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Antifungal property of *Tridax procumbens* L. against three phytopathogenic fungi

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Abstract: Experiments were conducted to check the antifungal effect of *Tridax procumbens* L. leaf extract of two concentrations (0.1% and 0.01% Methanolic extract) in PDA medium on *Helminthosporium oryzae,Rhizoctonia solani* and *Pyricularia oryzae.* The radial growth (R.G.) of these fungal pathogens were significantly effected by 0.1% concentration of extract. The leaf extract has shown higher efficacy in the percent inhibition of radial growth of *H.oryzae* with respective values of *R.solani* and *P.oryzae.* In 0.1% and 0.01% concentrations, the comparative magnitude of percent inhibition was found more in *H.oryzae* and *R. solani* as compared to other two pathogens over their respective control. Upon separation of the Methanolic extracts by Column Chromatography and TLC, the partial purified compound was found as flavonoid derivative compound.
Keywords: *Tridax* leaf extract and flavonoid derivative.

INTRODUCTION

Tridax procumbens L. is a small herb of Fam. Asteraceae. Its leaves cooked as a vegetable(Chatterjee,2001); they are also eaten by cattle(*Wealth of India,1988*). It is commonly known as 'Ghamra' in Hindi and in English popularly called 'Coat button' because of appearance of flowers(Vyas *et.al*, 2004). Aerial parts of TP Reported Immunomodulatory effects(Vyas *et.al*, 2004). Aerial parts shows Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats(Thiruvengadam *et. al.*,2005). Leaves are reported Hemostatic activity(Dhake *et.al*.,2008), effect on Blood pressure and Heart Rate in rats(Salahdeen et. al.,2004) and Anti-diabetic activity(Bhagwat et. al.,2008). The occurrence of β-sitosterol-3-O-β-D-xylopyranoside (Albert and Saxena,2005), lipid constituents(Gupta and Verma,1988), and saturated and unsaturated Fatty acid(Gabhe and Gadre,1988) from *Tridax procumbens.*

Several fungal pathogens are present in the nature which cause many harmful diseases to the essential and beneficial plants. These pathogens are not only related to tissue damage, nacrosis and rottening to the plant parts but reduces the yield performances also. As medicinal plants are evolved with the pathogens, these pathogens are destroyed by the antimicrobial principles extracted from these plants. In the present work, three such pathogen's activity was investigated by the external application of extracts from Calendula leaf.

Helminthosporium oryzae Breda de Haan of the family Pleomassarinaceae causes brown spot of rice. It is a serious disease of rice causing a massive damage of flag leaf, mature leaves and some parts of stem.

Rhizoctonia solani (teleomorph: *Thanatephorus cucumeris*) is a plant pathogenic fungus which causes sheath blight of rice and other related species.*R. solani* does not produce spores, hence it is identified only from mycelial characteristics. Its hyphal cells are multinucleate. Also,it produces white to deep brown mycelium when grown on artificial medium. The hyphae are 4-15 μm wide and tend to branch at right angles. A septum near each hyphal branch and a slight constriction at the branch are diagnostic.

Magnaporthe grisea (T.T. Hebert) M.E. Barr is the causal organism of fungal blast of rice leaf. *M. grisea* is an ascomycete fungus. It is an extremely effective plant pathogen as it can reproduce both sexually and asexually to produce specialized infectious structures known as appresoria that infect aerial tissues and hyphae that can infect root tissues.

MATERIALS AND METHODS

Antifungal activity of *Tridax* extract was studied by poisoned food technique (Nene and Thapliyal,1993). The pure culture of three fungal pathogen were obtained from

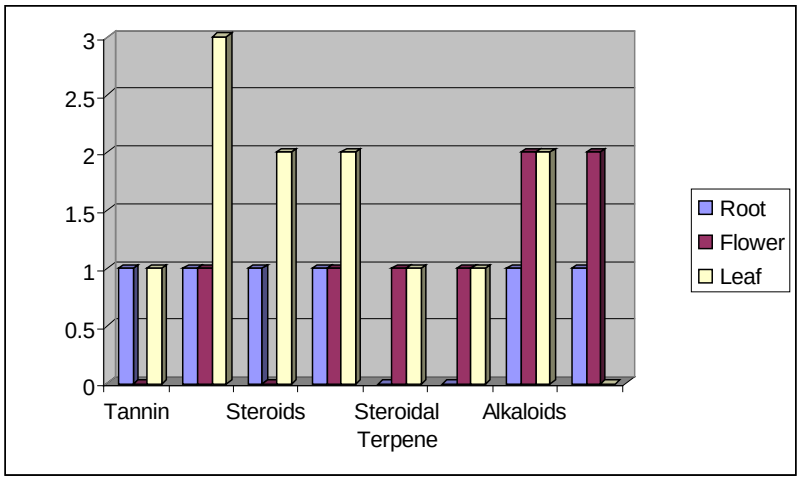

Fig 1: Qualitative Elucidation Of Various Bioactive Compounds In Different Parts

Table 1 : Qualitative presence of various bioactive compounds from different parts of test plant

