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ISOLATION AND IDENTIFICATION OF HELINDICINE ALKALOID FROM THE LEAVES OF *HELIOTROPIUM INDICUM*

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ABSTRACT

Heliotropium indicum Linn extracted with ethanol and alkaloid fraction was separated on silica gel column using n-hexane, chloroform, ethyl acetate and methanol. extract was concentrated and subjected to TLC, found mixture of three compounds, they were separated by column chromatography. Compound on the bases of spectral analysis identified as a Helindicine alkaloid. Compounds were identified by IR, NMR and mass spectrum. Compound on the bases of spectral analysis identified as a Helindicine alkaloid. Helindicine is a pyrrolizidine alkaloid with unusual structural features.

K**eywords:** Alkaloid, pyrrolizidine alkaloid, spectral analysis, antineoplastic, antitumor, antibacterial, antifertility, chromatography, indicine.

INTRODUCTION

Heliotropium indicum Linn, commonly known as "Indian heliotrope" is a herb with pale violet flowers belonging to the family Boraginaceae and is very common in India with a long history of traditional medicinal uses in many countries in the world, and also found throughout in India. The plant is reported to possess anti-bacterial, anti-tumor, anti- fertility, wound healing and anti-inflammatory activities.

The leaves are used in fever[1.] The ethanolic extract of stem has yielded an n-oxide of alkaloid indicine, which is reported to possess antineoplastic activity[2,3] and one more alkaloid retaronecanol $C_{18}H_{15}ON$ has been isolated in minor quantity. while roots of the plant is used in asthma and also in cough [4.] the flowers of plant are reported to possess anticancerous activity specially used in abdominal tumor[5.]

Pyrrolizidine alkaloid are considered of greatpharmalogical, biological and chemotaxonomic interest[6-8]. These meta bolites have been isolated from a wide variety of plants, especially from genera belonging to the boraginaceae family[9-11]. The genus *Heliotropium* , a well-known source of such alkaloids [12-14] and other minor compounds , such as flavonoids and geranyl aromatic derivatives , is constituted of about 250 species represented by herbs and shrubs, distributed throughout the India In the a search for biologically active compounds from the EtOH extract from leaves of *Heliotropium Indicum* L. popularly known as fedegosa ,found abundantly in the region of northeast in India was investigated this species is widely used in folk medicine in the treatment of skin disease[15], and as a powerful expectorant[16] based on

a literature survey ,the investigated species has been the subject of several previous studies , leading to the isolation of Helidicine alkaloid[8,17].

MATERIAL AND METHODS:

The leaves of the plant were collected from the local herb supplier. The plant material was authenticated by the botany department of the college. Photograph of the leaves of the *Heliotropiun indicum*:

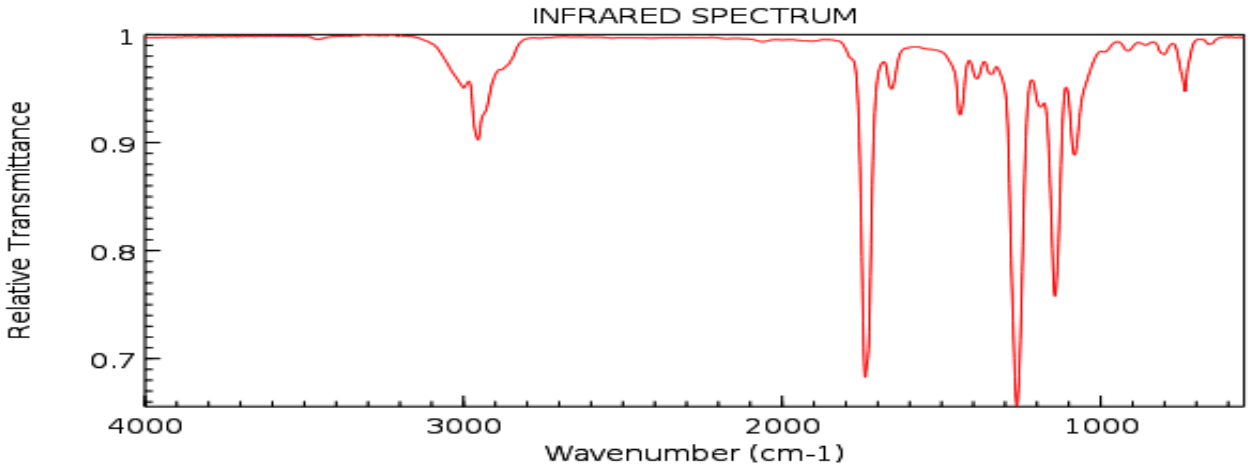
EXTRACTION AND ISOLATION:

