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Arid Zone Plants of Rajasthan. I. Physico-chemical and Antimicrobial Studies of *Heliotropium subulatum*

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Abstract - Triterpenoids have been isolated from hexane fraction of *Heliotropium subulatum* Hochst. ex DC. and were found to be active against the selected microorganisms, Gram +ve and Gram -ve bacteria and fungi.

Key words - *Heliotropium subulatum*; physico-chemical studies; triterpenoids; antimicrobial activity.

H. subulatum Hochst. ex DC. (syn. *H. zeylanicum* Clarke; Boraginaceae) is an annual herb and widely distributed in arid zone of Rajasthan. Its ethanolic extract exhibited anticancer activity against human epidermoid carcinoma of nasopharynx in tissue culture as well as P_{388} lymphocytic leukemia in mice. This extract also demonstrated hypoglycaemic activity in albino rats. However, it was devoid of antiprotozoal, antiviral, CNS, CVS, antispasmodic and diuretic effects.[1]

The preliminary investigations of aerial parts of plant species demonstrated pyrrolizidine alkaloids,[2-3] such as heliotrine and subulacine N-oxide[4] where heliotrine showed *in vivo* and *in vitro* ganglion blocking activity.[5]

Materials and Methods

Plant material - The plant material of *H. subulatum* collected (May-June, 1997) from Agriculture Research Station, Durgapura, Jaipur, and authenticated from Herbarium, Department of Botany, University of Rajasthan, Jaipur, India, was used.

Physico-chemical studies - The powder of this plant species was subjected to various chemical treatments[6] separately, exhibiting differential behaviour which might be due to their diversity in chemical constituents. Further the ethanolic extract was fractionated with various solvents sequentially and each fraction was tested for varied chemical constituents such as carbohydrates, proteins, alkaloids, flavonoids, triterpenoids and tannins and also their antimicrobial efficacy.

The powdered plant material was also ignited in an oven (500℃, 3 h), kept in desicator overnight and weighed to calculate ash value (%).

Extraction and characterization - Dried and powdered plant material (500 g) was defatted in petroleum ether for 24 h. The extract was filtered and the residue was Soxhlet extracted in ethanol for 36 h. The ethanolic extract (1.26%) fractionated with hexane (yield:48.54%) and examined[7] on tlc (silica gel G; heptane:benzene:alcohol-100:100:1; spray 20% $SbCl_3$) which demonstrated eight spots corresponding to the

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reference compounds. Using preparative tlc, the spots were isolated and identified on the basis of their physical, chemical and comparative spectral studies[8,9] viz. β-sitos terol (I, 0.35%); stigmasterol (II, 0.48%); β-amyrin (III, 0.38%); friedelan 3β-ol (epifriedelenol) (IV, 0.45%); cycloartenone (V, 0.29 %); β-amyrin acetate (VI, 0.14%); friedelin (VII, 0.21%) and epifriedenyl acetate (VIII, 0.19%).

Antimicrobial activity

Microorganisms used – Pure cultures of bacteria-*Escherichia coli, Staphylococcus aureus, Bacillus thuringiensis* and *Klebsiella pneumoniae* (obtained from S.M.S. Medical College, Jaipur) grown on Nutrient Broth medium by incubating at 37℃ for 48 h, and fungi-*Aspergillus niger, A. flavus, Rhizoctonia phaseoli* and *Penicillium crysogenum* (from Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur) were grown on Potato Dextrose Agar (PDA) medium and incubated at 27℃ for 48 h.

Bioassay – Antibacterial and antifungal activities were evaluated by disc diffusion method,[10] where the test microorganims and growth medium were mixed thoroughly to ensure uniform distribution. The paper discs (Whatman No. 1, 6 mm in diameter) of sequential extracts (10 mg/disc) and isolated compounds (1 mg/disc) were placed in bacterial and fungal cultures, using gentamycin and mycostatin as standards (1 mg/disc) for bactericidal and fungicidal testings respectively. The inhibition zones were recorded and compared with the respective reference once to calculate the activity index (AI).

Results and Discussion

During physico-chemical studies the powder of the plant species showed varied colours (Table I) and the presence of various primary and secondary metabolites under evaluation (Table II).

