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Antimicrobial Activity of Pyrrolizidine Alkaloids from *Heliotropium ellipticum*

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Pyrrolizidine alkaloids, known for their significant antitumor activity, have been isolated from the aerial parts of *H. ellipticum* LEDEB. Europine (0.120%), heliotridine (0.152%), lasiocarpine (0.136%) and lasiocarpine-*N*-oxide (0.090%) have been identified. Antimicrobial activity of the isolated alkaloids against selected pathogenic bacteria and fungi has been investigated for the first time.

Keywords——*Heliotropium ellipticum*; Boraginaceae; europine; heliotridine; lasiocarpine; lasiocarpine-*N*-oxide; antibacterial activity; antifungal activity

Heliotropium ellipticum LEDEB., (Boraginaceae) is a herbaceous weed and widely distributed in the state of Rajasthan. Genus *Heliotropium* has been known to possess a number of medicinal properties and these are chiefly attributed to pyrrolizidine alkaloids.[1-3] These alkaloids besides hepatotoxic[4-6] also possess antitumor activity[7,8] against a number of tumor system. A large number of *Heliotropium* species have been investigated[9-21] for their pyrrolizidine alkaloids and different biological properties.[22] However, *H. ellipticum* remained untouched so far and hence, in the present study isolation of pyrrolizidine alkaloids and their antimicrobial testings have been reported.

Four pyrrolizidine alkaloids, europine (0.120%), heliotridine (0.152%), lasiocarpine (0.136%) and lasiocarpine-*N*-oxide (0.090%) have been isolated and identified on the basis of mp, infrared (IR) and mass spectral data (see Table I and Experimental).

Varied amounts of europine from *H. digynum*,[18] *H. europaeum*[17] and *H. marifolium*,[19] heliotridine from *H. eichwaldi*[20] and *H. lasiocarpum*, both in free and as its *N*-oxide, have been reported from different species of *Heliotropium*.[12,18,19,21]

While investigating the antimicrobial activities of the isolated alkaloids, although, the maximum inhibitory activity was demonstrated by europine (12 mm) against *Escherichia coli* and lasiocarpine against *Fusarium moniliforme* (11 mm) as compared to lasiocarpine-*N*-oxide and heliotridine (see Table II), but, unexpectedly it was not much exhibited as compared to the crude ethanolic extract against the test organisms.

TABLE I. Yields, Melting Points and Spectral Data of the Isolated Pyrrolizidine Alkaloids

Compounds	Yield (%)	mp (°C)	IR ($CHCl_3$) νcm^{-1}	MS m/z (M^+)
Europine	0.120	115	3500 (br), 1690, 1250	329
Heliotridine	0.152	116—118	3480 (br), 1710	223
Lasiocarpine	0.136	95—96	3480, 3460, 3400, 1735, 1710, 1280	396
Lasiocarpine-*N*-oxide	0.090	133 (dec.)	3300 (br), 1680, 1180	—

TABLE II. Antimicrobial Activity of Ethanolic Extract and the Isolated Pyrrolizidine Alkaloids from *Heliotropium ellipticum* LEDEB.

Test organisms	Zone of inhibition (mm±S.E.)[a]				
	Ethanolic extract	Europine	Heliotridine	Lasiocarpine	Lasiocarpine-*N*-oxide
Bacteria					
E. coli	8.00±0.09	10.00±0.22	—	12.00±0.34	9.00±0.89
E. cloacae	—	12.00±0.67	—	10.00±0.41	9.00±0.70
S. aureus	—	—	—	—	—
S. faecalis	—	—	—	—	—
Fungi					
A. flavus	9.00±0.83	7.00±0.76	—	—	—
C. lunata	10.00±0.59	—	—	—	8.00±0.47
D. tetramera	±	8.00±0.62	7.00±0.51	—	—
F. moniliforme	—	11.00±1.34	—	—	7.00±0.54
C. albicans	8.00±0.43	—	—	—	—

a) Mean of three replicates±S.E.; (±) trace activity; (—) not measurable activity.

From the results, it is evident that the efficacy of the crude extract against the test organisms is either due to the pyrrolizidine alkaloids present and/or the activity might be further enhanced by other metabolites, on identification, present in the whole extract.

