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RESEARCH ARTICLE

Antifungal activity of *Terminalia chebula* extract against wilt fungi *Fusarium oxysporum* an important plant pathogen

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ABSTRACT:
Terminalia chebula, a deciduous plant, endogenous to India has medicinal value. Its fruits are known for its cure to many ailments in human beings. This study is to test the biological activity of these compounds tested against plant pathogenic fungi *Fusarium oxysporum* which cause wilt in economically important crops. Wilt is a devastating disease which destroys the infected plant and cause economic loss to farmers. Panama disease in bananas is a type of wilt disease which is very prevalent in India. This fungi has broad host range from tomato, cotton etc. Outbreak reported in many parts of India. Standard culture of *Fusarium oxysporum* ITCC -6246, *Fusarium oxysporum f. sp. ciceri* ITCC 3636 and *Fusarium oxysporum f. sp. lycopersici* MTCC 10270 were used to detect the potential chemical compound in dried fruit by macro-dilution. Each organic solvent (Water, Ethanol, Chloroform, Benzene) has different extraction capacity based on the polarity of chemical compound in plants. These chemical compounds extensively studied for their antioxidant property and preliminary screening of chemical compound constituents in all extract. Mycelium growth inhibition tested on solid medium and zone of inhibition measured in mm diameter. Spores of *Fusarium* sp. present in soil for many months in dormant form. Under suitable climatic conditions this will germinate and cause infection in plants. Rate of spore germination inhibition in liquid medium measured and calculated for every solvent extract. Rate of inhibition was calculated and analysed statistically by t test. The efficient solvent extract determined for bulk extraction of biologically active compound from seeds.

KEYWORDS: Phytochemical, Fungicides, Plant pathogen, Macro-dilution, Plant diseases.

INTRODUCTION:
Phytochemicals are plant based substances produced by the plant for the plant to protect itself from the adverse condition and from impact of mircoorganisms, animals, insects etc[1,2,3]. They are considered non-nutritive but biologically active compound. Phytochemicals are well known for its beneficial[4,5,6,7,8] effect but some are destructive. They are distinct to each plant even specific to its part. Varied climatic condition in India influence the production of secondary phytochemical in plants. Phytochemicals are not only used as medicine but also used as nutritional supplements, enhance taste, flavour and colour.

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Phytochemicals based on their chemical nature are divided in to many categories, carbohydrates, lipids, phenolic compounds, terpenoids and alkaloids[9,10,11]. Each of these compound has varying degree of antioxidant property[12], biological activity and it is remedy for many ailments. Pharmaceutical products like suspension prepared with plant based antioxidant to improve self-life.

Phenolic compound contain benzene rings with simple hydroxyl group to highly complex side chain. These secondary metabolite broadly classified under flavonoids, phenolic acids, xanthons, lignans and anthocyanins. Phenolic compounds helps to pollinate, protect from animals and antimicrobial effect[13,14,15,16]. Terpenoids otherwise known as isoprenoids impart flavour and fragrance known to have antimicrobial activity, it helps the plant to interact with environment and tolerance to adverse condition eg. Vitamin A. Alkaloids natural base contain nitrogen, reserve nitrogen acts as growth stimulator and mainly involved in plant defence. They are soluble in water under acidic, in neutral or basic condition soluble in lipids[17]. These molecules have wide range of application[18].

In this study inhibitory activity of extracted compound from seed of *Terminalia chebula* tested for spore germination of plant pathogenic fungi, *Fusarium oxysporum* (Wilt in Tomato, Banana, Guava), *Fusarium oxysporum f. sp. ciceri* (Wilt in Chickpea) and *Fusarium oxysporum f. sp. lycopersici* (Wilt in tomato).

Management of these plant diseases is a complex activity as there is always need of new compound to control the growth[19] of fungi targeting either mycelium or spore germination without affecting the ecosystem[20]. Many scientist are interested in phytochemicals to control plant disease, tested many compounds against plant pathogenic microorganisms by in vitro found capable of controlling the growth[21].

