JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2008. Vol. **2**(2), p. 563-567

Antifungal Activity of Some Medicinal Herbs Extracts against *Fungi imperfectii*

Naveen Sharma, Jashoda Kumari, Madhu Rathore, Ankita Sharma and Kanika Sharma

Microbial Research Laboratory, Department of Botany, University College of Science, M.L.S University, Udaipur - 313 001, India.

(Received: 12 June 2008; accepted: 24 July 2008)

The antifungal activity of crude extracts of some medicinal herbs was assayed against plant pathogenic *Fungi imperfectii* such as *Drechslera graminea, Alterneria solani, Sclerotium rolfsii, Drechslera halodes, Fusarium moniliforme, Rhizoctonia solani, Curvularia lunata* and *Fusarium solani*. The results were evaluated by the diameter of zone of inhibition. Percent extractive value was also evaluated. The results were found against 50% alcoholic extract was good for inhibition of some fungus.

Key words: *In vitro*, antifungal activity, medicinal herbs, *Fungi imperfectii.*

Man is dependent on plants for food hence; destruction of crop plants due to infection by fungal pathogens has always been an area of prime concern. Scientists all over the world are involved in finding methods or developing techniques for control of plant diseases. Chemical control is the most common and prevalent method of disease control. Synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective. The flip side of this is that synthetic chemicals are harmful for human as well as soil health. They deteriorate soil fertility and characteristics, cause pollution, enter the food chain and cause several deleterious effects on human health and biosphere. Therefore, focus is shifting towards alternative strategies for control of fungal diseases in addition to well known disease management methods such as crop rotation, use of resistant cultivars, planting disease free seed, biological control etc. A search for an environmentally safe and economically viable strategy for the control of diseases has led to an increased use of plant based products in agriculture. Plant product preparations and bio-agents do not leave any toxic residues and therefore can effectively replace synthetic fungicides.

* To whom all correspondence should be addressed.
Tel.:+91-9314794504
E-mail:-naveenbiotechster@gmail.com

Use of plants as a source of medicine is as old as humanity. As the focus of the world is shifting toward natural products and their analogues, the demand of herbal medicine is also increasing and several plants have been screened for activity. Antifungal activity of plants or their extracts as well as essential oils have been studied by several workers (Ahmed *et al.,* 1999; Fiori *et al.* 2000; Guleria & Kumar, 2006; Thapliyal *et. al*. 2000; Cavaleiro *et al.,* 2006). In the present study, extracts of some medicinal herbs *Artemisia Absinthis, Origanum Majorana, Artemisia meritima, Lippia nodiflora* and *Andrographisa paniculata* will be screened for antifungal activity against certain deuteromycetous plant pathogenic fungi. Antifungal properties of these plants and another have been reported by several workers (Datar *et al.,* 1999; Yadav *et al.,* 2003; Jain & Sharma, 2006).

MATERIAL AND METHODS

Collection of plant material

All five medicinal herbs *i.e Artemisia absinthise, Origanum majorana, Artimisia meritima, Lippia nodiflora* and *Andrographis paniculata* were collected in the month of December, 2006 from Herbal Park, Rajasthan College of Agriculture, Maharana Pratap Agriculture University, Udaipur, India. Whole plant parts were shade dried at room temperature and finely ground in an electrical grinder and passed through sieve of mesh size 60 so as to obtained fine powder, which was used to prepare extract.

Preparation of crude extraction

100% alcoholic, 50% alcoholic as well as 100% aqueous extract was prepared by dissolving 20 g dried and powdered plant material in 100 ml of solvent (alcohol/water) for 48 hrs (Shadomy & Ingroff, 1974). The mixture was then filtered and supernatant was evaporated under reduced pressure using a rotary evaporator. The dried residue was used as extract, which were stored in an airtight jar refrigerator. Crude extract and fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator. The dried extract and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula and given in Table 1.

