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HPTLC FINGER PRINT PROFILE OF ROOT EXTRACTS OF *MARTYNIA ANNUA* LINN.

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ABSTRACT: The objective of the present study is to evaluate phytochemical composition and HPTLC finger print profile of medicinally useful plant *Martynia annua* L. (Martyniaceae) root extracts. The CAMAG HPTLC system was used for the finger print profiling of root extract using the mobile phase chloroform: Methanol (9:1 v/v). The profile showed that the root extract of *Martynia annua* exhibited several peaks with different R_f values when visualized at 254 nm and 366 nm. At 254 nm a total 7 peaks were observed and at 366 nm 9 peaks were observed. The result of HPTLC analysis of *Martynia annua* root extracts shows that the maximum number of chemical constituents present in alcoholic root extract of *Martynia annua* in comparison to hydro-alcoholic and aqueous root extract of *Martynia annua* in given solvent system of chloroform and methanol. Further bioactivity guided fractionation and analysis of isolated chemical entity can reveal the active constituents in the different root extract of *Martynia annua*.

INTRODUCTION: Herbal drugs got from medicinal plants which in turn synthesize complex organic constitutes with frequently unknown organically active constituents. The herbal drugs are mostly prepared from the crude extracts, not standardized or analyzed for the content of the dynamic ingredients. Quality control is intricate, as many factors can influence the final product, *i.e.* growth conditions of the plant, parts of the plant utilized, extraction of the plant, volume of extract used in the final preparation and various others. All of these factors can affect the level of active compound and therefore the competence of the herbal formulation [1]. Modern medicine has evolved from folk medicine and traditional system only after detailed chemical and pharmaceutical screening; plants remain a major source of therapeutic compounds.

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Synthetic drugs cause's side effects as a consequence, people are more approving to use natural compounds obtained from plants [2]. There are nearly 1250 Indian medicinal plants, which were used for formulating therapeutic preparation according Ayurveda and additional traditional system of medicine [3]. Phytochemical analysis of folklore plants has contributed a number of compounds with different pharmacological activities.

Standardization of the plant material is need of the day as many pharmacopoeia containing monographs of the plant materials describe only the physicochemical characters. Assessment of complete and accurate physicochemical values of herbs used for therapeutic purpose provides a scientific basis of its utility. Hence, the present technique describing the identification as well as quantification of active ingredients in the plant material may be functional for proper standardization of herbs and its formulations [4–6]. Medicinal plant species, have limited choices of the source of information for identification. So there is restricted information, which is not sufficient to identify the new medicinally valuable plant and

those medicinal plant species which are closely related, similarly identification of the adulterant plant species [7]. Chromatographic finger printing can be used to identify the plant, determine active ingredients or markers and detect impurities or contaminants such as herbicides [8]. High performance thin layer chromatography (HPTLC) is frequently used as an alternative to HPLC for the authentication and quantification of plant products because of its accuracy, simplicity, cost-effectiveness and rapidity [9].

HPTLC methods are faster, reproducible and reliable. Integrating HPTLC with digital scanning profiling gives accurate quantifiable analysis and R_f values of samples by in situ scanning densitometry assisted by the creation of easily detectable by post chromatography chemical reactions as necessary as well as documentation of separation in the form of chromatography with fractions represented as peaks with define parameters counting observance (Intensity), Rf height and area [10]. HPTLC plates has higher surface area thereby allowing for quicker and clearer sample separation due to extra consistent and considerably smaller particle size of the adsorbent [11].

Chromatographic fingerprint is a logical option to meet the need for more effectual and powerful quality assessment to TCHM (Chinese traditional herbal medicine) and ITM (Indian Traditional Medicine). The optimized chromatographic finger print is not only an alternative analytical instrument for authentication, but also an approach to express the assorted patterns of chemical ingredients disseminated in the herbal drugs. HPTLC finger print analysis has developed into the most important assessment technique for quality control of herbal medicines because of its reliability and simplicity. It can use as an instrument for authentication, identification and quality control of herbal drugs [12].

