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Antifungal Evaluation and Phytochemical Analysis of Plant Leaf Extracts Against Plant Pathogenic Fungi.

Botany

KEYWORDS : Plant Extracts, Fungal Pathogens, Antifungal activity, Phytochemical analysis.

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ABSTRACT

The antifungal activity of three different medicinal plants namely., Lawsonia inermis L., Eucalyptus globulus L., Vitex negundo L., were tested against plant pathogenic fungi Fusarium oxysporum (F.o), Fusarium solani (F.s), Alternaria solani (A.s), Rhizoctonia solani (R.s) and Colletotrichum lindemuthianum (C.l). The plant leaves were extracted with cold water and methanol. Among the different plant tested, the methanol extracts of L. inermis showed maximum antifungal activity against all pathogens, where as Aqueous extract of V. negundo showed least inhibition activity against all pathogens. Phytochemical analysis of both plant extracts were carried out.

INTRODUCTION:

Agriculture is the backbone of nation's economy, growth and development. Various groups of pathogens are known to cause losses to agricultural yield all over the world including India. Pathogenic fungi alone cause nearly 20% reduction in the yield of major food and cash crops (Agrios 2000). Different species of Alternaria, Fusarium, Curvularia, Rhizoctonia, and Colletotrichum are most common associates of vegetables all over the world, causing pre and post infections and considerable quality losses. Fungicides have been used for over 200 years to protect plants against plants against fungal disease (Brent and Hollomon, 2007). Since World War II, traditional agricultural practices have been replaced by the use of synthetic chemicals for the management of plant pathogens, pests and weeds. This has, no doubt, increased crop production but with some deterioration of environmental quality and human health (Cutler & Cutler 1999). In addition to the target pathogen, pesticides may also kill various beneficial organisms and their toxic forms can persist in soil (Hayes & Laws 1991). The increasing incidence of resistance among pathogens towards synthetic chemicals is also a cause for serious concern. Because of these problems there is a need to find alternatives to synthetic pesticides. Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale et al. 2004).

MATERIALS AND METHODS:

Infected vegetable were collected from different fields.

Isolation of Pathogens:

The Infected parts of the vegetables were cut into small pieces, surface sterilized with 0.1% mercuric chloride solution for 30 seconds. Then these pieces washed three times by distilled water to remove the disinfectants and blotted dry and then transferred to solid potato dextrose agar plates aseptically by sterile forceps. The inoculated plates were incubated at 25oC for 4-6 days and examined daily for the growth of fungus. The cultures were identified according to their cultural and morphological characteristics using standard literature.

Collection of Plant Materials:

The leaves of three plants namely Eucalyptus globulus L., Lawsonia inermis L., Vitex negundo L., were collected from the campus of Govt. Institute of Science, Aurangabad. The collected leaves were surface sterilized with 0.1% mercuric chloride and then washed with distilled water, 2-3 times separately and shade dried, finely powdered and used for the experimental work.

Phytochemical Analysis:

Qualitative phytochemical analysis of the crude powder of the 03 plants collected was determined as follows:

i) Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2ml filtrate + 2ml FeCl3, blue-black precipitate indicated the presence of Tannins.

ii) Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2ml filtrate + 1% HCl + steam, 1ml filtrate + 6 drops of Mayors reagents/ Wagner's reagent/ Dragendroff reagent, creamish precipitate/ brownish-red precipitate/ orange precipitate indicated the presence of respective alkaloids.

iii) Saponins (frothing test: 0.5ml filtrate + 5ml distilled water); frothing persistence indicated presence of saponins.

iv) Cardiac glycosides (Keller-Kiliani test: 2ml filtrate + 1ml glacial acetic acid + FeCl3 + conc. H2SO4) green-blue color indicated the presence of cardiac glycosides.

v) Steroids (Liebermann-Burchard reaction: 200mg plant material in 10ml chloroform, filtered); a 2ml filtrate + 2ml acetic anhydride + conc. H2SO4. Blue-green ring indicated the presence of terpenoids.

vi) Flavonoid (200mg plant material in 10ml ethanol, filtered); a 2ml filtrate + conc. HCl + magnesium ribbon pink-tomato recolor indicated the presence of flavonoids

Preparation of leaf extracts:

Aqueous extract:

The fresh leaves of healthy plants were collected and washed thoroughly with tap water and air dried. Ten grams of plant tissue was ground using pestle and mortar by adding equal amount (10ml) of sterilized distilled water (1:1 w/v). The extract was filtered through muslin cloth. The supernatant was taken as standard plant extract solution (100%). Further, the extract was diluted by adding sterilized water to get different concentrations. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination.

Solvent extract:

Extracts were prepared in methanol at room temperature by simple extraction method (Deshpande et al., 2004). Collected plant parts were shade dried and ground to a fine powder using grinder mixer. Dried powder of plant parts (10g) was mixed with 100 ml solvent in 250 ml conical flask. The flasks were plugged tightly with cotton and wrapped with papers. All conical flasks were kept on shaker for 24 h then it was allowed to stand for five hours to settle the plant materials. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 45°C in vacuum evaporator to make the final volume 1/5th of the original volume. It was stored at 4°C in airtight bottles for further studies.

