.20	77.50	100
		E_2	70.30	85.00	100
		A	-	-	15.00
	Leaf	E_1	58.00	78.50	100
		E_2	60.20	75.40	95.40
		A	58.00	77.60	96.40

E1: Free Flavonoids; E2: Bound Flavonoids; A: Alkaloid extract

% Mycelial inhibiton=

$$\frac{\text{Mycelial inhibition in control- mycelial inhibition in extract}}{\text{Mycelial inhibition in control}} \times 100$$

Table 2: Inhibition of spore germination of *Fusarium oxysporum* by different plant extracts.

Plant	Part	Extract	% inhibition of spore germination		
			2.5 mg/ml	5 mg/ml	10 mg/ml
Jatropha curcas	Root	E_1	20.00	45.20	54.50
		E_2	15.50	38.70	45.45
		A	30.11	58.8	63.60
	Stem	E_1	60.2	76.00	86.36
		E_2	-	-	9.09
		A	12.80	30.20	59.09
	Leaf	E_1	10.80	36.20	54.50
		E_2	7.30	31.11	45.45
		A	60.00	65.70	80.20
	Seeds	E_1	76.00	80.85	90.20
		E_2	6.40	12.50	22.70
		A	80.50	100	100
Abrus precatorius	Root	E_1	38.70	54.50	63.60
		E_2	20.30	30.11	45.45
		A	16.80	21.60	31.81
	Stem	E_1	12.00	18.20	30.00
		E_2	85.21	95.11	100
		A	38.27	42.50	54.50
	Leaf	E_1	80.97	88.40	96.70
		E_2	88.40	95.45	100
		A	49.00	58.63	69.70

E_1: Free Flavonoids; E_2: Bound Flavonoids; A: Alkaloid extract

$$\%\text{Spore Inhibition} = \frac{\text{Spore inhibition in control} - \text{Spore inhibition in extract}}{\text{Spore inhibition in control}} \times 100$$

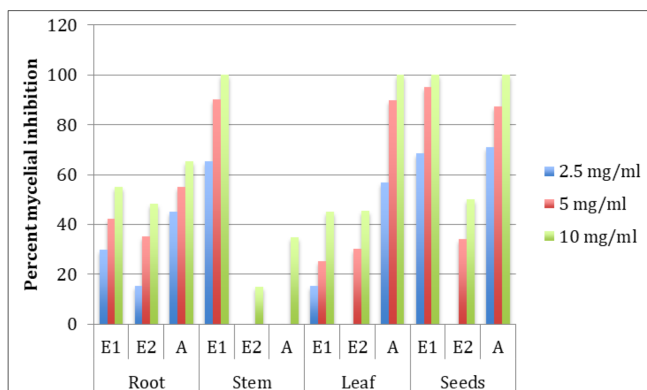

Fig 1: Percent mycelial inhibition by the various extracts of *J. curcas.*

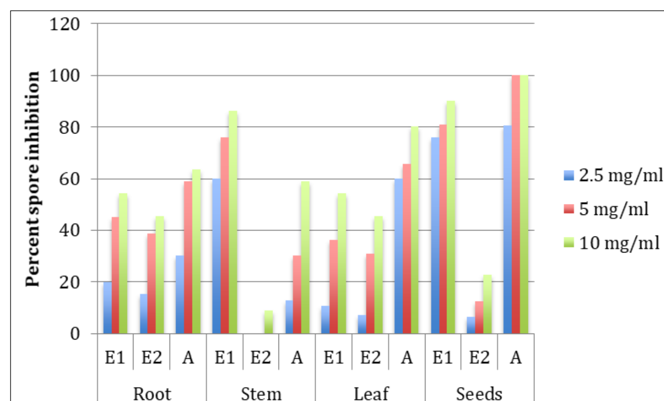

Fig 3: Percent spore inhibition by the various extracts of *J. curcas.*

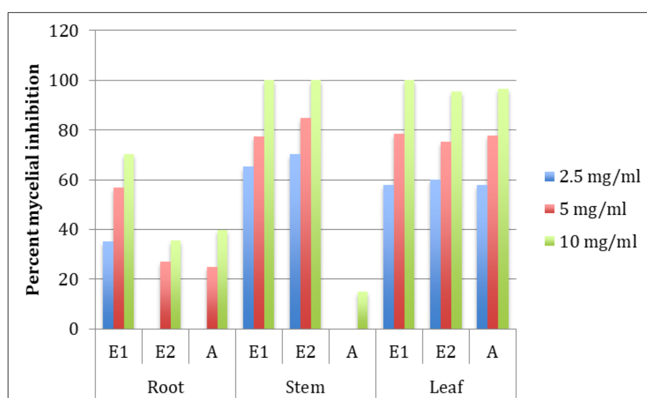

Fig 2: Percent mycelial inhibition by the various extracts of *A. precatorius.*

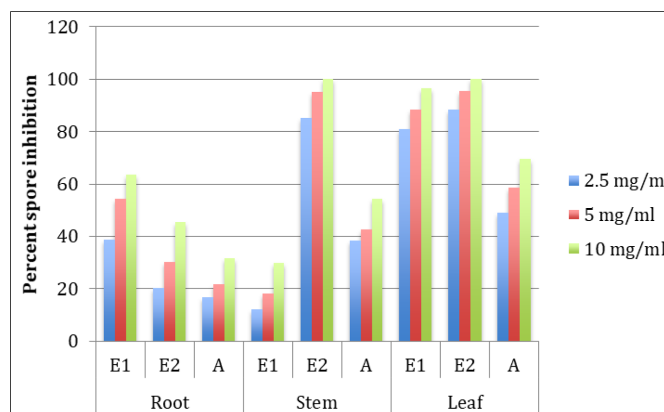

Fig 4: Percent spore inhibition by the various extracts of *A. precatorius.*

Conclusion

Present study establishes antifungal potency of alkaloids and flavonoids obtained from *Jatropha curcas* and *Abrus precatorius.* All the extracts that were detected to have strong potential to inhibit mycelia of the test fungi were also recorded to have significant spore inhibition potency. It indicates that active component/s of the extracts possess capacity to inhibit the fungus systemically.

Present investigation paves the path for further chemical exploration of active test extracts and suggests chemical profiling of the most potent extracts (i.e. free and bound flavonoids from *A.precatorius*, alkaloids and free flavonoids from *J. curcas*) to find out active compounds present in the extracts. Isolation, identification and characterization of active extracts are always desirable to establish new novel compounds. In the light of the fact that microorganisms are rapidly developing resistance to existing synthetic drugs, exploration of new herbal bioactive compounds is always welcoming step.

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