Martynia annua is an upright short-lived herbaceous plant. The roots are white in colour with charactestic odour. *Martynia annua* belongs to family Martyniaceae and it is commonly found in dense cluster on roadsides, degraded moist and dry deciduous forest, waste lands and over-grazed pasture. It is a weedy foreign species native to tropical and sub-tropical region of Mexico, Central America, Burma, West Pakistan and naturalized throughout India. Its excellent dispersal mechanism has helped it spread throughout the tropical world as a weed [13].

In folklore practices decoction of *Martynia annua* plant is given in pneumonia and cold fever. The poultice of roots used in snake bite for external application. Roots of *Martynia annua* are boiled in milk and taken as a tonic. In Tribal Pockets of Satpura Plateau in Madhya Pradesh, Root paste of *Martynia annua* is used to treat Cancer and rheumatism [14]. The juice of the leaves is used as a gargle for sore throat and the leaf paste for wounds of domestic animals [15].

The unripe fruits of *M. annua* found to have antioxidant activity [16] and the ash of fruits mixed with coconut oil are used to cure burns [17]. The roots are also used as local sedative and antidote to scorpion stings [18]. Seed oil is used for abscesses and treating itching and skin infections. The seeds of *M. annua* are used for prevention of graying of hair [19]. The whole plant is used for fever, hair loss, scabies and abscess on the back [20]. In view of the above findings in literature we tried to examine the plant *Martynia annua* for HPTLC finger print profile by taking the root part of the plant and identify the presence of number of phyto-compounds.

MATERIALS AND METHODS:

Collection of the Plant Material: The fresh plant was collected from Government Ayurved College Campus, Gwalior (Madhya Pradesh). Preserved this plant as herbarium in departmental repository and was authenticated from Regional Ayurveda Research Institute for Metabolic Disorders (RARI) Bangalore (Karnataka). Its authentication number is Authentication / SMPU/RARIMD/BNG/2017-18, Bengaluru, Dated 26/02/2018. The roots were washed thoroughly two to three times with running tap water and once with sterile distilled water and immediately sprayed with alcohol. The root material was then dehydrated under shade. After complete aeration, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable airtight container for further use.

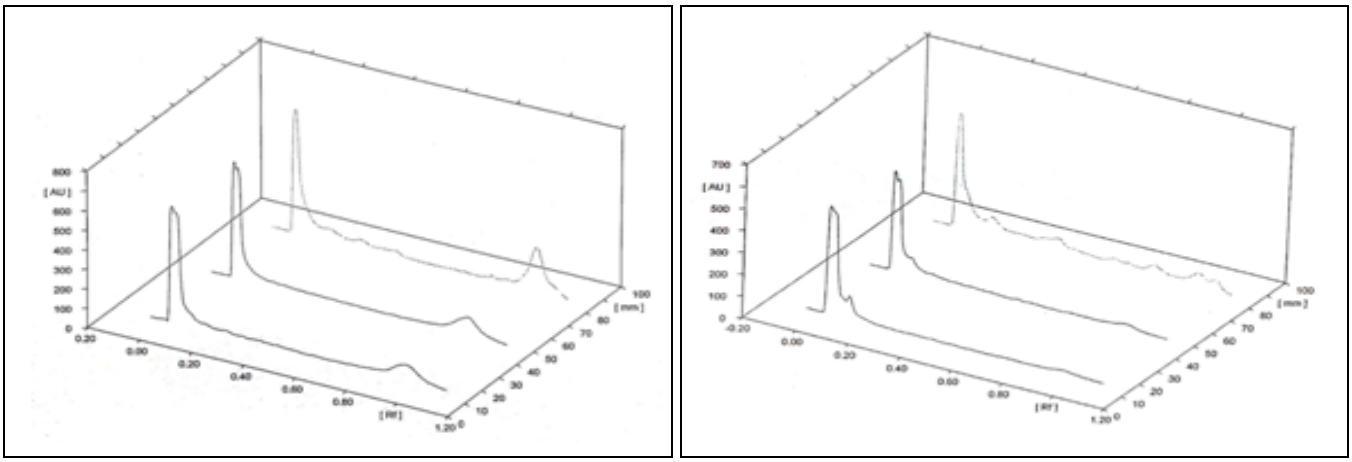
Preparation of the Extracts: Extraction is the general process for separation of active constituents by the use of different solvents. The coarse powder of shade-dried plant root were successively extracted in Soxhlet extractor using the solvents such as alcohol (ethanol), Hydro-alcohol (distill water: alcohol) (50:50) and distill water.

