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BIOACTIVE COMPOUNDS PROFILING AND STRUCTURE ELUCIDATION OF *MARTYNIA ANNUA* L. HERB

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ABSTRACT: The medicinal herbs used by native people of Mexico since ancient times for numerous therapeutic purposes. The plant is inborn to Mexico but is now well familiarized throughout India on wastelands. In India *Martynia annua* L. is a well recognized small herbaceous annual plant. The present study was carried out with HPTLC, and the results showed that there were many photochemical in *Martynia annua* L. Pre-coated HPTLC graded plates and autosampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. From HPTLC studies, it has been found that Petroleum-ether, acetone, and methanol extracts contain a mixture of compounds. Therefore, it is established that the pharmacological activity shown by them was due to the cumulative effect of all the compounds in a composite. Total eleven compounds identified; the most prevailing compounds were, Oleic acid (30.61%), 2, 5-dihydroxybenzoic acid (36.3%), 1-Hexyl-2-nitrocyclohexane (31.01%), and Cynidin-3-galactoside (34.14%). Other components were Apigenin-7-A-D, glucoside (4.4%), Pelargonidin-3, 5-diglucoside (12.9%), Ethanol, 2-(2-aminoethoxy) - (16.78%), n-Hexadecanoic acid (17.13%), Eicosanoic acid (18.55%), Chlorogrnic acid (25.07%) and Apigenin (27.07%) also found by GC-MS analysis.

INTRODUCTION: *Martynia annua* L. (Martyniaceae) is one of the medicinal herbs used by native people of Mexico since ancient times for numerous therapeutic purposes. The plant is inborn to Mexico but is now well familiarized throughout India on wastelands [1-3]. In India *Martynia annua* L. is a well-recognized small herbaceous annual plant. It is commonly known as Devil's claw, or Cat's claw denotes the inner woody capsule, which splits open at one end into two curved horns or claws [4-5]. In Ayurveda, the plant is known as Kakanasika in Sanskrit. In Hindi, it is called Bichhu, and in Gujarati, it is known as Vinchudo, which is being used in Indian traditional medicines for epilepsy, inflammation and applied locally for tuberculosis glands of camel's neck [6-8]. The Martyniaceae family has three genera, and these genera have sticky, hairy leaves, orchid-like flowers, and woody, beak-shaped pods. The seeds of the yellow-flowered Ibicella-lutea, which is native to South America, are not commercially available in United Status, although the species occurs as an occasional weed in California's Central Valley.

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Introduced members of the Martynia and Proboscidea genera are often found growing as weeds in the South-western United Status [9]. So *Martynia annua* L. belongs to Mexico and Central America. It is naturalized throughout tropical regions of the world. In the present study, profiling of bioactive constituents using various hyphenated

techniques *i.e.*, GCMS as well as HPTLC techniques for *Martynia annua* L.

MATERIALS AND METHODS:

Plant Material Collection and Authentication: Whole plant (leaf, stem and root) of *Martynia annua* were collected from the field area of Bhopal district M. P. India. For identification and taxonomic authentication, the specimen was submitted in the Department of Botany, Specimen No. 103/Saif/Sci/Clg-Saifia College, Bhopal, India. Its authenticity was confirmed and authenticated by Dr. Saba Naaz. Collected plant materials were shade-dried and coarsely powdered.

Preparation of Extract: Shade-dried and coarsely powdered 100 gm powder from bark of *Martynia annua L.* were soaked in petroleum ether, acetone and methanol 500 ml of solvent/drug mass ratio 5:1 separately. It was kept at room temperature for 48 h with intermittent mixing. The extracts of plants obtained after 48 h of soaking was filtered using Whatman paper. The extracts, which was thus obtained, was evaporated to make it into the powder form to re-dissolve in respective solvents.

