International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Fungi-toxic Properties of Leaf Extracts of Some Herbaceous Wild Plants

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Abstract: ***In the present investigation fungi-toxic properties of leaf extracts of five herbaceous wild plant species namely, Ageratum conozoides, Blumea erianth, Chrozophora rottleri, Galingsoga parviflora and Phyllanthus niruri were evaluated in vitro against two phyto-pathogens viz. Alternaria solani and Rhizoctonia solani. Leaf extracts were prepared in three solvents i.e. Ethenol, Methanol and Hot water. Extracts of Ageratum conozoides and Chrozophora rottleri, showed complete (100%) mycellial inhibition of A. solani as well as R. solani at 10 % concentration which was comparable with respective fungicide at 100 ppm. Irrespective of plant species, extracts prepared in ethanol was most effective in arresting the mycelial growth of the pathogens whereas, superior phytotoxic activity was observed in Ageratum conozoides extracts and other four plants' toxicity was found in corresponding manner - Chrozophora rottleri > Galingsoga parviflora > Blumea eriantha > Phyllanthus niruri.***

Keywords: Fungi-toxic, Phyto-toxicity, Leaf Extracts, Herbaceous wild plants, Mycelial inhibition.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman 2001). Herbal Medicine is also known as botanical medicine or phyto-medicine, lately phyto-therapy has been introduced as more accurate synonym of herbal or botanical medicine. In the early twentieth century herbal medicine was prime healthcare system as antibiotics or analgesics were not as yet discovered. With the advent of allopathic system of medicine, herbal medicine gradually lost its popularity among people, which is based on the fast therapeutic actions of synthetic drugs (Singh 2007).

Weeds both endemic and exotic are posing threat to biological system, especially physiological and genetic systems. They greatly interfere with the growth of other plant species and microbes around them. This coupled with the production of numerous seeds has contributed to the rapid spread of these weeds and possess a threat to environment. Toxic properties of chemical constituent of such weeds may provide another aspect as antimicrobial activity.

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency (Ge et al. 2002; Nair and Chanda 2005; Neogi et al. 2008). Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world (Cohen 2002; Hancock 2005).

A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina et al., 2005; Romero et al., 2005). Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts (Low Dog, 2009).

Aims of present investigation was to identify the preliminary phytochemical screening of the various leaf extracts like ethanol, methanol and hot water of leaf of herbaceous wild plants (*Ageratum conozoides, Blumea erianth, Chrozophora rottleri, Galingsoga parviflora and Phyllanthus niruri*) collected within the areas of Bilaspur town of Chhattisgarh and to asses antifungal activity of their leaf extracts.

2. Material and Methods

2.1 Isolation of pathogen

A. solani and R. solani were isolated from naturally infected plant parts. Infected plant materials were washed cut into 5 mm segments and surface disinfected in 0.5% Sodium hypochloride solution for 5 min followed by washing in three changes of sterile distilled water. The segments were dried in between sheets of sterile filter paper and plated on fresh sterilized Potato Dextrose Agar (P.D.A.) medium impregnated with Streptocycline (100 ppm), and incubated for 7 days at 26 ±1°C. Pure culture was obtained by sub-culturing three times and maintained on PDA slants in the refrigerator until required.

2.2 Collection and preparation of the plant extract

Fresh leaves were collected from the college campus and local fields. The plant materials used in the present study and their feature details are given in the Table-1. Fresh plant leaves were washed and surface sterilized (Mercuric

Chloride, 0.01%) and chopped finely in a blender before the extraction (Kurucheve et al., 1997). The plant materials were plunged in required quantity of solvent (1:1 w/v) in a glass beaker, heated over a hot plate at 90-100°C (45-50min) for hot water and kept overnight at room temperature for organic solvent (ethanol, methanol and hot water) extraction respectively. The pulp of the plant tissue along with the extraction solvent were then squeezed and filtered through 3 layers of muslin cloths followed by low speed centrifugation (5000 rpm for 5 min) to get the clear supernatant (Priya and Ganjewala, 2007). The residual solvents were evaporated at room temperature. Plant extracts thus obtained was crude standard stock solution (100%) (Tiwari et al., 2005).