All three root extracts obtained were concentrated and dried under vacuum, packed and stored in refrigerator until further use.

HPTLC Fingerprinting Profile: The HPTLC fingerprint profile of the all root extract of *M. annua* was carried out using CAMAG HPTLC system (Muttenz, Switzerland) operational with a sample applicator Linomat-V, twin tough plate development chamber, TLC Scanner, winCATS software & Hamilton (Reno, Nevada, USA). A constant application rate of 10 µl of sample was applied on 8mm wide band using Camag Linomat-V automated applicator with the nitrogen flow providing a dosage speed of 150 nl/s from syringe on Pre- coated silica gel aluminum plates 60 F254 10 × 10 cm with 0.2 mm thickness (Merck, Germany, Catalogue No .1.05554). After sample application, plates were developed inside Camag twin through glass tank pre-saturated with the mobile phase chloroform: Methanol (9.1 v/v) for 20 min. The plate was developed horizontally in Camag horizontal developing chamber (10 cm × 10 cm) at the room temperature.

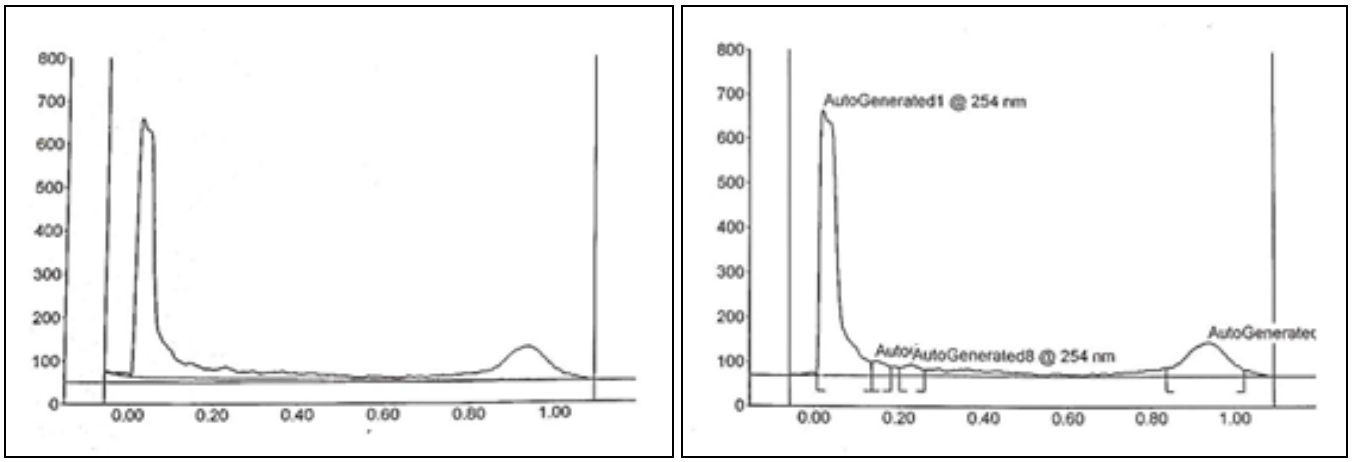
The plate was developed up to distance of 8 cm, after development, dried for 10 min. employing hot gun. After aeration, the plates were heated at 110 °C for 10 min in a pre-heated oven.

The plates were scanned within 10 min, using densitometric TLC scanner with win CATS software in the remission mode at 254 and 366 nm. The peaks were detected and their R_f values and peak areas were calculated.

