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Antimicrobial and Antioxidant Potentials of *Verbesina encelioides* (Cav.) Benth. and Hook. Fil ex Gray

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Abstract: Methanol, cold water and hot water extracts from fresh roots of *V. encelioides*, a weed, were studied for their putative antimicrobial activities against select microorganisms (Bacteria: *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli*, *Pseudomonas aeruginosa* and Fungi: *Aspergillus niger*, *Candida albicans*, *Penicillium crysogenum*, *Tricophyton rubrum*) by disc diffusion method at different concentrations (2.5, 5 and 10 mg disc^{-1}) and for antioxidant potential by DPPH method. All the test extracts exhibited potential antimicrobial activity but hot water extract showed appreciable activity (IZ 23 mm) against *P. aeruginosa* and *P. crysogenum*. Hot water extract demonstrated 20.04% inhibition of DPPH at 80 µg concentration.

Key words: *V. encelioides*, antimicrobial activity, antioxidant activity, DPPH

INTRODUCTION

Verbesina encelioides Benth. and Hook. Fil. ex Gray (Family: Asteraceae, Vern. Golden Crownbeard), a weed, introduced from the Eastern United States shows aggressive and dominant growth abilities. This plant can tolerate wide range of climatic conditions including drought and high temperature (Kaul and Mangal, 1987).

It is known that the weeds are important source of medicines for indigenous people (Stepp and Moerman, 2001). Some work concerning its toxicity (Kingsbury, 1964; Oelrichs *et al*., 1981; Eichholzer *et al*., 1982), allelopathy (Inderjit and Dakshini, 1999) and chemistry e.g. terpenoids (Tiwari *et al*., 1978) and sesquiterpenes (Joshi *et al*., 1983) has been carried out. Earlier we have studied flavonol glycosides (Glennie and Jain, 1980), primary metabolites (Jain and Purohit, 1985), triterpenoids and pharmacological evaluation for antimicrobial, antiviral, anti-tumor, hypoglycaemic and anti-implantation efficacies (Jain *et al*., 1988, 2007a, b) in this plant. In the present study, fresh roots of *V. encelioides* have been investigated for their antimicrobial and antioxidant potentials.

MATERIALS AND METHODS

Plant Materials

Whole plants of *V. encelioides* were collected from the fields of University in the month of April, 2005, a voucher specimen (Herbarium No. 12977) of which is on deposit at the Herbarium University, Department of Botany, Jaipur, India.

Preparation of Extract

Fresh roots (1 kg) of *V. encelioides* were ground to small pieces and percolated in methanol and cold water (at 35°C) in succession for 12 h. Later, the residue was again extracted with water for 24 h

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by using Soxhlet apparatus. The resultant extracts were dried using rotary evaporator. Each of the extract was used for their antimicrobial bioefficacy, while the hot water extract was used for antioxidant potential only.

Antimicrobial Activity

Sources of Microorganisms

Pure cultures of test bacteria, *Bacillus subtilis (*ATCC 6633), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25668) were obtained from the IMTECH, Chandigarh, India. These cultures were grown and maintained on Nutrient Broth (NB) medium at 27°C for 48 h. Similarly, test fungi, *Aspergillus niger* (ATCC 322), *Candida albicans* (ATCC 4718), *Penicillium crysogenum* (ATCC 5476) and *Tricophyton rubrum* (ATCC 2327) obtained from IARI, New Delhi, India, were cultured on Potato Dextrose Agar (PDA) medium at 37°C for 48 h. These cultures were used for antimicrobial screening.

Bioassays

Antimicrobial assay of the extracts (methanol, cold water and hot water) were performed by disc diffusion method using NB and PDA medium (Gould and Bowie, 1952). Whatman No. 1 filter paper discs were enriched with different concentrations (2.5, 5 and 10 mg) of each extract which was dissolved in appropriate solvents. The density of microorganisms was adjusted as per McFarland 0.5 standard. One hundred microliter suspension of each microorganism was inoculated in the petriplates containing NB or PDA medium. The discs of test extracts were used and these plates incubated (SEW, New Delhi, India) at 37°C for 24 h. The diameter of the inhibition zone (mm) was measured (Antibiotic zone scale; Hi media) and in each case the Activity Index (AI) was also calculated. Three replicates were used and the average value was recorded. A parallel negative control and streptomycin (10 mcg $disc^{-1}$) and ketonocozole (10 mcg $disc^{-1}$) as positive controls were used.

Antioxidant Activity

2, 2- Diphenyl-1- picrylhydrazyl (DPPH; molecular formula $C_{18}H_{12}N_5O_6$) and quercetin were obtained from Hi media, India. The method used by Fogliano *et al*. (1999) was adopted with suitable modifications to our particular circumstances. Methanolic solution of DPPH (0.002 g/10 mL) and ascorbic acid (as positive control) was used.