Table 1: General description of herbaceous wild plants and its importance

Sl. No.	Vernacular name	Botanical name and Family	Habits & Habitat	Economic Importance	Images
1	Stonebreaker	*Phyllanthus niruri* L. Phyllanthaceae	Mesophytic, tropical wild plants, commonly found in Barren / Pasture/ Agriculture fields.	Generally used for problems of the stomach, genitourinary system, liver, kidney and spleen	
2	Suryavarti	*Chrozophora rottleri* Euphorbiaceae	Wet places like waste areas, along roads, in stream beds. Soil: clay (mud).	Juice of the fruit is given in cases of cough and colds.	
3	Goatweed	*Ageratum conyzoides* L. Asteraceae	Its growth in dirty areas, Aggressive colonizer. Troublesome weed in gardens.	As a medicinal plant, limited uses due to its toxicity. It is also an insecticide and nematicide.	
4	Potato weed	*Galinsoga parviflora* Asteraceae	Weed of moist places, plantations and high elevation grasslands.	When rubbed onto the body, the plant is useful in treating nettle stings, The juice of the plant is applied to treat wounds.	
5	Nimurdi	*Blumea eriantha* Asteraceae	Aggressive colonizer. Abundant along railway tracks, road sides and degraded lands.	Anti-diabetic	

2.3 *In – vitro* fungi-toxicity of crude extract

Fungi-toxic efficacy of plant extracts were evaluated in vitro, adopting poisoned food technique (Grover and Moore, 1962). Crude plant extract stock solution were used at three concentrations (5%, 10%, 20%) prepared by in mixing aseptically 5ml, 10ml and 20ml of stock solution in 100ml of semisolid sterilized potato dextrose agar medium (Tiwari et al., 2005) at a temperature of 40°-45°C. Potato dextrose agar medium alone, with different solvents and with fungicide Carbendazim (Bavistin) at 100ppm concentration served as positive, solvent and negative control respectively. Plates with gelled medium were inoculated in the center with 7mm diameter mycelial disk of 3 days old fungal culture. All the plates were then incubated at 26 ±1°C and mycelial growth was recorded after 96 hrs of incubation. Colony diameter is recorded twice perpendicularly. Percent inhibition of mycelial growth was calculated using the formula given below (Verma and Kharwar, 2006). % inhibition = 100 X Mycelial growth (control) - Mycelial growth (treatment) / Mycelial growth (control)

2.4 Experimental design and statistical analysis

All the experiments were arranged in completely randomized design with three replications. Data were analyzed with the help of software and means were compared by least significant difference (L.S.D.) at 5% level. Average percent Mean mycelial inhibition of solvent extracts and the trial means of medicinal plants were calculated for average.

3. Result and Discussion

A significant (P≤0.05) variability was observed in antifungal activity within different plant extracts at varying concentrations against two fungal pathogens i.e. *Alternaria solani* and *Rhizoctonia solani*. As the concentration of plant extracts increased, the affectivity against pathogens was also increased significantly (Table-2 and Fig. -1 & 2). Complete inhibition (100%) of mycelial growth was recorded in some plant extracts prepared in different solvents and also observed in respective fungicide (Carbendazim at 100 ppm) treatment. Inhibition of mycelial growth was not observed in solvent alone treatment as well as in control treatment.

Greater efficacy of *A. conozoides* in terms of mycelial inhibition at 5 % concentration was recorded for extracts prepared in ethanol (84.3%) followed by methanol (79.6%) against *A. solani* whereas, against *R. solani* it was observed 83.23%, which was at par (P≤0.05) with methanol (78.8%). Extracts prepared in hot water (24.6%) showed least activity against *R. solani* and *A. solani* respectively (Table-2). Complete mycelial inhibition (100%) was exhibited by extracts prepared in ethanol and methanol at 5% and 94.7% in hot water at 20% concentration against *R. solani*. Extracts of *C. rottleri* at 5% concentration prepared in ethanol (83.4%) was found significantly (P≤0.05) superior over other solvents followed by methanol (79.2%) against *A. solan.*