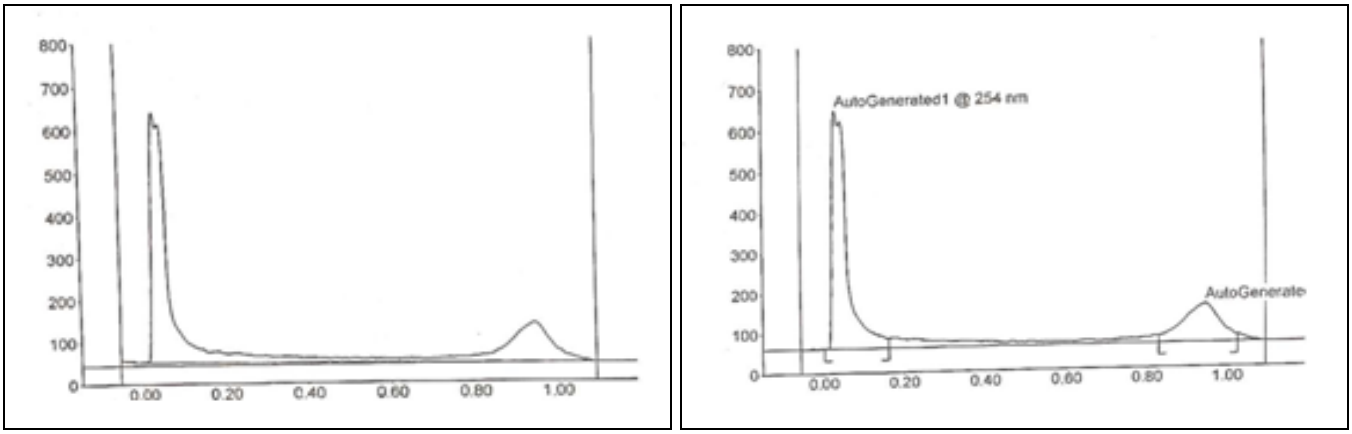


ALL TRACK @ 254 NM **ALL TRACK @ 366 NM**

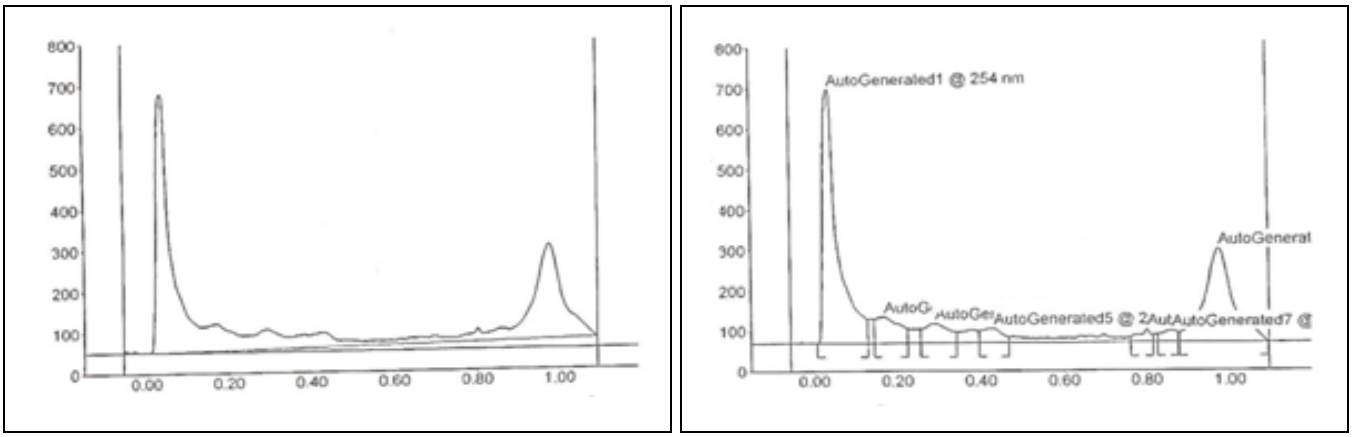
FIG. 1: CHROMATOGRAM OF ROOT EXTRACTS OF *M. ANNUA* AT 254 NM AND 366 nm