Bioactive Compound Identification Using GC-MS: The most effective fraction of the acetone, the most effective fraction of the acetone extract of the leaf was used for the identification of bioactive constituents by GC-MS analysis of the purified isolated compounds was recorded by direct inlet method [10]. Analysis by GC/MS was performed using a GC Clarus 500 Perkin Elmer with mass indicator Turbo mass Gold equipped with a fused silica capillary column Elite –1 (100% methyl polysiloxane). The fraction was pyrolyzed at 610 °C and then introduced to the GC column. The transfer line was held at 200 °C, and the source temperature was maintained at 200 °C, and ionization energy was set at 70 eV. Helium was employed as carrier gas (1 mL mG1). The GC oven temperature was programmed: The column held initially at 110 °C mG1 (isothermal) and then increased by at 9-280 °C mG1 minG1 (isothermal).The name and molecular weight of the components of the test materials were ascertained.

Bioactive Compound Identification Using HPTLC: HPTLC method is a modern, sophisticated, and automated separation technique derived from TLC [11]. Pre-coated HPTLC graded plates and autosampler was used to achieve precision, sensitive, significant separation, both qualitatively and quantitatively. High-Performance Thin-Layer Chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost-effectively. HPTLC method offers a high degree of selectivity, sensitivity, and rapidity combined with single-step sample preparation. It is a reliable method for the quantization of the nano-grams level of samples. Thus, this method can be conveniently adopted for routine quality control analysis. It provides a chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials [12].

RESULTS AND DISCUSSION: The results pertaining to GC-MS analysis of the acetone extract of leaves of *Martynia annua* L. lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached to Gas Chromatography. The various components present in the leaves of *Martynia annua* L. that were detected by the GC-MS are shown in **Table 1.**

The mass spectra of all the phytochemicals identified in acetone extract of leaves of *Martynia annua* L. Total eleven compounds identified in **Table 1**; the most prevailing compounds were Oleic acid (30.61%), 2, 5-dihydroxybenzoic acid (36.3%), 1-Hexyl-2-nitrocyclohexane (31.01%) and Cynidin-3-galactoside (34.14%). Other components were Apigenin-7-A-D, glucoside (4.4%), Pelargonidin-3, 5-diglucoside (12.9%), Ethanol, 2-(2-aminoethoxy) - (16.78%), n-Hexadecanoic acid (17.13%), Eicosanoic acid (18.55%), Chlorogenic acid (25.07%) and Apigenin (27.07%) also found by GC-MS analysis.

The peaks in the chromatogram were integrated and were compared with the database of the spectrum of known components stored in the GC-MS library. **Table 1** showed the compound Apigenin-7-A-D, glucoside ($C_{21}H_{18}O_{11}$) at the relative peak abundance at 18 m/z, reported with CAS No.29741-09-1 and molecular weight 446.36 g/mol. **Table 1** showed the compound Pelargonidin-3,5-diglucoside ($C_{27}H_{31}O_{15}$) at the relative peak abundance at 28 m/z, reported with CAS No.

17334-58-6 and molecular weight 595.53 g/mol. In compound Ethanol, 2 - (2 - aminoethoxy) - ($C_4H_{11}NO_2$) at the relative peak abundance at 30 m/z, reported with CAS No. 929-06-6 and molecular weight 103 g/mol. While compound n-Hexadecanoic acid ($C_{16}H_{32}O_2$) at the relative peak abundance at 43 m/z, reported with CAS No.57-10-3 and molecular weight 256g/mol. In compound Eicosanoic acid ($C_{20}H_{40}O_2$) at the relative peak abundance at 57 m/z, reported with CAS No.29741-09-1 and molecular weight 506-30-9 g/mol. Whereas compound Chlorogrnic acid ($C_{16}H_{18}O_9$) at the relative peak abundance at 73 m/z, reported with CAS No.327-97-9 and molecular weight 354.31 g/mol. In compound Apigenin ($C_{15}H_{10}O_5$) at the relative peak abundance at 97 m/z, reported with CAS No.520-36-5 and molecular weight 270.24 g/mol. In contrast, it showed the compound Oleic acid ($C_{18}H_{34}O_2$) at the relative peak abundance at 55 m/z, reported with CAS No.112-80-1and molecular weight 282 g/mol. In compound 1 – Hexyl – 2 - nitrocyclohexane ($C_{12}H_{23}NO_2$) at the relative peak abundance at 83 m/z, reported with CAS No.118252-04-3 and molecular weight 213g/mol. In compound Cynidin-3-galactoside ($C_{21}H_{21}O_{11}$) at the relative peak abundance at 129 m/z, reported with CAS No. 27661-36-5 and molecular weight 449.33 g/mol. In compound 2, 5-dihydroxybenzoic acid ($C_7H_6O_4$) at the relative peak abundance at 104 m/z, reported with CAS No.490-79-9 and molecular weight 154 g/mol.