Table 2: Fungitoxicity of leaf extract in different concentration (5%, 10% & 20%) of herbaceous wild plant species in different solvent (Ethanol, Methanol and Hot water) as measured by percent mycelia inhibition (Mean ± SD) of *Alternaria solani* and *Rhizoctonia solani.*

Fungal Strains	Extracts	Percent Mycelial Inhibition (%)														
		Ageratum conyzoides			*Blumea eriantha*			*Chrozophora rottleri*			*Galinsoga parviflora*			*Phyllanthus niruri*		
		5%	10%	20%	5%	10%	20%	5%	10%	20%	5%	10%	20%	5%	10%	20%
Alternaria solani	Ethanol	84.3 ±0.31	100.0 ±0.02	100.0 ±0.0	39.7 ±0.67	68.5 ±0.36	87.9 ±0.06	83.4 ±0.27	100.0 ±0.0	100.0 ±0.0	29.4 ±0.51	64.45 ±0.62	98.7 ±0.04	26.3 ±0.74	61.41 ±0.56	81.4 ±0.29
	Methanol	79.6 ±0.61	100.0 ±0.01	100.0 ±0.0	29.1 ±0.57	62.2 ±0.61	81.9 ±0.02	79.2 ±0.32	100.0 ±0.0	100.0 ±0.0	26.1 ±0.47	59.2 ±0.71	93.9 ±0.09	25.3 ±0.74	57.2 ±0.63	76.2 ±0.18
	Hot water	24.6 ±0.71	39.2± 0.44	57.6 ±0.57	19.6 ±0.62	38.6 ±0.55	45.0 ±0.71	30.1 ±0.69	45.6 ±0.65	60.1 ±0.70	16.1 ±0.72	35.6 ±0.45	53.0 ±0.61	9.6 ±0.82	33.6 ±0.75	41.0 ±0.41
	Trial Mean	57.4 ±0.65	81.2 ±0.45	95.8 ±0.52	43.3 ±0.44	55.2 ±0.71	77.35 ±0.81	54.5 ±0.63	71.6 ±0.67	86.37 ±0.46	39.7 ±0.51	68.4 ±0.46	78.5 ±0.68	42. 7 ±0.35	52.3 ±0.54	71.7 ±0.73
	$LSD_{0.05}$	1.8	2.3	3.6	1.4	1.7	2.4	1.9	2.7	3.3	1.2	1.6	2.8	1.1	1.7	2.5
Rhizoctonia solani	Ethanol	83.23 ±0.21	100.0 ±0.0	100.0 ±0.0	66.73 ±0.52	81.3 ±0.41	96.7 ±0.02	77.3 ±0.31	100.0 ±0.0	100.0 ±0.01	66.7 ±0.59	86.8 ±0.64	99.91 ±0.01	58.7 ±0.49	69.43 ±0.67	90.02 ±0.31
	Methanol	78.8 ±0.32	100.0 ±0.0	100.0 ±0.0	57.8 ±0.69	75.0 ±0.71	91.9 ±0.01	69.6 ±0.71	100.0 ±0.0	100.0 ±0.02	63.8 ±0.49	81.0 ±0.74	98.9 ±0.01	51.6 ±0.69	64.2 ±0.71	84.12 ±0.41
	Hot water	13.4 ±0.64	55.4 ±0.44	94.7 ±0.57	17.4 ±0.61	49.2 ±0.45	57.7 ±0.51	17.5 ±0.69	55.0 ±0.65	89.6 ±0.70	20.4 ±0.71	52.3 ±0.45	62.3 ±0.61	20.1 ±0.56	43.2 ±0.39	49.7 ±0.61
	Trial Mean	56.8 ±0.72	83.2 ±0.65	97.8 ±0.54	43.7 ±0.46	57.2 ±0.71	78.6 ±0.81	52.5 ±0.71	71.6 ±0.67	88.7 ±0.39	44.7 ±0.71	68.4 ±0.46	81.3 ±0.63	41. 5 ±0.38	55.3 ±0.57	74.7 ±0.62
	$LSD_{0.05}$	1.9	2.4	3.8	1.4	1.8	2.4	1.8	2.1	3.7	1.4	1.9	2.5	1.2	1.5	2.8
Control		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solvent alone		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fungicide		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