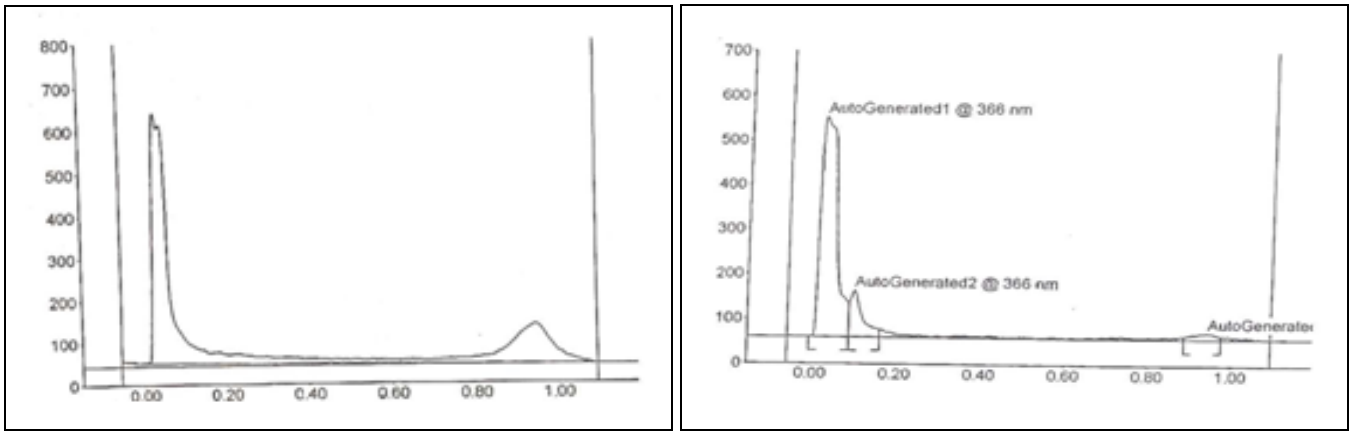
(A) TRACK 1: ACQUOUS ROOT EXTRACT OF *MARTYNIA ANNUA* (254 nm)



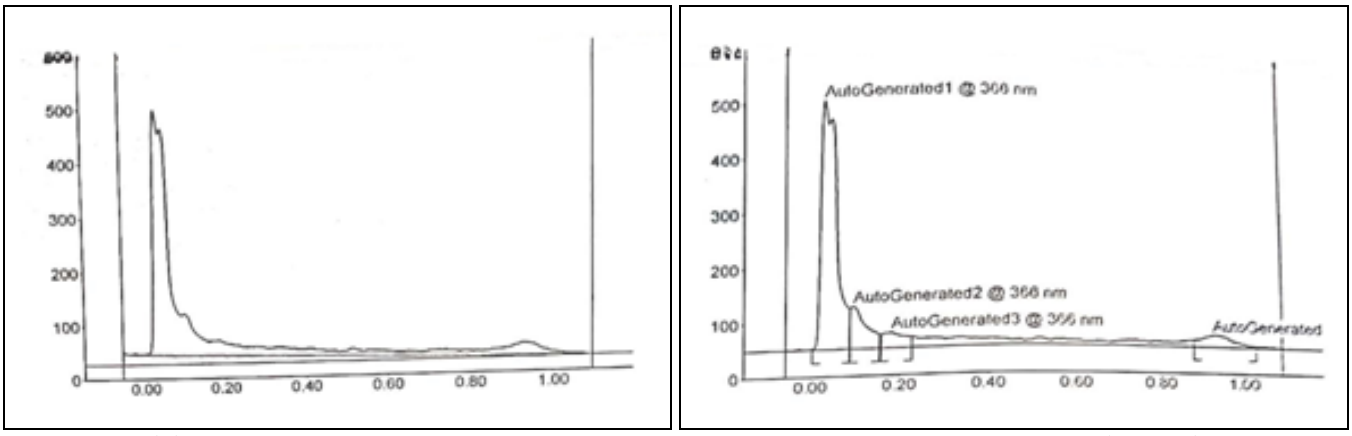
(B) TRACK 2: HYDRO-ALCOHLIC ROOT EXTRACT OF *MARTYNIA ANNUA* (254 nm)