Assay of antifungal activity of crude extracts

Crude extract is a mixture of all secondary metabolites present in the plant. 50% alcohol fraction of herbs was found to be most potent against all plant pathogenic fungi. Aqueous extract and 100% alcohol extract found inhibitory effect to be lesser. Positive Control (Thirum) and negative control (50% DMSO + Distilled water) were used as a standard anti fungal. All other fractions produced significant zones of the inhibition but these were very less. Disc or agar well diffusion method (Collee *et al*., 1996) is commonly used to determine antimicrobial sensitivity. This method depends on the inhibition of fungal growth as an indicator of activity and is measured as a function of the diameter of inhibition zone. The activity of extract is always compared with that of the currently used antifungal standard. Several workers studied

Table 1. Percent extractive of whole plant

S. No.	Name of Plant	Crude Extracts (In percentage)		
		100% Alcohol	50% Alcohol	100% Aqueous
1.	*Artemisia absinthes*	4.45	4.62	4.82
2.	*Origanum majorana*	2.44	2.60	3.10
3.	*Artemesia meritima*	2.75	2.20	2.25
4.	*Lippia nodiflora*	4.80	5.05	4.50
5.	*Andrographis paniculata*	4.50	4.15	3.54

sensitivity of microbes against plant extracts by agar well method. Soundararaj *et al.* (1999) tested sensitivity of different bacteria against different fraction of *Vitex negundo*

RESULTS AND DISCUSSION

Result of percent extract of various extracts are given in Table 1. In case of *Lippia nodiflora* 50% alcoholic crude extract have maximum percent extract observed. Maximum percent extract in case of *Artemisia absinthes* and *Origanum majorana* was observed with 100% aqueous extract. Similarly the antifungal activity of crude plant extract against these plant pathogenic fungi *imperfectii* such as *Drechslera graminea, Alterneria solani, Sclerotium rolfsii, Drechslera halodes, Fusarium moniliforme, Rhizoctonia solani, Curvularia lunata* and *Fusarium solani*. 50% alcoholic crude extract showed the maximum antifungal activity against *A. solani, D. graminea, Drechslera halodes and Rhizoctonia solani*. No and very less activity was observed against *Fusarium solani, Fusarium moniliforme, Sclerotium rolfsii and Curvularia lunata.* Maximum inhibition zone was found against *Rhizoctonia solani* against all herbs. Fungi were found to be susceptible to thirum which produce a zone of inhibition ranging from 29 to 32mm. Solvent control and negative control were found to be ineffective and the culture showed the luxurious growth. Similarly aqueous

Table 2. Antifungal activity of whole plant extracts against *Alternaria solani* and *Drechslera graminea*

S. No.	Plant Name	Crude extarct									
		50% Alcoholic 10mg/ml		Abs. Alcohol 10mg/ml		AqueousExtract 10mg/ml		Control Thiram		50%DMSO	
		As	*Dg*	*As*	*Dg*	*As*	*Dg*	*As*	*Dg*	*As*	*Dg*
1.	*Artemisia absinthis*	31	24	NA	NA	20	12	33	31	NA	NA
2.	*Origanum majorana*	17	24	NA	18	15	NA	32	30	NA	NA
3.	*Artemesia meritima*	21	18	26	22	NA	NA	33	31	NA	NA
4.	*Lippia nodiflora*	NA	17	26	18	NA	8	30	29	NA	NA
5.	*Andrographis panniculate*	28	25	NA	24	NA	11	29	31	NA	NA
	SD	12.18	3.78	14.24	9.53	9.75	5.85	1.82	0.89	NA	NA

Key:-Values include cup borer diameter (10.00mm) and mean of three replicates, All values are in mm;
NA =Not Activity;Th =Thirum ;DMSO=Diemethylsulfaxide; *As:Alterneria solani; Dg:Drechslera graminea*

Table 3.Antifungal activity of whole plant extracts against *Drechslera halodes* and *Rhizoctonia Solani*

S. No.	Plant Name	Crude Extarct									
		50% Alcoholic 10mg/ml		Abs. Alcohol 10mg/ml		AqueousExtract 10mg/ml		Control Thiram		50%DMSO	
		Dh	*Rs*	*Dh*	*Rs*	*Dh*	*Rs*	*Dh*	*Rs*	*Dh*	*Rs*
1	*Artemisia absinthis*	22	18	25	21	NA	NA	32	32	NA	NA
2.	*Origanum majorana*	NA	26	NA	20	NA	12	30	30	NA	NA
3.	*Artemesia meritima*	24	23	NA	16	NA	7	32	32	NA	NA
4.	*Lippia nodiflora*	28	18	19	9	NA	NA	32	32	NA	NA
5.	*Andrographis paniculata*	29	19	NA	26	NA	9	30	30	NA	NA
	SD	11.87	3.56	12.24	6.35	0	5.41	1.10	1.10	0	0