The extracts of B. eriantha, G. parviflora, and P. niruri in ethanol at 5% concentration shown mycelial inbition 39.7%, 29.4% and 26.3% against Alternaria solani, whereas it was shown lesser than mycelial inbition against Rizoctonia solani i.e. 66.73%, 66.7% and 58.7%. Similarly methanol extracts of these three plants was observed more mycelial inhibition more against R. solani than A. solani. However, hot water extract against both fungi was observed at par (P≤0.05). At 10% concentration significantly (P≤0.05) greater mycelial inhibition of R. solani was exhibited by extracts prepared in ethanol followed by methanol and hot water (Table 2). Complete mycelial inhibition (100%) was recorded for A. conozoides and C. rotlari extracts prepared in ethanol and methanol against R. solani and A. solani.

The present study was also in correlation with the previous report of Tiwari et al. (2007) which concluded that out of 950 plant extracts evaluated against R. solani, 153 at 10%, 141 at 5% and 79 at 1% concentration was effective in checking the mycelial growth. Similarly Nwachukwu and Osuji, (2008) reported the potential antifungal efficacy of Cassia alata (Candle bush) and Dennetia tripetala (Pepper fruit) leaf extracts on S. rolfsii.

Average value of percent mycelial inhibition by different solvents indicated that plant extracts prepared in ethanol were superior in exhibiting percent mycellial inhibition of followed by methanol and hot water (Fig-1 & 2). Several workers like Sengül et al. (2005) and Ozturk and Ercisli (2007) reported that methanol was a better solvent for

consistent extraction of antimicrobial substances from medicinal plants whereas, Masoko and Eloff (2006) found that acetone and methanol extracted more chemical compounds of antimicrobial potency from the leaves than other organic solvents. These observations can be rationalized by the fact that phytochemical compound dissolve in different solvents based on their polarity. In true fact most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent by Parekh et al., (2006) in the preparation plant extract for antimicrobial assay.