Plant part	Tannin	Flavonoid	Steroids	Terpenoids	Steroidal Terpene	Cardiac Glycoside	Alkaloids	Saponin
Root	+	+	+	+	-	-	+	+
Flower	-	+	-	+	+	+	++	++
Leaf	+	+++	++	++	+	+	++	-

Table 2: Effect of different concentration of tridax extract on percentage inhibition in radial growth of the test fungi

Treatment	*Helminsporium oryzae*				*Rhizoctonia solani*				*Magnaporthe grisea*			
	R.G.		Percent inhibition		R.G.		Percent inhibition		R.G.		Percent inhibition	
	4^{th} Day	8^{th} Day	4^{th} Day	8^{th} Day	4^{th} Day	8^{th} Day	4^{th} Day	8^{th} Day	4^{th} Day	8^{th} Day	4^{th} Day	8^{th} Day
Control	2.2*	3.2	-	-	3.6	4.9	-	-	4.0	7.2	-	-
0.1%	0.8	1.2	63.6	62.5	1.4	2.0	61.1	59.1	1.6	3.0	60.0	58.3
0.01%	1.4	2.	36.3	37.5	2.2	2.8	38.8	42.8	2.4	4.2	40.0	41.6

*All the readings are based on average of triplicates

Indian Type Culture Collection (ITCC), Chandigarh. For the preparation of plant extract, fresh leaf sample of *Tridax* were collected and washed with tap water followed by sterile water. These samples were then processed with Chloroform at 1 ml/g tissue (1:1 v/w) with an electric grinder and filtered through a double layered cheese cloth with sterile distilled water. The extracts thus obtained were subjected to low speed centrifugation (5000g for 10 min) and the clear supernatants were collected. This formed the standard plant extract solution (100%). All these plant extracts are known to retain their fungal toxicity after a thermal treatment at 70° C for 10 min (Kurucheve *et. al.*,1997). Potato Dextrose Agar (PDA) medium sterilized at 15 lb for 20 min. in autoclave was added to the requisite quantity of leaf extract to get 0.1 and 0.01% final concentration. The leaf extract was thoroughly mixed by stirring with PDA. Twenty (20) ml medium of each concentration was poured into separate

sterilized Petriplate in triplicates with their respective control. After solidification, small disc(6mm) of fungas culture grown on PDA medium for 8 days was cut with sterile cork borer and inoculated in the center of Petriplates under asceptic condition. All the plates were incubated at 25±2°C. The radial growth of fungas after 4 and 8 days of incubation were recorded for each treatment (Sharma *et al*.,2007). The present inhibition over control was calculated and the mean values for each variable treatment i.e., fungi and treatments were computed for comparison. Percent inhibition was calculated by using following formula –

Percent Inhibition = (R.G. of colony in control - R.G. of colony in extract) X100
Radial Growth(R.G.) of colony in control

Activity guided chromatographic fractionation of the chloroform root extract:

Chloroform leaf extract being potent extract was subjected for chromatographic separation. Chromatographic extract (5.0g) was subjected to column chromatographic separation [length: 120 cm; diameter: 4 cm, stationary phase: silica gel(120g)] and eluted with [MeOH:H_2O (1:1)]. After the removal of solvent, a light brown mass was obtained and monitored by Thin Layer Chromatography (TLC) using solvent system [EtOAc: HCOOH: H_2O (8:1:1)]. The development of chromatogram in iodine chamber showed three spots. The brown mass was re-chromatographed using column [length: 100 cm; diameter: 3 cm, stationary phase: silica gel(75g)] and eluted with different composition of solvent mixture [$CHCl_3$: MeOH]. Different fractions of 20ml were collected and subjected to TLC to ensure their purity using [EtOAc: $CHCl_3$:CH_3OH(8:1:1)] solvent system. Fractions of same R_f values (fraction 14-31; R_f = 0.71 labeled as FCH^1, fraction 44-58; R_f = 0.83 labeled as FCH^2 and fraction 86-94; R_f = 0.90 labeled as FCH^3) were mixed. Removal of solvent furnished a white (FCH^1), pale yellow(FCH^2) and dark yellow(FCH^3) compound.