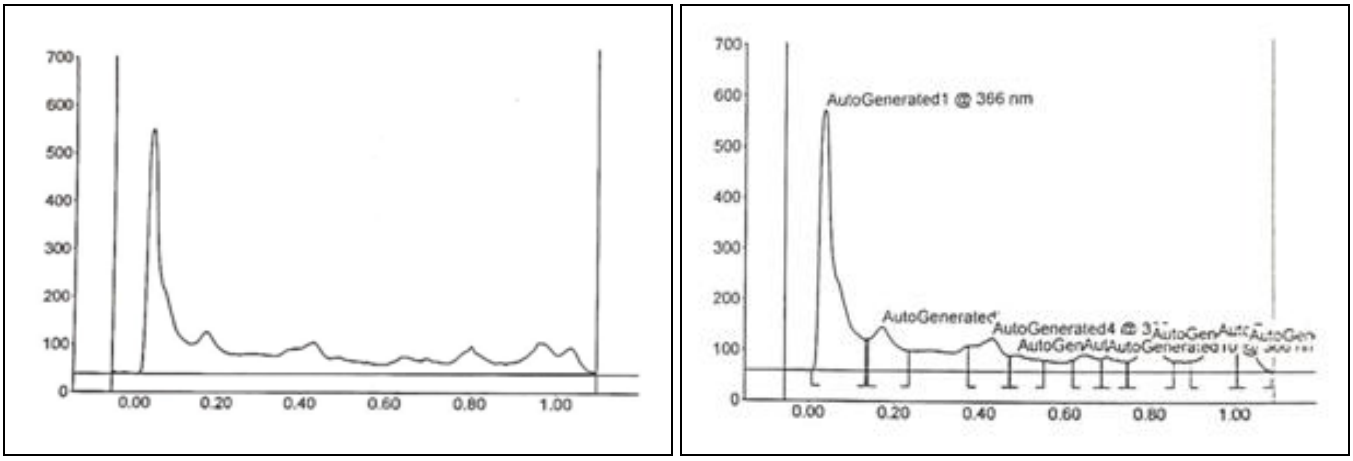
(C) TRACK 3: ALCOHLIC ROOT EXTRACT OF *MARTYNIA ANNUA* (254 nm)



(D) TRACK 4: ACQUOUS ROOT EXTRACT OF *MARTYNIA ANNUA* (366 nm)



(E) TRACK 5: HYDRO-ALCOHLIC ROOT EXTRACT OF *MARTYNIA ANNUA* (366 nm)



(F) TRACK 6: ALCOHLIC ROOT EXTRACT OF *MARTYNIA ANNUA* (366 nm)

FIG 2: HPTLC DENSITOGRAM OF *M. ANNUA* ROOT EXTRACTS AT 254 NM (A) (B) AND (C) AND AT 366 nm (C) (D) AND E)

TABLE 1: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10 µl ACQUOUS ROOT EXTRACT OF *M. ANNUA*, AT 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.01	7.0	0.03	594.2	81.27	0.14	30.3	15198.5	71.59
2	0.14	30.4	0.15	33.5	4.58	0.18	20.6	680.2	3.20
3	0.20	18.7	0.23	25.8	3.54	0.26	13.1	656.6	3.09
4	0.84	19.4	0.94	77.6	10.62	1.02	14.7	4696.9	22.12

TABLE 2: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10 µl HYDRO-ALCOHLIC ROOT EXT. OF *M. ANNUA*, AT 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.00	1.4	0.03	590.2	86.24	0.16	24.7	13639.2	72.02
2	0.83	18.1	0.95	94.2	13.76	1.03	17.3	5300.1	27.98

TABLE 3: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10µl ALCOHLIC ROOT EXTRACT OF *M. ANNUA*, AT 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.01	0.9	0.04	626.7	59.01	0.13	59.8	13642.1	46.27
2	0.15	59.6	0.17	64.6	6.09	0.23	35.8	2292.5	7.77
3	0.26	34.4	0.29	48.2	4.53	0.34	26.6	1763.6	5.98
4	0.40	29.3	0.43	35.4	3.33	0.47	13.1	1058.7	3.59
5	0.76	11.4	0.80	30.5	2.87	0.82	16.7	532.7	1.81
6	0.83	18.3	0.86	27.9	2.63	0.88	23.7	656.0	2.22
7	0.88	23.8	0.98	228.7	21.53	1.09	1.0	9541.2	32.36