So far, ethanolic extract of *H. bacciferum* and *H. subulatum* have been tested against one or the other microorganisms[22] but no active principles have been isolated. Therefore, in the present investigation the exhibited antimicrobial activity of the pyrrolizidine alkaloids isolated from the ethanolic extract of *H. ellipticum* is first of its kind and noteworthy.

A great deal of attention has been paid on the antitumor properties of pyrrolizidine alkaloids and heliotrine, lasiocarpine, monocrotaline, spectabilline and senecionine have been reported to be highly active against Walker-256 (intramuscular) tumor system.[8] Besides this, some of the alkaloids are responsible for malignant tumor of liver, skin and intestine in rats. The toxicity of the pyrrolizidine alkaloids containing plants to monogastric animals[23] increases by high ratio between the tertiary bases and their *N*-oxides. Therefore, the occurrence of europine, heliotridine, lasiocarpine and lasiocarpine-*N*-oxide might be responsible for the mild poisonous properties of *H. ellipticum*.

Experimental

All melting points are uncorrected. IR spectra were measured with Perkin-Elmer 337 spectrophotometer and mass spectra were run on a Hitachi RMC-7 spectrometer. Thin layer chromatography (TLC) and preparative TLC (pTLC) were performed with Silica gel G. Spots were located by spraying with Dragendorff's reagent followed by heating or by I_2 vapors.

Plant Material——Whole plants of *H. ellipticum* were collected from the fields in the month of August, 1986 and authenticated from the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Microorganisms——Pure cultures of *Escherichia coli, Enterobacter cloacae, Staphylococcus aureus, Streptococcus faecalis* and *Candida albicans* obtained from S.M.S. Medical College, Jaipur, India, and of *Aspergillus flavus, Curvularia lunata, Drechslera tetramera* and *Fusarium moniliforme* from the Laboratory of Microbiology, Department of Botany, University of Rajasthan, Jaipur, India, were used as test organisms.

Isolation of Alkaloids——The air-dried and powdered aerial parts (500 g) of the plant material were defatted with light petroleum ether and extracted with ethanol for 48 h in a Soxhlet apparatus. The viscous mass (9.7%) thus obtained was extracted with 500 ml of 5% H_2SO_4[17] and filtered. The filtrate was basified with ammonia and fractionated by extracting sequentially with ether (fr. E) and chloroform (fr. C). The aqueous layer was evaporated to

dryness and the dried residual mass was re-extracted with petroleum ether (fr. P). These fractions were then examined by co-chromatography ($CHCl_3$–MeOH–NH_4OH (85 : 14 : 1)). Seven spots (A→G; *Rf* 0.08, 0.20, 0.30, 0.50, 0.52, 0.80, 0.90) in fr. C and three spots (A→C; *Rf* 0.23, 0.61, 0.82) in fr. E were observed but fr. P did not yield any Dragendorff's positive spot. Out of the observed spots in the fr. C, spot C (*Rf* 0.30), D (*Rf* 0.50), E (*Rf* 0.52) and G (*Rf* 0.90) coincided with those of authentic europine, heliotridine, lasiocarpine and lasiocarpine-*N*-oxide used as markers. However, the remaining spots could not be identified due to their poor yields. These compounds were isolated by pTLC using the above solvent system (each developed two times) to obtain pure compounds, crystallized with methanol–acetone and then subjected for mps, IR and mass spectral studies and compared[24] with those of previously isolated samples, as recorded above, and by direct comparison by TLC, *etc.*

Media and Cultivation of Organisms——The selected bacteria were grown in Nutrient Broth medium and incubated at 37 °C for 48 h. Each bacterial culture was maintained by transferring to fresh medium every 48 h. However, fungi were grown on potato dextrose agar (PDA) medium by incubating at 27 °C for 48 h and maintained by periodic subculturings on fresh medium.

Antimicrobial Assay——For the antimicrobial assays, the *in vitro* paper disc diffusion method was adopted.[25] The different organisms were pre-seeded separately using a sterile swab over previously sterilized culture medium plates and the zones of growth inhibition were observed around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter) which were containing 4 mg of plant extract (0.1 g/ml) or 2 mg of the isolated alkaloids (0.1 g/ml of the respective solvent) or streptomycin (10 mg/ml) or mycostatin (100 units/ml) as reference compounds separately. Such treated discs were air-dried at room temperature to remove any residual solvent which might interfere with the determination.

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