Antifungal bioassay of the chloroform fractions:

The compounds(FCH^1, FCH^2 and FCH^3) recovered from chloroform extracts were screened for antifungal activity against *Helminthosporium oryzae,Rhizoctonia solani* and *Pyricularia oryzae.* Different fractions were taken in concentration range (25-600 µg/ml). Compound FCH^2 was found to be more effective against *Helminthosporium oryzae, Rhizoctonia solani* and *Pyricularia oryzae* and considered as bioactive principle.

Acid hydrolysis of the compound(FCH^2):

Compound (FCH^2) was taken in 20% of ethanolic sulphuric acid in a round bottomed flask fitted with a reflux condenser. The reaction mixture was heated for about 8 hours on water bath. The content of the flask were extracted with water and aqueous layer was shaken with solvent ether in separating funnel. The ethereal layer was separated, washed and dried over anhydrous Sodium Sulphate. After removal of solvent, aglycone (FCH^2A) was obtained.

RESULTS AND DISCUSSION

For the presence of more amount of aromatic smell in leaf, root and flower and as per the inhibitory effects of *Tridax* leaf extract against three phytopathogenic fungi, different chemical tests were carried out on the aqueous extract and on the powdered specimen using standard procedures to identify the constituent as described by Sofowara(1993), Trease and Evans (1989), Harborne(1973) and Edeoga *et al*.(2005) hypothesising the presence of various bioactive compounds. Among the all bioactive compounds, the flavonoids showed maximum inhibition over three pathogenic fungi which is mostly found in leaves.

From the data presented in table, it is clear that after 4 days, 0.1% concentration Calendula leaf extract caused 63.6%, 61.1% and 60.0% inhibition of radial growth of *H. oryzae,R. solani* and *P. oryzae* respectively and 0.01% concentration gave 36.3%, 38.8% and 40.0% inhibition in the same order. Maximum inhibition was observed in *H. oryzae* in 0.1% concentration of leaf extract and *P. oryzae* in 0.01% concentration in same extract. After 8 days, 0.1% concentrated Calendula leaf extract caused 62.5%, 59.1% and 58.3% inhibition of radial growth of *H. oryzae,R. solani* and *P. oryzae* respectively and 0.01% concentration gave 37.5%, 42.8% and 41.6% inhibition in the same order. Maximum inhibition was observed in *H. oryzae* for 0.1% concentration of leaf extract and in *R. solani* 0.01% concentration in same leaf extract.

The higher level of percent inhibition was recorded in 0.1% concentration of Calendula leaf extract and followed by 0.01% concentration. Thus the maximum inhibition in 0.1% concentration was observed in *H. oryzae* as compared to *R. solani* and *P. oryzae*. This was essentially due to better efficacy of Calendula leaf extract against *H. oryzae* followed by *R. solani* and *P. oryzae* and the results supported the view that 0.1% concentration is more inhibitory than 0.01% concentration extract for the above same pathogens.

The compounds (FCH^1, FCH^2 and FCH^3) recovered from chloroform extract were screened for antifungal bioassay for reduction in colony diameter. Compound FCH^2 exhibited significant antifungal activity against all three fungi and was qualitatively analysed. FCH^2 positively responded to Molisch and Shinoda tests indicating the presence of flavonoidal glycoside type structural moiety.

Elucidation of the nature of bioactive principle(FCH^2)

Aglycone(FCH^2) was bioassayed for its antifungal properties against all three fungi. No appreciable change was observed in the antifungal activity of glycosidal flavonoid(FCH^2) and hydrolysed aglycone (FCH^2A). The aglycone was recrystallized from methanol into light yellow crystals (m.p. 193-195^{0}C) and ensured for its purity. The compound (FCH^2A) responded to all the characteristic tests of flavonoids/ isoflavonoids.

Various workers reported the fungitoxic nature of other plant species for same or other microbes (Bagul and Patel,2001; Bansal and Gupta,2000; Bhowmik and Chowdhary,1982; Biswas *et al*.,2002; Shivpuri *et al.*,1997; Amaresh *et al*., 2002; Daya,1997; Wadhawani *et al*.,1986; Gowdar and Reddy,2006; Sharma *et al*.,2005; Sharma *et al*.,2007; Dixit *et al*.,1978; Dubey *et al.*,1983; Grover and Moore,1962; Kishore *et al.*,1988; Mishra and Dubey,1994; Pandey and Dubey,1997; Phukan,2004; Rizvi *et al.*,1980; Sinha and Saxena,1987; Tewari *et al*.,2004; Tripathi and Dixit,1977; Tripathi *et al.*,1978; Verma and Dubey,1999) suggesting researches on the potential of herbal antimicrobials including those from *Tridax procumbens* Linn. and work on the identification of active ingradients is in progress.

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