TABLE 1: PHYTOCONSTITUENTS IDENTIFIED IN ACETONE EXTRACT OF *MARTYNIA ANNUA* L. BY GC-MS

S. no.	Compound Name	Retention time	Molecular weight (g/mol)	Formula	CAS
1	Apigenin-7-A-D,glucuronide	4.4	446.36	$C_{21}H_{18}O_{11}$	29741-09-1
2	Pelargonidin-3,5-diglucoside	12.98	595.53	$C_{27}H_{31}O_{15}$	17334-58-6
3	Ethanol,2-(2-aminoethoxy)-	16.78	103	$C_4H_{11}NO_2$	929-06-6
4	n-Hexadecanoic acid	17.13	256	$C_{16}H_{32}O_2$	57-10-3
5	Eicosanoic acid	18.55	312	$C_{20}H_{40}O_2$	506-30-9
6	Chlorogrnic acid	25.07	354.31	$C_{16}H_{18}O_9$	327-97-9
7	Apigenin	27.07	270.24	$C_{15}H_{10}O_5$	520-36-5
8	Oleic acid	30.61	282	$C_{18}H_{34}O_2$	112-80-1
9	1-Hexyl-2-nitrocyclohexane	31.01	213	$C_{12}H_{23}NO_2$	118252-04-3
10	Cynidin-3-galactoside	34.14	449.33	$C_{21}H_{21}O_{11}$	27661-36-5
11	2,5-dihydroxybenzoic acid	36.2	154	$C_7H_6O_4$	490-79-9

HPTLC Analysis:

HPTLC analysis, at short UV 254 nm **Fig. 1** and at long UV 366 nm **Fig. 2** different spots were found in all three extracts *i.e.,* methanol extract, acetone extract, and petroleum ether extracts which indicates the presence of different phytocomponents.

HPTLC fingerprint patterns have been evolved for different extracts of *Martynia annua* L. Plant. There were total of nine extracts of stem, root, and leaves, which applied accurately on HPTLC precoated aluminum plates with mobile phase Toluene: Chloroform: Acetone: Petroleum ether: methanol (4:3.5:2.5: 0.5: 0.5 v/v).