Among sequential extracts, varied antimicrobial activity (chloroform extract against *K. pneumoniae*-10 mm and *P. chrysogenum*-9 mm; petroleum ether extract against *B. thuringiensis*-11 mm) was recorded.

Amongst the isolated compounds, increased bioefficacy of β-amyrin and β-amyrin acetate was recorded against the test bacteria and of β-sitosterol and β-amyrin against the fungi. From the results it is evidenced that *H. subulatum* which is

Table I. Behaviour of the drug (*H. subulatum*) with different reagents

Treatment	Colour after treatment (in daylight)
Powdered drug+1 or 2 drops of H_2SO_4	Dark
+ACOH	Yellow
+1N HCl	Light yellow
+5% I_2 solution	Red
+few drops of NH_3+$K_4Fe(CN)_6$	Golden yellow
+10% NaOH followed by a few drops of $CuSO_4$	Yellowish brown
+40% NaOH+few drops of 10% $Pb(CH_3COO)$	Yellow
+CH_3COOH+H_2SO_4	Yellow
+HNO_3 excess of NH_3 solution	Yellowish Red
+CH_3COOH+few drops of $FeCl_3$ and H_2SO_4	Dark yellow

Table II. Physico-chemical tests in *H. subulatum*

	Extractives				
	PE	C_6H_6	$CHCl_3$	EtOH	Aqueous
Total % by weight	1.150	0.970	1.215	2.388	5.722
Physical appearance and consistency	Greenish yellow, sticky	Yellow sticky	Greenish brown, sticky	Green, sticky	Brown, viscous
Carbohydrates	+	–	–	–	+++
Proteins	–	–	–	–	++
Alkaloids	–	–	++	+	–
Tannins	–	–	–	–	+++
Flavonoids	–	–	–	–	–
Triterpenoids	+++	++	+	–	–

Total % of extractives=11.445. Total % of ash value=6.95. Relative intensity of the tests=+/++/+++.

Table III. Antimicrobial activity of *H. subulatum*

Microorganisms		Sequential extracts			Isolated compounds							
		PE	C_6H_6	$CHCl_3$	I	II	III	IV	V	VI	VII	VIII
A. Bacteria												
E. coli	IZ*	10	09	08	18	+	12	09	07	17	05	07
	AI	0.71	0.64	0.57	1.20		0.80	0.60	0.40	1.13	0.33	0.46
S. aureus	IZ*	09	10	06	09	12	18	11	09	12	05	08
	AI	0.56	0.71	0.37	0.50	0.75	1.12	0.68	0.50	0.75	0.31	0.50
B. thuringiensis	IZ*	11	08	07	11	14	17	13	08	09	04	11
	AI	0.78	0.57	0.50	0.61	0.77	0.94	0.72	0.44	0.50	0.22	0.61
K. pneumoniae	IZ*	08	07	10	12	13	16	09	10	16	14	12
	AI	0.66	0.55	0.83	0.85	0.92	1.14	0.64	0.71	1.14	1.00	0.85
B. Fungi												
A. niger	IZ*	05	04	06	07	05	06	04	04	06	04	04
	AI	0.55	0.44	0.66	0.50	0.41	0.50	0.33	0.33	0.50	0.33	0.33
A. flavus	IZ*	06	06	08	08	06	08	05	06	08	06	08
	AI	0.60	0.60	0.80	0.80	0.60	0.80	0.50	0.60	0.80	0.60	0.80
P. crysogenum	IZ*	04	05	09	06	05	07	08	05	07	06	07
	AI	0.57	0.71	1.28	0.66	0.55	0.77	0.88	0.55	0.77	0.66	0.77
R. phaseoli	IZ*	07	06	05	05	+	09	06	08	06	05	06
	AI	0.63	0.54	0.45	0.55		1.00	0.66	0.88	0.66	0.55	0.66

*Total inhibition area of disc (in mm); Activity index (AI)=Inhibition area of test sample/Inhibition area of standard; +=negligible activity. Abbreviations used: PE=Petroleum ether; C_6H_6=Benzene; $CHCl_3$=Chloroform. Values are mean of triplicate readings.

a weed in the arid zone of Rajasthan, synthesizes not only triterpenoids but also exhibited appreciable antimicrobial activity, later, which could be put to its increased utility.

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