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Antimycotic Activity of Important Medicinal Plants against Wilt Pathogen of Pigeon Pea and Leaf Spot Pathogen of Tomato

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Abstract

Aqueous and ethanolic leaf extracts of six different medicinal plants such as *Adhatoda vasica Nees, Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don., *Cymbopogon citratus* (DC.) Stapf., *Eucalyptus globules* Labill. and *Ocimum sanctum* L., were tested for their antifungal activity against *Fusarium oxysporum* f.sp. *udum* Butler, a wilt pathogen of pigeon pea [*Cajanus cajan* (L.) Millsp.] and *Alternaria solani*, a leaf spot pathogen of tomato (*Lycopersicon esculentum* Mill.). The leaf extracts (aqueous and ethanolic) prepared from six different plant leaves at different level of concentrations i.e. 5,10, 15 & 20% were incorporated in glucose nitrate liquid medium. The results were indicated that the ethanolic leaf extracts found good inhibitory activity than aqueous leaf extracts against plant pathogenic fungi tested. The ethanolic leaf extract prepared from *Azadirachta indica* was showed better efficacy against wilt pathogenic fungus and extract from *E. globules* found good inhibitory activity against leaf spot pathogen of tomato.

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Antimycotic activity, Medicinal plants, Wilt and Leaf Spot Pathogens.

Introduction

Cajanus cajan is one of the major legume (pulse) crops of tropics and sub tropics. It is grown in many developing countries, it fixes atmospheric nitrogen in the soil[1]. Wilt disease of *Cajanus cajan* caused by F. *oxysporum* f.sp. *udum* is an important soil borne disease, which causes major yield losses in susceptible cultivars throughout the pigeon pea growing areas[2,3]. Tomato is second most important solanaceous vegetable crops after potato. It is widely cultivated in 140 countries of the world.[4] The tomato crop infected by so many fungi, bacteria, viruses and nematodes. This causes several diseases and yield loss in crop. Among the fungal diseases, leaf spot disease caused by A. *solani* is the most important one[5,6] because of this there is great reduction in the quantity and quality of fruit yield. We are using synthetic fungicides to control these diseases. However, there is a need of alternative managing methods because of the negative public

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perceptions about the use of artificial chemicals. It have been studied that, the plant-derived products have low mammalian toxicity and they shows less environmental effect[7]. The crop yield loss due to pathogens has become important thing in agriculture. Using of chemicals based products to control these pathogens has resulted in problems like increased resistance for chemicals in target pathogens, residual effect of chemicals in agri-based products, and environmental pollution. There are 45,000 plant species in India and among them; several thousands have been reported as they possess medicinal properties. It is also reported as plants act as a good source for secondary metabolites, such as alkaloids, terpenoids, flavanoids, tannins, phenols, steroids etc., [8]. There are reports on 12,000 phytocompounds have been isolated from the different plant species [9]. In many cases, these substances protect plants species against invasion by microorganisms, insects and herbivores. Crude extracts prepared from some important medicinal plants are used to control some of the plant pathogens[10]. Plant based pesticides and plant metabolites shows minimal environmental impact to consumers when compare with chemical pesticides. Hence these are better alternatives at the place of synthetic pesticides[11]. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails[12,13,14,15]. The aim of the present study was to evaluate the antimycotic activity of aqueous and ethanolic leaf extracts of some important medicinal plants against wilt pathogen of pigeon pea and leaf spot pathogen of tomato.

Materials and Methods

Collection of Plant Material

Fresh and disease free leaves of six important medicinal plants such as *Adhatoda vasica Nees*, *Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don., *Cymbopogon citratus* (DC.) Stapf., *Eucalyptus globules* Labill. and *Ocimum sanctum* L., were collected from University campus area and thoroughly washed under tap water and surface sterilized with 0.01% $HgCl_2$ (mercuric chloride) for

Table 1: Antifungal activity of some important medicinal plant leaf extracts (aqueous & ethanolic) against *Fusarium oxysporum* f.Sp. *udum* Butler.