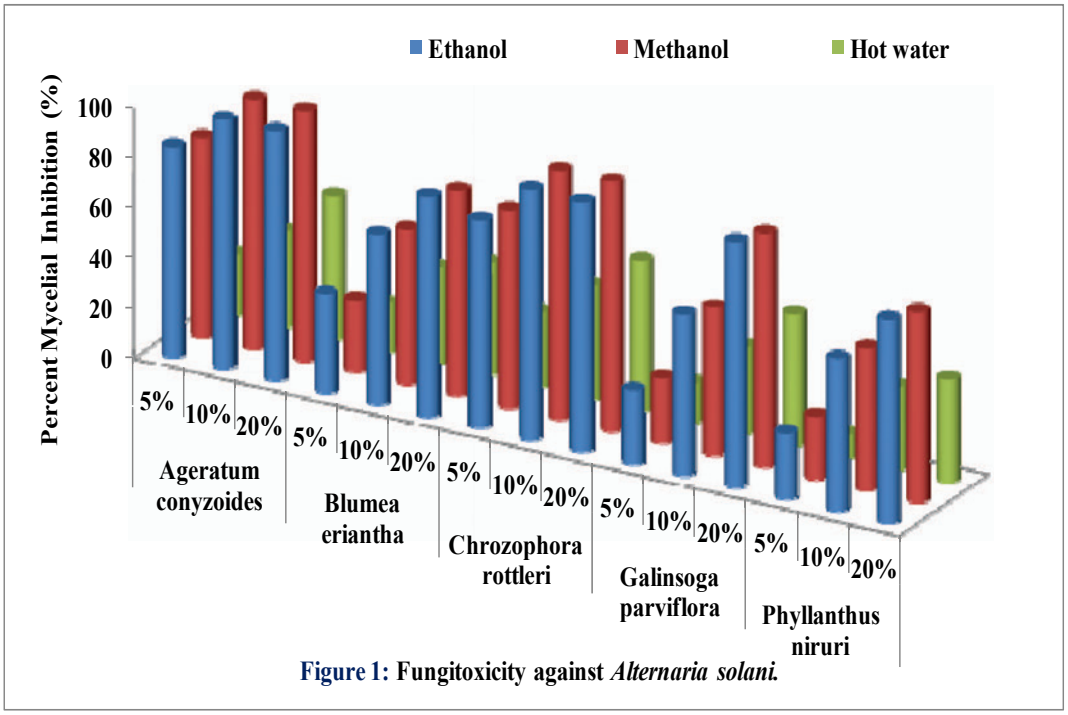

Figure 1: Fungitoxicity against *Alternaria solani.*

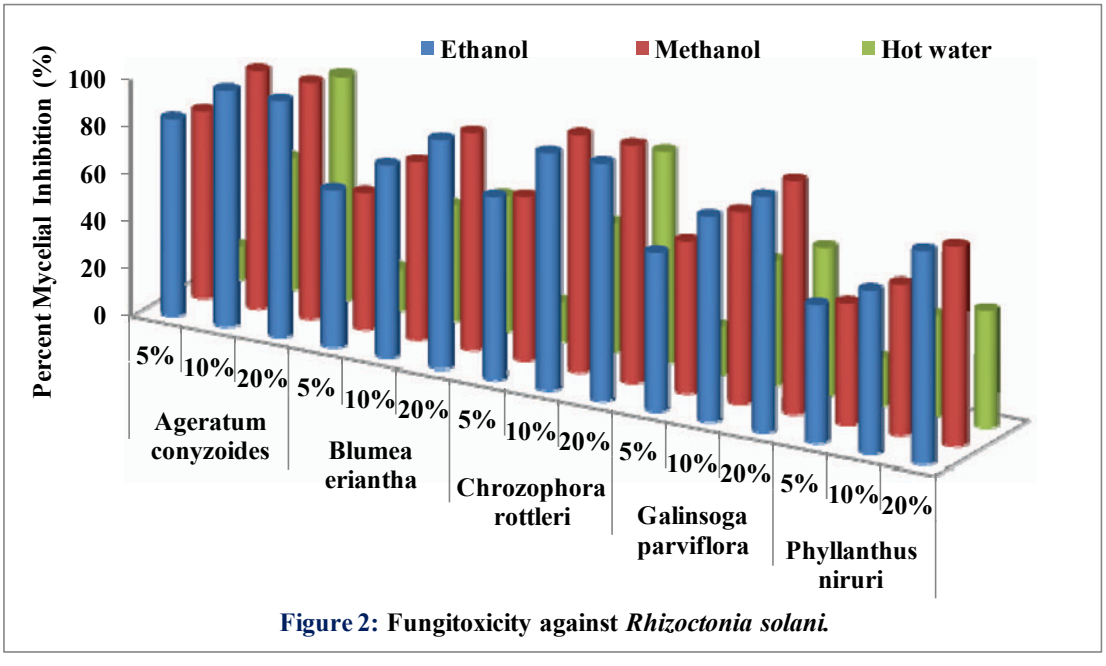

Figure 2: Fungitoxicity against *Rhizoctonia solani.*

4. Conclusion

Findings of the present investigation reveals that the leaf extracts of such herbaceous wild plant species have potential to yield potent fungicide compound targeted to phyto-pathogenic fungi. On the basis of present findings it may be concluded that to reduce the dependency on the synthetic pesticides / fungicides as well as a cost effective alternative, so many wild plant extracts are surely to be used to control plant pathogens.

Following the light of this remarkable finding, proper screening of such wild plant species and characterization of their extracts as the effective compound responsible for significant fungi-toxic property is necessary, which may lead to the formulation significant pesticides of plant origin. In

this way the result is highly considerable for development of antifungal herbal product by further research.

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