Dried and powdered leaves of the plant (1.5 Kg.) were exhaustively extracted with ethanol. The extract was evaporated in vacuum to yield a crude extract (110 g.) which was subjected to the TLC and found three spots resulting extract is a mixture of the three compounds [18]. The alkaloid fraction was separated on silica gel column using n-hexane, chloroform, ethyl acetate and methanol, the acetone soluble fraction (1.5 g) was chromatographed on silica gel using step wise gradient solvent system of hexane-ethanol (1:1). Ethanol-acetone (1:1), acetone-methanol (1:1) and methanol to obtain 10 fractions, each of 60 ml. The fraction one was again subjected to TLC and found singe compound, and then chromatographed on a column using methanol as eluent to yield (70 mg) compound.

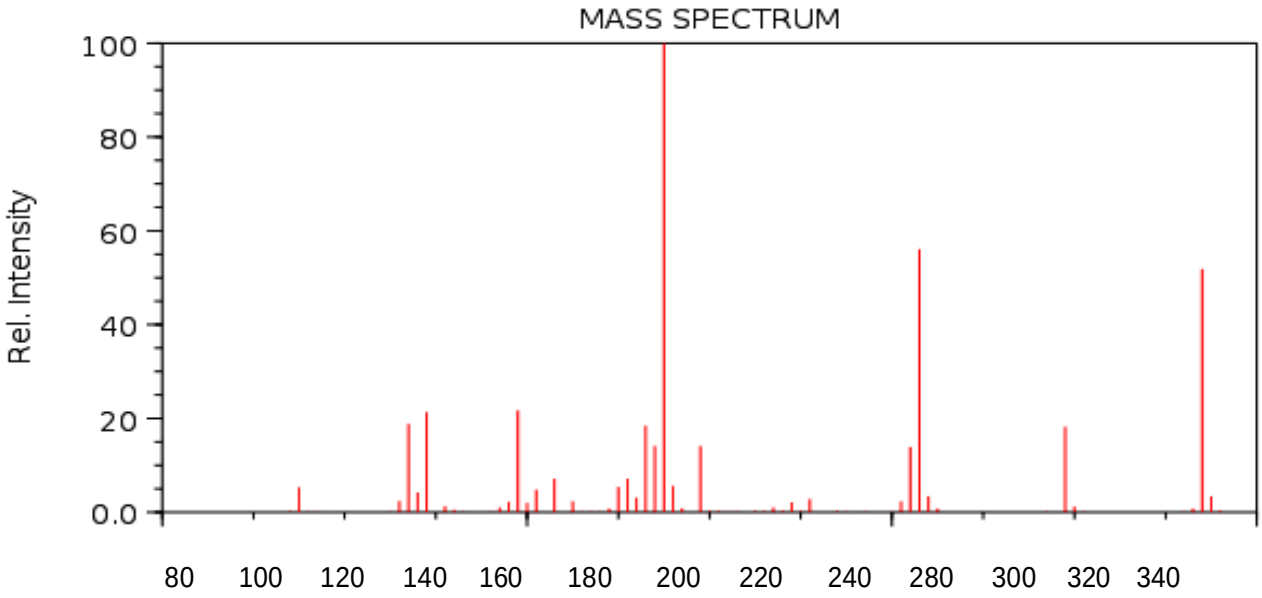
IDENTIFICATION OF THE COMPOUND:

Compound is a colorless resine; $(\alpha)^{20}_{D}$ - 0.64˚ (0.05, CH_3OH);

IR($CHCl_3$) v max in cm^{-1}: 3435,1730,1640,1245,1030.