MATERIALS AND METHODS:

Sample collection and processing:

Terminalia chebula dried fruit sample were collected from Chennai, Tamil Nadu. Surface washed with sterile distilled water and air dried. These fruits were broken and divided into two parts, only seeds collected and powdered. Hundred grams of powdered *Terminalia chebula* seed materials were weighed into separate sterile conical flasks. The samples were extracted using 500 ml of aqueous, ethanol, chloroform and benzene separately and left for 48 hours at room temperature. The resultant suspensions were filtered into sterile conical flasks. These extracts were air dried and the residue was collected[15]if needed suspend in sterile double distilled water.

Preliminary phytochemical analysis:

Phytochemical composition[22, 23] of sample tested for sugars, amino acids, anthroquinins, coumarins, saponins, tannins, phenolic compounds, flavonoid, terpenoids and glycosides[24] results were tabulated (Table 1).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay:

DPPH radical scavenging assay was performed to test presence of antioxidants[4,13,14,16]. In brief, 0.135 mM DPPH was prepared in methanol. Different concentration of extract (5, 10, 20, 40, 80, 160 and 320 µg/ml) was mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexes thoroughly and kept at room temperature for 30 min. Ascorbic acid was used as the reference standard. The Absorbance of the mixture was measured at 517 nm. The ability of plant extract to scavenge DPPH radical and control was calculated from the following formula: respectively.

Rate of DPPH inhibition = [(absorbance of control - absorbance of test)/(absorbance of control)] ×100

Preliminary study for antifungal activity by well diffusion:

Seven day old culture of *Fusarium oxysporum* seeded by spread-plate method on Mueller-Hinton agar. Approximately wells of uniform size (0.65 cm) were made with a cork-borer onto the plates inoculated with test organisms. Crude plant extracts of 250 µl, 500 µl, 500µl and 1000µl were respectively added into each well aseptically. Triplicates were used as replicates for each treatment. Inoculated plates were incubated at room temperature until the fungal growth of the control plates reached the edge of the plate. Zone of inhibition measured and mean calculated for further analysis.

Antifungal activity by dilution method:

Standard fungal plant pathogen were selected to test the ability of seed extract for their potential antifungal capabilities. *Fusarium oxysporum* ITCC -6246, *Fusarium oxysporum f. sp. ciceri* ITCC 3636 and *Fusarium oxysporum f. sp. lycopersici* MTCC 10270 were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India and Indian type culture collection (ITCC), New Delhi, India. Maintained the strains on Potato dextrose agar at 4°C. Fifty mL of sterile distilled water was added to 7 day old culture grown on potato dextrose agar (PDA) and scraped gently, filtered through Buchner funnel under sterile condition. The filtrate was adjusted to $1x10^5$ spores/ml and used for further study. 100µl of conidial suspension, 100µl of *Terminalia chebula* (ethanol, chloroform, benzene, water) extracts of seed with various concentrations ranging from 1.0, 2.0, 5.0 mg/mL were added separately and incubated in a shaker at 28°C with 120 rpm for 10 days. For control, test tube received 100µl of conidial suspension and 100µl of sterile distilled water. Optical readings were taken at 600 nm at every 2 days interval up to 10 days. All procedures described above were carried out under sterile conditions. Experiments were done in triplicate mean was calculated, rate of inhibition was calculated by (aborbance of control-abosrbance of test sample)/ (aborbance of control × 100).

RESULTS AND DISCUSSION:

1. Phytochemical constituents of extracts:

Various chemical constituents expressed as high, medium, low and nil for four solvent extract.