S. No.	Name of plant	Average dry weight (mg) of mycelia mat after 7 days of incubation at different concentrations							
		Aqueous leaf extracts				Ethanolic leaf extracts			
		5%	10%	15%	20%	5%	10%	15%	20%
1	*Adhatoda vasica*	360 (-16.129)	300 (3.225)	280 (9.677)	200 (35.48)	300 (3.225)	280 (9.677)	210 (32.258)	150 (51.612)
2	*Azadirachta indica*	320 (-3.225)	260 -16.129	200 (35.483)	170 (45.161)	250 (19.354)	200 (35.483)	110 (64.516)	70 (77.41)
3	*Catharanthus roseus*	340 (-9.677)	290 (6.451)	250 (16.354)	200 (35.483)	300 (3.225)	240 (22.580)	180 (41.935)	140 (54.838)
4	*Cymbopogon*	370 (-19.354)	310 (0.00)	280 (9.677)	220 (29.032)	310 (0.00)	290 (6.451)	220 (29.032)	160 (48.387)
5	*Eucalyptus globules*	320 (-3.225)	290) (6.451	260 (16.129)	210 (32.258)	280 (9.677)	250 (16.354)	220 (29.032)	200 (35.480)
6	*Ocimum sanctum*	340 (-9.677)	310 (0.00)	280 (9.677)	200 (35.483)	290 (6.451)	260 (16.129)	170 (45.161)	130 (58.064)
7	**Control**				**310 (0.00)**				

* Data in parenthesis represents the percent of inhibition.

1 to 2 min and washed with sterile distilled water 2-3 times then dried shade. The fine powder was prepared from dried leaves in electric blender.

Preparation of Aqueous Leaf Extracts

The leaf powder was extracted with sterile distilled water at room temperature (25±2°C) at various concentrations (5, 10, 15 and 20%) i.e. 5 g in 100 ml, 10 g in 100 ml, 15 g in 100 ml and 20 g in 100 ml. Extracts were filtered through three layered cheese cloth, after through Whatman's filter paper No.1 and stored in refrigerator for further uses.

Preparation of Ethanolic Leaf Extracts

The leaf powder was extracted with 80% ethanol at various concentrations (5, 10, 15 and 20% i.e. 5 g in 100 ml, 10 g in 100 ml, 15 g in 100 ml and 20 g in 100 ml. Then extracts were filtered through three layered cheese cloth, after through Whatman's filter paper No.1. The ethanol part was evaporated by boiling of extracts and then the volume of extract was adjusted to 100 ml with distilled water and stored in refrigerator (at 4°C) in pre-sterilized flasks for further uses.

Test Fungi

The wilt pathogen of pigeon pea i.e. *F. oxysporum udum* and leaf spot pathogen i.e. *A. solani* were isolated from infected parts of host plants i.e. roots of pigeon pea and leaves of tomato. The pure cultures were maintained on Potato dextrose agar medium.

Bioassay of Leaf Extracts

Bioassay of plant extract was done in double strength Glucose nitrate (Basal medium) liquid medium. 10 ml of basal medium (1:1) was mixed with same amount of leaf extract (different concentrated) in a conical flask. The flasks were sterilized in autoclave at 121°C, 15 lbs pressure for 15 min. Same quantity of distilled water was added to the 10 ml of basal medium serve as control. The antibacterial substance (streptomycin) was added to the poisoned medium to avoid bacterial contamination. 5 mm inoculums discs were obtained from 7 day old healthy growing fungal cultures of above pathogens were aseptically transferred in to flasks containing medium and plant extracts (to control also), then the flasks were incubated at room temperature (25±2°C) for seven

Table 2: Antifungal activity of some important medicinal plant leaf extracts (aqueous & ethanolic) against *Alternaria solani* (Ell & Mart.) Grout.

S. No.	Name of plant	Average dry weight (mg) of mycelia mat after 7 days of incubation at different concentrations							
		Aqueous leaf extracts				Aqueous leaf extracts			
		5%	10%	15%	20%	5%	10%	15%	20%
1	*Adhatoda vasica*	330 (-17.857)	300 (-7.142)	260 (7.142)	240 (14.285)	260 (7.142)	220 (21.428)	180 (35.714)	160 (42.857)
2	*Azadirachta indica*	300 (-7.142)	260 (7.142)	250 (10.710)	200 (28.571)	250 (10.710)	230 (17.857)	200 (28.571)	130 (53.571)
3	*Catharanthus roseus*	320 (-14.285)	290 (-3.571)	250 (10.710)	210 (25.00)	260 (7.142)	200 (28.571)	170 (29.285)	150 (46.428)
4	*Cymbopogon citratus*	330 (-17.857)	290 (-3.571)	260 (7.142)	230 (17.857)	280 (0.00)	240 (14.285)	200 (28.571)	170 (29.285)
5	*Eucalyptus globules*	300 (-7.142)	250 (10.710)	200 (28.571)	170 (39.285)	200 (28.571)	180 (35.714)	120 (57.142)	50 (82.142)
6	*Ocimum sanctum*	310 (-10.710)	280 (0.00)	240 (14.285)	200 (28.571)	240 (14.285)	210 (25.00)	180 (35.714)	160 (42.857)
7	**Control**				**280 (0.00)**				