Antifungal activity:

The plant extracts were evaluated in vitro through Poison food technique (Nene and Thapliyal, 2000). The filtrate was considered as standard extract solution (100%). Required concentrations were obtained by adding appropriate amount of sterile distilled water. 2 ml of each test extract was dispensed in petriplates (9 cm) with the help of sterilized pipettes. After pipetting the extract solution in petriplates, 20 ml of molten potato dex-

trose agar (PDA) was poured in petriplates containing the extract solution. The plates were gently rotated to ensure uniform distribution of extract in the media. After solidification, discs of 6mm cut from 7 days old cultures of targeted fungal pathogens were inoculated at the center of petriplates aseptically. Three replications were maintained for each treatment. The media without extract served as check. The plates were incubated at 27 ± 1°C till the complete growth was observed in control plates. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition.
C = Colony diameter in control.
T = Colony diameter in treatment.

RESULT:

In the present investigation three plant extracts were evaluated under in vitro condition against five fungal pathogens to know the fungitoxic nature of their extracts. Among them aqueous leaf extract of Lawsonia inermis showed maximum inhibition (89.67 %) followed by extract of Eucalyptus globulus (73.06%), extract of Vitex negundo (43.93%). Where as in case of methanol extracts, Maximum inhibition showed by Vitex negundo (88.79%) followed by Eucalyptusb globulus (86.75%), extract of Lawsonia inermis showed maximum inhibition (81.49%). In aqueous extract antifungal activity was observed against all the test fungi and the extent of percent of mycelia growth inhibition was varied from 24.24% to 92.63% at 100 % concentration.

Table 1: Qualitative Phytochemical Analysis of the extracts:

Plant Species	Extracts	Tannins	Alkaloids	Saponins	Cardiac glycosides	Steriods	Flavodoids
Lawsonia inermis	H2O	+	+	+	-	-	+
	MeOH	+	+	-	+	+	+
Eucalyptus globulus	H2O	+	+	-	-	-	+
	MeOH	+	+	+	-	+	+
Vitex negundo	H2O	+	+	-	-	-	+
	MeOH	+	+	+	+	-	+

(+) indicates presence while (–) indicates the absence of the components.

Table 2: Inhibitory effect of plant extracts 100% (v/v) on mycelia growth of five fungal Pathogens of vegetables.

Plant Species Extract		F. oxysporum		F. solani		A. solani	
		Mycelial Growth (mm)	% inhibition over control	Mycelial Growth (mm)	% inhibition over control	Mycelial Growth (mm)	% inhibition over control
Lawsonia inermis	Water	66	92.63	66	92.63	13	82.33
	MeOH	86	90.36	13	85.15	73	91.85
Eucalyptus globulus	Water	30	65.73	13	75.95	13	81.16
	MeOH	13	85.10	12	85.89	73	90.94
Vitex negundo	Water	42	42.89	48	45.75	35	24.24
	MeOH	93	89.50	14	83.90	66	91.60
Control	-	86	0.00	88	0.00	73	0.00

Values expressed in mean of triplicates.

Table 3: Inhibitory effect of plant extracts 100% (v/v) on mycelia growth of five fungal Pathogens of vegetables.

Plant Species ExtracMycelial Growth (mm)		R. solani		C. lidemuthianum	
		% inhibition over control	Mycelial Growth (mm)	% inhibition over control	
Lawsonia inermis	Water	66	92.63	10	88.16
	MeOH	66	90.36	66	92.63
Eucalyptus globulus	Water	25	68.37	22	74.11
	MeOH	13	85.10	11	86.75
Vitex negundo	Water	41	53.04	41	53.75
	MeOH	10	88.12	73	90.87
Control		87	0.00	86	0.00

Values expressed in mean of triplicates.

DISCUSSION:

On the basis of the results obtained it can be suggested that L. Inermis, E. globulus and Vitex negundo has a broad spectrum antifungal activity against various plant pathogenic fungi. Saha (1997) reported that, leaf extract of Lawsonia inermis completely controlled the growth of Drechslera oryzae, Sclerotium oryzae, S. rolfsii and Rhizoctonia solani at 20% (w/v) concentration. Antifungal and antibacterial activity of Eucalyptus globulus may be due to the presence of eucalyptin, eucalyptol and elligatannin compounds (Gutierrez et al. 1999). As compared to aqueous extracts organic solvent extracts was found to be more effective. Antimicrobial activity of plants is due to the presence of secondary metabolites which are extracted in organic solvents due to their solubility (Cowan.,1999). Alkaloids, steroids, quinines and tannins are highly soluble in these solvents (Kokate et al., 1990.). In our findings, aqueous leaf extract of V. negundo showed highest activity of 43.93% whereas its methanol extract showed Maximum inhibition of 88.79%. It has been reported that methanol is a better solvent for consistent extraction of antimicrobial substances from plants as compared to other solvent such as aqueous, ethanol and hexane (Lin et al., 1999). Phytochemical analysis of these six extracts revealed the

presence of the alkaloids, flavonoids, tannins, cardiac glycosides and terpenoids. These bioactive molecules are reported to give resistance to plants against pests and pathogenic infections (Dixon., 2011; Wang et al., 1989).

CONCLUSION:

In conclusion, the results obtained from this study indicated that chemical constituents from these plant species may be developed as potential biofungicides in agriculture. Further phytochemical studies are required to determine the types of compounds responsible for the antifungal effects of these species.

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