2. Antioxidant assay:

Ethanol extract shows maximum and benzene extract shows least antioxidant activity. Polar compounds from the dried fruit exhibit higher antioxidant activity than nonpolar compound extracted with benzene (Fig.1)

Table 1: Phytochemical analysis of *Terminalia chebula* seeds for the following constitutions

Plant constitutions	Seed extract with solvent			
	Aqueous	Ethanol	Chloro form	Benzene
Steroids	+	+++	++	-
Terpenoids	+++	-	++	+
Reducing sugar	-	+++	++	+
Sugars	-	-	-	++
Phenolic compound	++	+++	+	-
Flavonoids	-	-	-	-
Saponins	-	-	++	+++
Tannins	-	+	+++	+++
Amino acids	+	+++	++	-
Glycoside	-	+++	-	++
Coumarins	-	+++	+++	+
Anthroquinon es	+++	++	-	-

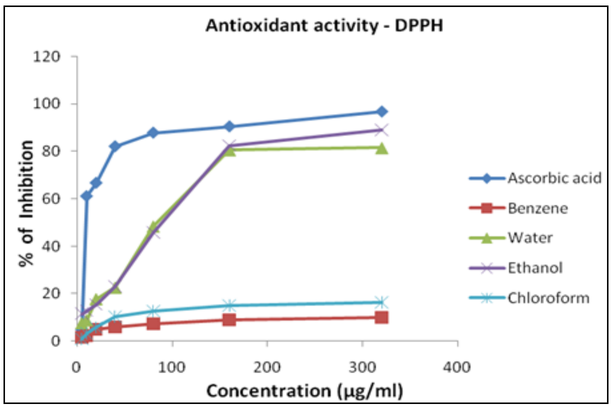

Fig 1: DPPH method of antioxidant assay of *Terminalia chebula* dried seed extract.

3. Well diffusion:

Mean value of diameter of zone of inhibition in mm of different solvent extract on *Fusarium oxysporum* ITTC 6246 in table 2.

Table 2 Zone of inhibition in mm against *Fusarium oxysporum* ITCC 6246 of different solvent extract

Solvent extract	250 µl	500 µl	750 µl	1000 µl
Water	0	0	0	0
Ethanol	13.06	14.63	14.76	21.03
Chloroform	12.06	13.2	15.06	18.1
Benzene	0.53	0.66	21.13	28.06

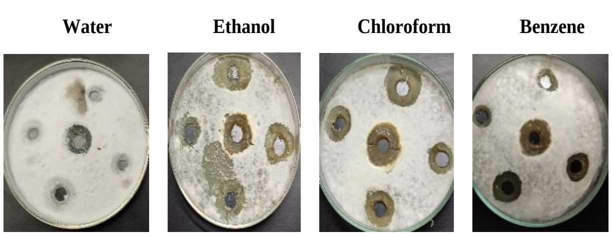

Fig 2: Zone of inhibition of various Organic solvent extract water, ethanol, chloroform and benzene against *Fusarium oxysporum* ITCC6246

4. Rate of inhibition:

Spore germination inhibition of *Fusarium oxysporum* ITCC -6246, *Fusarium oxysporum f. sp. ciceri* ITCC 3636 *and Fusarium oxysporum f. sp. lycopersici* MTCC 10270 absorbance measured in UV spectrophotometer, from mean value rate of inhibition tabulated (Table 3, 4 and 5) rate of inhibitory concentration (IC) range from 17 to 93. IC 70 considered as most efficient.

Table 3: Rate of inhibitory concentration of *Terminalia chebula* seed extract against *Fusarium oxysporum* ITCC -6246 using different solvent

Solvent	Days	Negative Control	1.0 mg/mL	2.0 mg/mL	5.0 mg/mL
	2	0.264	47.34	64.77	70.07
Water	4	0.408	40.44	51.71	62.5
	6	0.671	48.88	54.39	64.08
	8	0.898	36.63	41.75	51.33
	10	0.989	19.21	28.87	41.45
	2	0.264	66.66	80.68	91.28
Ethanol	4	0.408	66.17	75.98	85.04
	6	0.671	70.78	77.94	86.14
	8	0.898	69.59	77.83	84.74
	10	0.989	66.53	72.29	81.69
	2	0.264	59.09	74.24	85.6
Chloroform	4	0.408	57.35	63.72	77.69
	6	0.671	59.16	63.18	73.47
	8	0.898	47.88	57.34	65.36
	10	0.989	37.61	49.14	51.66
	2	0.264	57.95	66.28	74.62
Benzene	4	0.408	46.81	57.59	67.4
	6	0.671	52.45	58.56	65.57
	8	0.898	40.86	48.99	55.56
	10	0.989	25.37	35.08	43.07