Qualitative Assay

The rapid evaluation of antioxidant activity of hot water extract of roots was determined according to the DPPH method. In this procedure, the plant extract, quercetin and ascorbic acid as standard (20 mg) were dissolved in 1 mL methanol, out of which 1 μL in case was applied on TLC plates (Silica gel 60 $F_{254,}$ 20×20 cm). Later, these plates were sprayed with DPPH (0.002 g/10 mL) and exposed to daylight until discoloring of the background (6 h). Resulting yellow colour on the plates was determined as active antioxidant constituent. This method was also used for positive and negative control.

Quantitative Assay

For quantitative antioxidant assay, hot water extract (0.008 g) was dissolved in 10 mL of methanol and various concentrations (10, 20, 40, 60 and 80 μg) were prepared. Each 2.5 mL test extract was mixed with DPPH (0.002 g/10 mL) and allowed to stand for 30 min for the reaction to occur. The absorbance of the colour developed was measured at 517 nm by UV Spectrophotometer (Varian type Cary PCB 150 Water Peltier System with Standard Quattes). The negative control and positive controls (standard quercetin and ascorbic acid) were also used. Three replicates were run and the average absorption was noted for each concentration. Data was processed using EXCEL and inhibition of DPPH in percentage was calculated by following equation:

$$\% \text{ inhibition} = 1 - (A_1/A_2) \times 100$$

Where:
A_1 = Absorbance of the test sample
A_2 = The absorbance of control reaction

RESULTS AND DISCUSSION

The total yield of crude extracts was found to be variable (methanol- 0.536%, cold water- 0.990% and hot water- 0.400% on fresh weight basis). All the three extracts (methanol, cold water and hot water) demonstrated appreciable activity against most of the test bacteria and fungi. In antibacterial screening, the potential activity (IZ 23 mm) and (IZ 19 mm) was demonstrated by hot water extract at 10 mg disc^{-1} concentration against *P. aeruginosa* and *B. subtilis*, respectively (Table 1). Likewise, against fungi, pronounced activity (IZ 20, 21, 23 and 17) was recorded in hot water extract against *A. niger*, *C. albicans*, *P. crysogenum* and *T. rubrum*, respectively at the same concentration (10 mg disc^{-1}). It is noteworthy that 10 mg disc^{-1} the extracts exhibited greater efficacy as compared to the standards used.

The data of antioxidant activity of hot water extract as determined by DPPH method (Fig. 1) and the % inhibition at different concentrations is shown in Table 2. From the results, it is evident that

Table 1: Antimicrobial activity of *V. encelioides*

		Extracts (mg disc^{-1})								
		Methanol			Cold water			Hot water		
		2.5	5	10	2.5	5	10	2.5	5	10
Microorganisms		(mg)								
Bacteria										
B. subtilis	IZ[a]	±	8.00	9.00	-	-	-	7.00	11.00	19.00
	AI[b]	-	0.32	0.36				0.28	0.44	0.76
E. coli	IZ	7.00	8.00	9.00	-	8.00	10.00	7.00	8.00	10.00
	AI	0.35	0.40	0.45		0.40	0.50	0.35	0.40	0.50
E. aerogenes	IZ	-	9.00	15.00	±	10.00	11.00	8.00	11.00	15.00
	AI		0.50	0.83		0.55	0.61	0.44	0.61	0.83
P. aeruginosa	IZ	8.00.	11.00	17.00	-	9.00	16.00	9.00	17.00	23.00
	AI	0.50	0.68	1.06		0.56	1.00	0.56	1.06	1.43
Fungi										
A. niger	IZ	8.00	11.00	15.00	-	9.00	10.00	11.00	17.00	20.00
	AI	0.38	0.52	0.71		0.42	0.47	0.52	0.80	0.95
C. albicans	IZ	-	9.00	11.00	-	9.00	12.00	8.00	16.00	21.00
	AI		0.30	0.36		0.30	0.40	0.26	0.53	0.70
P. crysogenum	IZ	8.00	16.00	21.00	8.00	9.00	10.00	10.00	18.00	23.00
	AI	0.40	0.80	1.05	0.40	0.45	0.50	0.50	0.90	1.15
T. rubrum	IZ	7.00	9.00	10.00	7.00	10.00	15.00	7.00	9.00	17.00
	AI	0.28	0.36	0.40	0.28	0.40	0.60	0.28	0.36	0.68