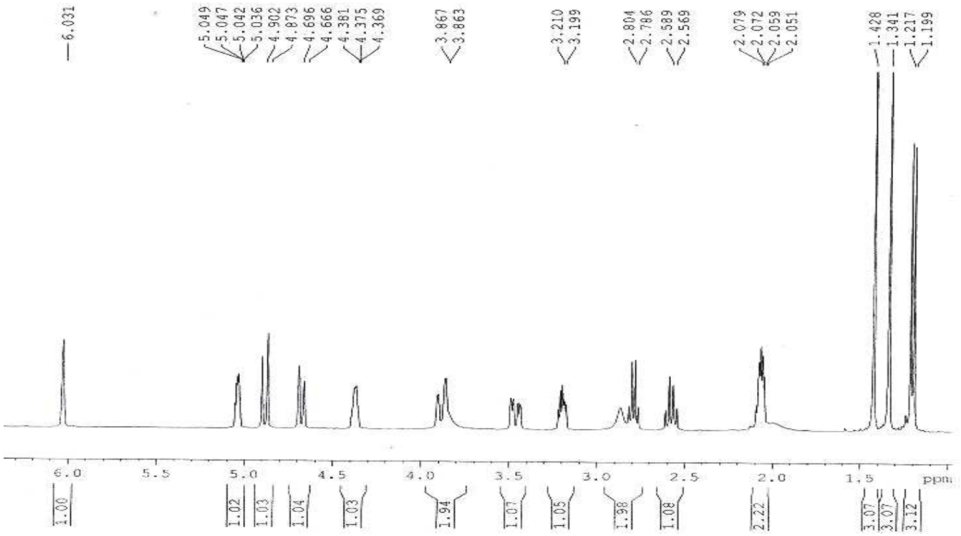
EIMS: (M^+,m/z 281.2); EI-MS m/z (rei.int); 281 (M),(35), 207 (100),191 (15),149 (37),135 (33), 109(34),97(73),95(55),83(57),81(46).



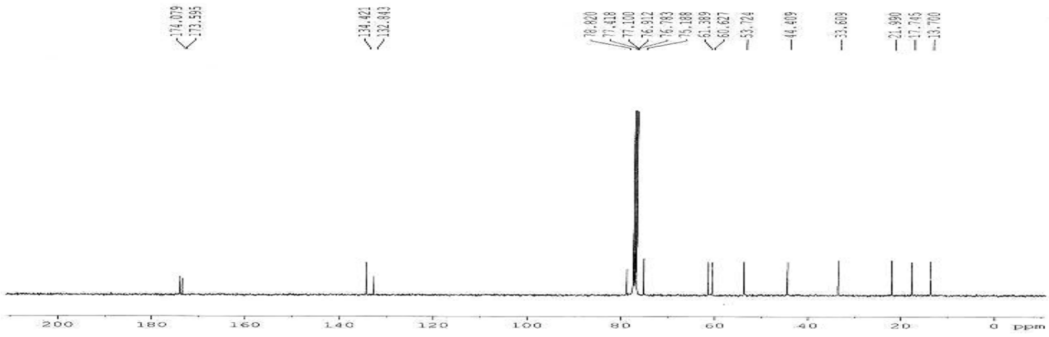
.^{1}H (400 MHz) and ^{13}C(100MHz) NMR spectral data for compound in CD, OD as solvent[19,20]

C	δC	δH	COMPOUND $^2J_{CH}$
1	134.2		H-2,H-8
2	125.3	5.94(br s)`	2H-3, H-8
3	62.5	4.40(br . j 15.0)	H-2
		3.92(br d, J 15.0)	
5	55.5	3.97(m)3.	
		3.34(m)	
6	36.7	2.15(2H,m)	H-5b
7	70.5	4.63(br s)	2H-6
8	80.5	4.909 br s)	
9	61.7	4.98(d ,J 13.8)	
1.	175.6		
2.	84.5		H-5’
3.	70.5	4.05(q. J 6.2)	3H-4’
4.	17.3	1.17(d, J6.2)	
5.	34.2	2.03(hept. J 7.00	3H-6’,3H-7’
6.	17.2	0.95(d, J 7.0)	H-5’
7.	17.6	0.93(d, J 7.0)	H-5’

All assignments were based on DEPT, COSY, HMQC, HMBC and NOESY experiments. Coupling constants (J) in Hz for hydrogen atoms were obtained of the 1D ^{1}HNMR spectra Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and ^{1}H-^{1}H COSY spectra.