Table 4: Rate of inhibitory concentration of *Terminalia chebula* seed extract against *Fusarium oxysporum f. sp. ciceri* ITCC 3636 using different solvent

Solvent	**Days**	**Negative Control**	**1.0 mg/mL**	**2.0 mg/mL**	**5.0 mg/mL**
	2	0.243	45.67	55.96	60.08
Water	4	0.314	25.47	35.35	53.18
	6	0.597	40.87	50.08	51.25
	8	0.863	33.83	42.52	53.88
	10	0.971	17.71	21.93	38.61
	2	0.243	76.13	80.24	93
Ethanol	4	0.314	51.27	58.28	71.65
	6	0.597	60.3	70.18	77.05
	8	0.863	63.26	72.88	80.99
	10	0.971	61.99	71.36	79.6
	2	0.243	60.08	74.07	79.01
Chloroform	4	0.314	37.26	45.54	58.91
	6	0.597	49.91	58.29	60.13
	8	0.863	45.19	51.68	59.32
	10	0.971	34.7	44.59	59.93
	2	0.243	51.02	63.37	69.65
Benzene	4	0.314	29.61	42.03	57.96
	6	0.597	44.89	54.27	62.98
	8	0.863	37.77	43.91	56.89
	10	0.971	25.12	35.42	47.16

Table 5: Rate of inhibitory concentration of *Terminalia chebula* seed extract against *Fusarium oxysporum f. sp. lycopersici* MTCC 10270 using different solvent

Solvent	Days	Negative Control	1.0 mg/mL	2.0 mg/mL	5.0 mg/mL
	2	0.239	50.62	63.59	71.96
Water	4	0.304	25	44.73	58.22
	6	0.447	27.29	37.13	50.11
	8	0.743	27.72	32.57	44.68
	10	0.963	18.06	27.62	41.95
	2	0.239	78.66	86.19	89.95
Ethanol	4	0.304	68.09	77.96	86.51
	6	0.447	70.02	82.55	86.12
	8	0.743	74.83	82.36	88.02
	10	0.963	65.31	77.46	88.36
	2	0.239	71.54	78.66	87.44
Chloroform	4	0.304	86.5	55.92	61.51
	6	0.447	45.63	50.55	61.74
	8	0.743	57.33	60.02	63.52
	10	0.963	46.62	51.92	62.82
	2	0.239	58.99	71.96	77.4
Benzene	4	0.304	37.82	50.32	61.18
	6	0.447	41.61	40.26	53.46
	8	0.743	40.64	38.49	47.91
	10	0.963	24.09	34.78	49.53

5. **Efficacy of crude seed extract.**

Around seventy percent of spore germination inhibition rate considered as significant activity of seed extract (mg/mL) against plant pathogenic fungi. Initial incubation with ethanol extract effectively inhibit *Fusarium oxysporum* ITCC -6246, *Fusarium oxysporum f. sp. ciceri* ITCC 3636 and *Fusarium oxysporum f. sp. lycopersici* MTCC 10270 spore germination. 1mg/mL Effective minimum inhibitory concentration of ethanol extract against *Fusarium oxysporum f. sp. lycopersici* MTCC 10270, t test performed in excel p value less than 0.05 confirms the significant antifungal activity.

CONCLUSION:

Terminalia chebula seed extract possess antifungal activity against *Fusarium sp.* Based on these analysis ethanol extract alternate for synthetic compound controlling plant fungal disease. Further chloroform extract contain biologically active phytochemical which will play major contribution in controlling mycelium growth. Phytochemicals found in these extract used to control wilt fungi spore germination as well as mycelial growth repectively.

CONFLICT OF INTEREST:

The author(s) declare no conflict of interest.

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