Key:-Values include cup borer diameter (10.00mm) and mean of three replicates, All values are in mm,
NA =Not Activity;Th=Thirum;DMSO=Diemethylsulfaxide; Dh;*Drechslera halodes*; Rs;*Rhizoctonia solani*

extract did not showed significant inhibition of all test fungi in case of *Alterneria solani*, *Drechslera halodes, Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii, Curvularia lunata, Drechslera graminea and Fusarium moniliforme*. Antifungal activity of various extracts against the different test fungi are listed in (table 2, 3, 4, 5). 50% alcoholic extracts of *Artemesia absinthis* and *Andrographis panniculata* was found to be inhibitory against some test fungi except *Fusarium spp., Curvularia lunata, Rhizoctonia solani and Sclerotium rolfsii*. It showed best activity against *Alterneria solani*, *Drechslera halodes* and *Drechslera graminea*.100% alcohol showed selective inhibition. Best activity of 100% alcohol of all test pathogens observed against *Rhizoctonia solani*.Maximum inhibition of this fungus was also observed with 100% alcoholic extract of *Andrographis penniculata*, *Rhizoctonia solani* was found to be most susceptible fungus. The 50% crude alcoholic extract is effective against all plant pathogenic fungi.

In conclusion, the present observation shows that the contribution of various secondary metabolites by plants which are present naturally. These secondary metabolites showed inhibitory effect and might be possible to enhance the therapeutic and antifungal efficiency of plant due to the present of different secondary metabolites in crude extracts such as carvacrol, triterpenoids, thymol, terpenes, linalool, camphor, thujene etc. So it can be suggest that the medicinal herbs are useful for making herbal biofungicides.

Table 4.Antifungal activity of whole plant extracts against *Fusarium solani* and *Curvularia lunata*

S. No.	Plant Name	Crude Extarct									
		50% Alcoholic 10mg/ml		Abs. Alcohol 10mg/ml		AqueousExtract 10mg/ml		Control Thiram		50%DMSO	
		Fs	*Cl*	*Fs*	*Cl*	*Fs*	*Cl*	*Fs*	*Cl*	*Fs*	*Cl*
1.	*Artemisia absinthis*	NA	19	NA	NA	NA	NA	32	32	NA	NA
2.	*Origanum majorana*	NA	NA	NA	NA	NA	NA	30	30	NA	NA
3.	*Artemesia meritima*	NA	18	NA	NA	NA	NA	32	32	NA	NA
4.	*Lippia nodiflora*	NA	NA	NA	NA	NA	NA	32	31	NA	NA
5.	*Andrographis paniculata*	NA	NA	NA	NA	NA	NA	30	29	NA	NA
	SD	0	10.14	0	0	0	0	1.10	1.30	0	0

Key:-Values include cup borer diameter (10.00mm) and mean of three replicates, All values are in mm;
NA =Not Activity;Th=Thirum;DMSO=Diemethylsulfoxide; Fs= *Fusarium solani*, Cl=*Curvularia lunata*

Table 5. Antifungal activity of whole plant extracts against *Sclerotium rolfsii* and *Fusarium moniliforme*

S. No.	Plant Name	Crude Extarct									
		50% Alcoholic 10mg/ml		Abs. Alcohol 10mg/ml		AqueousExtract 10mg/ml		Control Thiram		50%DMSO	
		Sr	*Fm*	*Sr*	*Fm*	*Sr*	*Fm*	*Sr*	*Fm*	*Sr*	*Fm*
1	*Artemisia absinthis*	19	19	NA	NA	NA	11	32	32	NA	NA
2	*Origanum majorana*	14	24	17	19	NA	17	30	30	NA	NA
3	*Artemesia meritima*	17	16	14	14	NA	NA	32	32	NA	NA
4	*Lippia nodiflora*	NA	NA	13	NA	NA	NA	30	32	NA	NA
5	*Andrographis paniculata*	NA	NA	NA	NA	NA	NA	29	29	NA	NA
	SD	11.24	11.14	11.03	9.21	0	7.96	1.34	1.41	0	0

Key:-Values include cup borer diameter (10.00mm) and mean of three replicates, All values are in mm NA =Not Activity;
Th=Thirum; DMSO=Diemethyl sulfoxide; Sr=*Sclerotium rolfsii*, Fm:*Fusarium moniliforme*

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