TABLE 4: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10 µl ACQUOUS ROOT EXTRACT OF *M. ANNUA*, AT 366 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	-0.01	0.4	0.03	492.3	80.67	0.09	77.4	11930.3	83.10
2	0.09	78.5	0.10	103.3	16.92	0.16	16.7	1889.3	13.16
3	0.89	7.0	0.94	14.7	2.41	0.98	7.9	536.1	3.73

TABLE 5: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10 µl HYDRO-ALCOHLIC ROOT EXTRACT OF *M. ANNUA*, AT 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.00	1.9	0.03	451.9	77.63	0.09	75.6	9701.9	72.09
2	0.09	75.9	0.10	78.0	13.40	0.16	26.2	1734.7	12.89
3	0.16	26.1	0.18	29.2	5.01	0.23	19.6	904.3	6.72
4	0.88	12.3	0.93	23.0	3.96	1.03	3.3	1117.1	8.30

TABLE 6: PEAK LIST AND RF VALUES OF THE DENSITOGRAM OF 10 µl ALCOHLIC ROOT EXTRACT OF *M. ANNUA*, AT 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.01	1.3	0.04	508.3	55.02	0.13	61.0	11913.7	45.45
2	0.14	62.3	0.17	86.1	9.32	0.24	39.7	8160.4	12.06
3	0.38	49.1	0.43	64.6	6.99	0.47	30.5	2586.3	9.87
4	0.47	30.7	0.49	32.7	3.54	0.55	21.6	1163.8	4.44
5	0.62	23.0	0.64	33.7	3.65	0.69	24.8	1045.8	3.99
6	0.69	25.6	0.70	30.4	3.29	0.75	20.2	768.0	2.93
7	0.75	20.6	0.80	54.5	5.90	0.86	20.0	1960.1	7.48
8	0.90	21.4	0.96	62.0	6.72	1.01	36.2	2396.1	9.14
9	1.01	36.5	1.04	51.4	5.57	1.09	2.6	1220.2	4.65

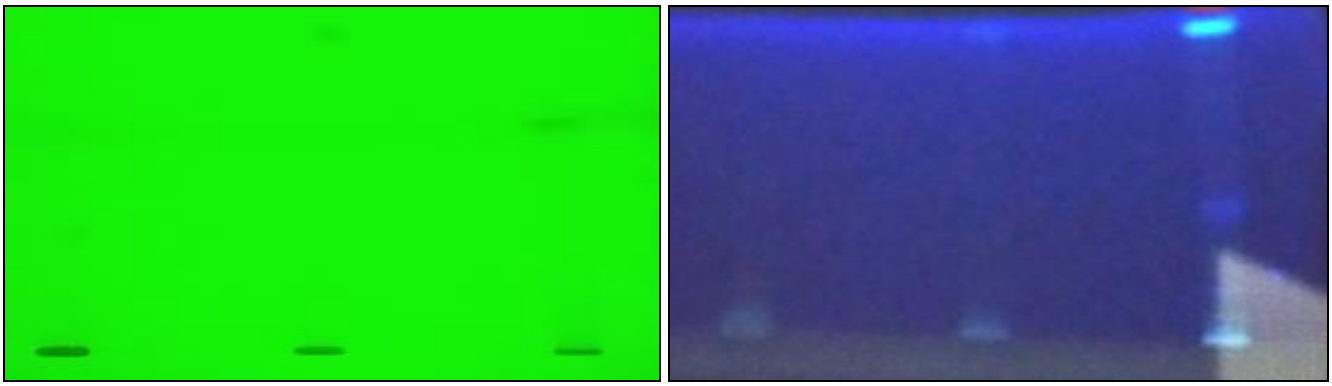


FIG. 3A: AT UV 254 nm **FIG. 3B: AT UV 366 nm**

FIG. 3: THE ROOT EXTRACTS OF *M. ANNUA* WERE SUBJECTED TO HPTLC ANALYSIS BY SPECIFIC SOLVENT SYSTEM CHLOROFORM: METHANOL (9:1 V/V) AND DETECTED UNDER UV AT 254 nm (A) AND 366 nm (B)