* Data in parentheses represents the percent of inhibition

days. After 7 days of incubation the mycelial mats were collected from triplicate samples for each treatment on pre-weighed filter paper. The mycelial mat was dried in oven at 60°C and the percentage of inhibition was calculated by the formula given below.

% of inhibition = Dry weight of mycelial mat in control – Dry weight of mycelial mat in test X 100 / Dry weight of mycelial mat in control

Results and Discussion

Antimycotic activity of medicinal plants such as *Adhatoda vasica*, *Azadirachta indica, Catharanthus roseus, Cymbopogon citratus*, *Eucalyptus globules* and *Ocimum* sanctum were tested against *F. oxysporum udum* wilt pathogen of pigeon pea and *A. solani* a leaf spot pathogen of tomato. All the ethanolic leaf extracts showed good inhibitory effect and reduced the growth of plant pathogenic fungi tested. Aqueous extracts prepared at low concentrations such as 5% and 10% enhanced the growth of pathogen; at 15% and 20% it reduced the growth of pathogenic fungi.

Ethanolic leaf extracts prepared from medicinal plants at various concentrations viz. 5%, 10%, 15% and 20% were showed good inhibitory activity against the pathogenic fungi. It was observed that, the inhibitory activity was more at high level of concentration (20%). Leaf extracts prepared from *A. indica* showed good inhibitory activity against F. *oxysporum udum* and E. globules against *A. solani*. It is revealed from the results predicted in the tables 1 and 2 that all the leaf extracts significantly inhibited the growth of fungal pathogens.

Plant extract forms that include ethanol extracts and its fractions, resins and essential oils have been reported to have antifungal activity and show a potential for the control of phytopathogenic fungi[16,17,18]. The antifungal efficacy of plant leaf extracts might be attributed due to the presence of antifungal compounds[8]. The diversity in the biocomposition of chemical components of plant extracts, i.e., the secondary metabolites of plants, even those obtained from the same species, may result in different responses, especially with regard to the potential for microorganism inhibition. Other associated factors include solubility, pH, volatility, diffusion characteristics in growth medium, and the type of microorganism under evaluation[19, 20]. Naziha et. al.[21] studied the antifungal activity of aqueous extracts prepared from neem (*Azadiracta indica*) plant leaves and reported that it was suppressed mycelial growth of *Alternaria solani* and Fusarium oxyspo¬rum, the causal organisms of leaf spot of tomato and wilt of pigeon pea and the level of suppression gradually increased with increasing concentration. Dellavalle, et.al,[22] studied the inhibitory effect of 10 different plant extracts against Alternaria spp. used in traditional Uruguayan medicine and they reported that the leaf extracts prepared from *Salvia sclarea*, S. *officinalis* and *Rosmsrinus officinalis* have the potentiality to control *Alternaria* spp. The studies made on the antimicrobial effect of some plant species such as *Azadirachta indica, Ocimum basilicum, Datura stramonium, Nerium oleander, Allium sativumwas* and *Eucalyptus chamadulonsis* for controlling *Alternaria solani* in-vitro and in-vivo and it have been observed that the leaf extracts of *D. stramonium*, *A. indica*, and *A. sativum* showed good inhibition of mycelial growth of *A. solani*[23]. The results obtained from the study correlated with the studies made by Jalander and Gachande[24], leaf extracts prepared from Datura sp. against *Fusarium oxysporum* f.s. *udum* and *Alternaria solani*; Farooq et. al.[25] with different weed extracts against *Macrophomina phaseolina* associated with charcoal rot of *Sesamum indicum*; Dissanayake and Jaysinghe[26] with selected plant extracts against pathogenic fungi.

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