Standard test drugs: streptomycin, ketonocozole, (10 mcg disc^{-1}); IZ[a] = Inhibition zone (in mm) including the diameter of discs (6 mm); AI[b] = Activity index= Inhibition area of the sample/Inhibition area of standard; - = No activity; ± = Trace activity

Table 2: Antioxidant activity of hot water extract of roots of *V. encelioides*

Concentration	% of inhibition (μg mL^{-1})		
(μg mL^{-1})	Hot water	Quercetin	Ascorbic acid
10	0.00	62.44	97.60
20	0.00	80.58	97.60
40	16.34	92.38	97.60
60	18.50	93.82	97.70
80	20.04	94.71	97.70

% inhibition = 1- (Absorbance of sample/Absorbance of control) ×100

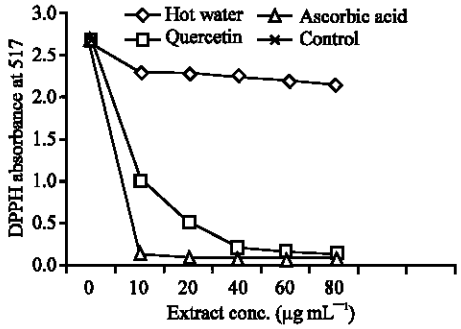

Fig. 1: Antioxidant potential of *V. encelioides*

the hot water extract at various concentrations (10, 20, 40, 60 and 80 µg mL^{-1}) exhibited antioxidant activity in a concentration dependent manner. At 80 µg mL^{-1} concentration, this extract showed 20.04% inhibition as compared to the standard quercetin (94.71%) and ascorbic acid (97.71%).

Our present findings further supports the earlier data (Jain *et al*., 1988) that hot water extract has the potential to synthesize several secondary compounds as chemical defense against herbivory of ecological functions. Further separation and characterization of the bioactive compounds is in progress and will be reported later.

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REFERENCES

Eichholzer, J.V., I.A.S. Lewis, J.K. MacLeod, P.B. Oelrichs and P.J. Vallely, 1982. Galegine and a new dihydroxyalkylacetamide from *Verbesina encelioides*. Phytochemistry, 21 (1): 97.

Fogliano, V., V. Verde, G. Randazzo and X. Retieni, 1999. A method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food. Chem., 47 (3): 1035-1040.

Glennie, C.W. and S.C. Jain, 1980. Flavonol 3, 7- diglycosides of *Verbesina encelioides*. Phytochemistry, 19 (1): 157-158.

Gould, J.C. and J.H. Bowie, 1952. The determination of bacterial sensitivity of antibiotics. Edinb. Med. J., 59 (4): 178-199.

Inderjit, C.A. and K.M. Dakshini, 1999. Allelopathic potential of *Verbesina encelioides* root leachate in soil. Can. J. Bot., 77 (10): 1419-1424.

Jain, S.C. and M. Purohit, 1985. Establishment of callus culture of three medicinally important plants and investigation of their metabolites. I. Herba Pol., 31 (1-2): 35-37.

Jain, S.C., M. Purohit and R. Sharma, 1988. Pharmacological evaluation of *Verbesina encelioides*. Phytother. Res., 2 (3): 146-148.

Jain, S.C., R. Jain, R. Singh and E. Menghani, 2007a. Galegine-Antimicrobial principle from *Verbesina encelioides*. Indian Drugs, 44 (1): 5-7.

Jain, S.C., R. Jain, R. Singh and E. Menghani, 2007b. Studies on *Verbesina encelioides*: Chemistry and bioactivity. Proceeding of Symp. on Plant Biotechnology: New Frontiers), CIMAP, Lucknow, India, pp: 439-445.

Joshi, K.C., P. Singh and C.L. Singhi, 1983. Chemical constituents of *Verbesina encelioides* and *Holmkioldia sanguinea*. J. Chem. Soc., 60: 905-906.

Kaul, M.L.H. and P.D. Mangal, 1987. Phenology and germination of Crownbeard (*Verbesina encelioides*). Weed Sci., 35 (4): 513-518.

Kingsbury, J.M., 1964. Poisonous Plants of the United States and Canada. Prentice - Hall, Inc., New Jersey, pp: 38-43.

Oelrichs, P.B., P.J. Vallely, J.K. MacLeod and I.A.S. Lewis, 1981. Isolation of galegine from *Verbesina encelioides*. J. Nat. Prod., 44 (6): 754-755.

Stepp, J.R. and D.E. Moerman, 2001. The importance of weeds in ethnopharmacology. J. Ethnopharmacol., 75 (1): 25-31.

Tiwari, H., P. Rao and V. Sambasiva, 1978. Constituents of *Verbesina encelioides*: Isolation of triterpenoids from a *Verbesina species*. Indian J. Chem., 16 (B): 1133.