RESULTS AND DISCUSSION: The densitogram shown in **Fig. 1** *M. annua* at 254 nm indicate that all sample constituents were separated. It is evident from **Table 1** *i.e.* peak list and R_f values of the densitogram of *Martynia annua* 10 µl Acquous root extract at 254 nm found 4 spots respectively. The following Max R_f 0.03, 0.15, 0.23 and 0.94 **Fig. 2A** indicating R_f values 0.03, 0.94, 0.15 and 0.23 were found to be more predominant as the percentage area was more with 71.59%, 22.12%, 3.20% and 3.09%, respectively. **Table 2** peak list and R_f values of the densitogram of *Martynia annua* 10 µl Hydro-alcohlic root extract shown in **Fig. 2** Track 2: *Martynia annua* at 254 nm found 2 spots respectively. The following Max R_f 0.03 and 0.95 **Fig. 2B** indicating R_f values were found to be more predominant as the percentage area was more with 72.02% and 27.98%, respectively.

Table 3 peak list and R_f values of the densitogram of *Martynia annua* 10 µl Alcohlic root extract shown in **Fig. 2** Track 3: *Martynia annua* at 254 nm found 7 spots, respectively. The following Max R_f 0.04, 0.17, 0.29, 0.43, 0.80, 0.86 and 0.98 **Fig. 2C** indicating R_f values 0.04, 0.98, 0.17, 0.29 and 0.43 were found to be more predominant as the percentage area was more with 46.27%, 32.36%, 7.77%, 5.98% and 3.59%, respectively. **Table 4** peak list and R_f values of the densitogram of *Martynia annua* 10 µl Acquous root extract shown in **Fig. 2D** Track 4: *Martynia annua* at 366 nm found 3 spots respectively. The following Max R_f 0.03, 0.10 and 0.94 **Fig. 2D** indicating R_f values 0.03, 0.10 and 0.94, were found to be more predominant as the percentage area was more with 83.10%, 13.16% and 3.73%, respectively. **Table 5** peak list and R_f values of the densitogram of *Martynia annua* 10 µl Hydro-alcohlic root extract shown in **Fig. 2E** Track 5: *Martynia annua* at 366 nm found 4 spots respectively. The following Max R_f 0.03, 0.10, 0.18 and 0.93 **Fig. 2E** indicating R_f values 0.03, 0.10, 0.93 and 0.18, were found to be more predominant as the percentage area was more with 72.09%, 12.89%, 8.30% and 6.72%, respectively.

Table 6 peak list and R_f values of the densitogram of *Martynia annua* 10 µl alcohlic root extract shown in **Fig. 2F** Track 6: *Martynia annua* at 366 nm found 9 spots respectively. The following Max R_f 0.04, 0.17, 0.43, 0.49, 0.64, 0.70, 0.80, 0.96 and 1.04 **Fig. 2F** indicating R_f values 0.04, 0.17, 0.43,

0.96, 0.70 and 1.04, were found to be more predominant as the percentage area was more with 45.45%, 12.06%, 9.87%, 9.14%, 7.48% and 4.65%, respectively. From the results we can say that the alcohlic roots extract of *Martynia annua* has been thoroughly investigated by HPTLC method and better separation was achieved. The visualization reagents enable to see the spots efficiently and the densitometry will able to quantify the constituents.

The experimental method allows checking phyto-constituents present in Alcohlic roots extract and their concentration.

CONCLUSION: Chromatography is essentially a group of techniques used for separation of the constituents of mixture by continuous distribution or adsorption of analyte between two phases. Among various chromatographic analytical techniques HPTLC has a firm place as a reliable method for analysing several samples of divergent nature and composition at the same time. HPTLC is a valuable tool for dependable identification, it gives chromatographic fingerprints that can be visualized and stored as electronic images which can be utilized several times without any errors and change.

The result of HPTLC analysis of *Martynia annua* root extracts shows that there are more bands. The maximum number of chemical constituents present in alcoholic root extract of *Martynia annua* in comparison to hydro-alcoholic and aqueous root extract of *Martynia annua* in given solvent system of chloroform and methanol.

Further, bioactivity guided fractionation and analysis of isolated chemical entity can reveal the active constituents in the different root extract of *Martynia annua*.

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CONFLICTS OF INTEREST: None

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