^{13}C-NMR SPECTRUM



^{13}C-NMR OF HELICIDIZINE ALKALOID

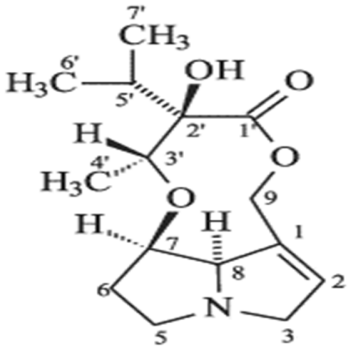
RESULT AND DISCUSSION:

Compound was isolated as colorless oil. Its molecular formula is C_{15} H_{23} NO_4, was deduced by HR-EIMS (m/z 281, 1627, M^+). The IR spectrum exhibited characteristic absorption of hydroxyl and carbonyl group at ν max 3435 and 1726 $^{cm-1}$, respectively. Its EI-MS gave a molecular ion peak at m/z 281, and a base peak at m/z 207(1a, $C_{11}H_{13}NO_{3)}$, along with unusual PA

fragmentation ions of at M/Z 149(1b,37%, $C_9H_{11}NO$), 135 (1c,33%C_8H_9NO) 115id,15%, $C_6H_{11}O_2$),109(1e,34%, $C_7H_{11}N$),95(1f,55%, C_6H_9N) and 81 (1g,46%, C_5H_7N).

The ^{13}CNMR spectrum displayed a total of fifteen carbon signals, while the DEPT experiment evidenced the presence of three methyl, four methylene and five methyne and consequently. three quaternary carbon atoms.

The ^{1}HNMR spectrum of compound exhibited signals at δ H 0.93 (d, J 7.0 Hz, H_3-7'),0.95(d, J 7.0 Hz, H_3-6'), and 2.03 (hept, J 7.0 Hz, H-5') characteristic of an isopropyl group, along with an oxymethyne group, showing a resonance at δH 4,05 (q, J6.2 Hz, H-3') and an additional methyl signal at δH 1.17 (d, J6.2 Hz, H_3-4'). These hydrogen signals were correlated with the carbon signals at Δc 17.6 (CH_3-7'). 17.2 (CH_3-6').34.2(CH-5').70.5(CH-3'), and 17.3(CH_3-4'). Respectively in the HMQC spectrum. Both H-3' and H-5'displayed ^{3}J-HMBC correlations with the ester carbonyl group at δ_C 175.6(C-1').These data are in agreement with a diflorate ester moity[20.] Adeshielded hydrogen signal at $\delta_{H\ 5.94(s,H-2)}$ in ^{1}HNMR spectrum ,together with two olefinic carbon signals at δ_c 175.6(C-1').,suggested the presence of a trisubstituted double bond , signals assigned to oxygenated carbon atoms were observed at δ_H 70.5(CH-7) and 61.7(CH_2-9),which showed correlation with the hydrogen signals at δ_H 4.63 (br s, H-7) and 4.98 (d,j 13.8 Hz, H-9a)and 4.80(d,j 13.8 Hz,H-9b), respectively .Additionally signals corresponding to three carbon atoms attached to nitrogen were also observed at $_{\delta}$C 80.5(CH-8),62.5(CH_2-3), and 55.5(CH_2-5).These data were all consistent with a retronecine moity [17,19].As expected the HMBC spectrum . the HMBC spectrum of compound revealed a correlation between H-7 and the oxymethyne carbon atom CH-3', as well as a correlation between H-3' and CH-7. The NOESY correlation between the oxymethine hydrogen atom CH-7 and the methyl hydrogens H_3-4', both in the α-position, confirm this deduction. The relative stereochemistry of the Helindicine alkaloid was established based on coupling constant values and the NOESY experiment.



STRUCTURE OF THE HELINDICINE ALKALOID

On the bases of spectral analysis of isolated compound identified as a above structure Helindicine alkaloid. Helindicine is a pyrrolizidine alkaloid with unusual structural features.

CONCLUSION:

The object of the research was to exploit leaves of the *Heliotropium Indicum* phytochemically. In the findings of the work a compounds were isolated and identified by using several physico-chemical techniques and identified as a natural product pyrrolizidine alkaloid, Helindicine alkaloid.

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