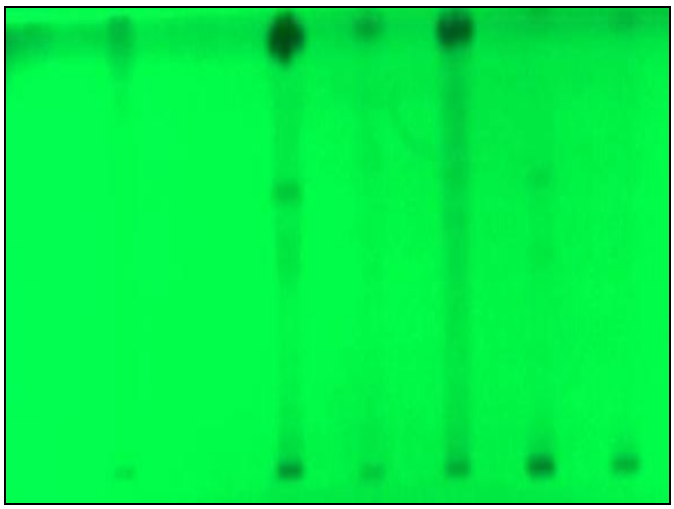

FIG. 1: HPTLC SCREENING AT 254 nm

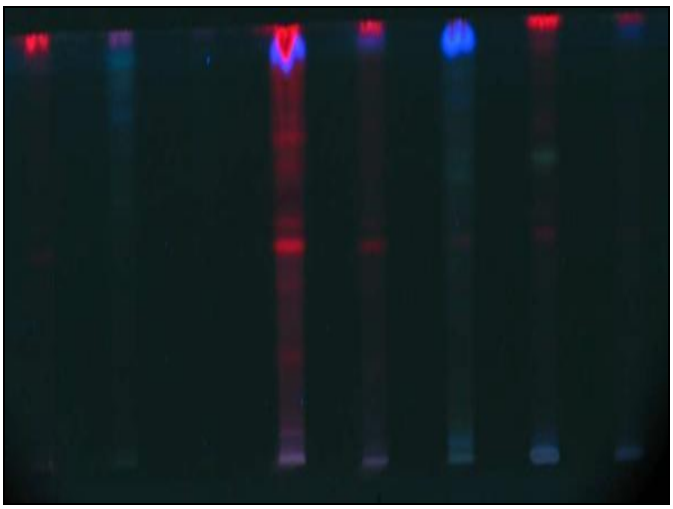

FIG. 2: HPTLC SCREENING AT 366 nm

TABLE 2: R_f VALUE OF PLANT PARTS OF *MARTYNIA ANNUA* L.

S. no.	Plant Parts	Extracts	No. of Peaks	R_f values	% Area
1	Leaf	P	2	0.64, 0.80	57. 06, 42.94
		A	5	0.40, 0.49, 0.56, 0.74, 0.78	18.17, 8.23, 65.63, 2.21, 5.76
		M	2	0.43, 0.58	22.66, 73.34
2	Stem	P	5	0.46, 0.51, 0.60, 0.79, 0.81	8.34, 25.78, 27.14, 21.30, 17.44
		A	3	0.58, 0.64, 0.79	15.64, 51.23, 33.13
		M	2	0.60, 0.77	56.20, 43.80
3	Root	P	1	0.81	100
		A	6	0.41, 0.52, 0.60, 0.66, 0.70, 0.79	11.68, 20.62, 18.83, 11.01, 13.38, 24.48
		M	2	0.26, 0.83	69.10, 30.90

*P-Petroleum ether, A-Acetone, M-Methanol

HPTLC analysis of leaves of *Martynia annua* L: The petroleum ether extract showed 2 peak area covering 57.06 and 42.94% cover area with R_f value 0.64 and 0.80 **Table 2**. While acetone extract showed 5 peak area covering 18.17, 8.23,65.63, 2.21 and 5.76% cover area with R_f value 0.40, 0.49, 0.56, 0.74 and 0.78 **Table 2**. The methanolic extract showed 2 peak area is covering 57.06 and 42.94 %cover area with R_f value 0.43 and 0.58 **Table 2.**

HPTLC Analysis of Stem of *Martynia annua* L: The petroleum ether extract showed 5 peak areas covering 8.34, 25.78, 27.14, 21.30, and 17.44 %cover area with R_f value 0.46, 0.51, 0.60, 0.79, and 0.81 **Table 2**). While acetone extract showed 3 peak areas covering 15.64, 51.23, 33.13% cover area with R_f value 0.58, 0.64 and 0.79 **Table 2**. And the methanolic extract showed 2 peak area covering 56.20 and 43.80% cover area with R_f value 0.60 and .77 **Table 2.**

HPTLC Analysis of Root of *Martynia annua* L: The petroleum ether extract showed only 1 peak area covering100 % cover area with R_f value 0.81 **Table 2**. While acetone extract showed 6 peak area covering 11.68, 20.62, 18.83, 11.01, 13.38and 24.48 % cover area with R_f value 0.41, 0.52, 0.60, 0.66, 0.70 and0.79 **Table 2**. The methanolic extract showed 2 peak areas covering 69.10 and 30.90 %cover area with R_f values 0.26 and 0.83 **Table 2**.

CONCLUSION: The results of the phytochemical analysis showed that the acetone extract of the selected plant parts showed the highest bands than petroleum ether and methanol extracts. Thus, the plant studied here can be seen as a potential source of medicinally useful drugs. The phytochemical description of the extracts, the identification of responsible bioactive compounds, and quality standards are necessary for future validation.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

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