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RESEARCH ARTICLE

HPTLC FINGERPRINT PROFILE OF STEROIDAL CONSTITUENTS OF *ACHYRANTHES ASPERA* LINN.

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ABSTRACT

The study was carried out with an aim to determine the steroidal profile of the medicinally important plant *Achyranthes aspera* Linn. from methanolic extract of root, stem and leaf. Preliminary phytochemical screening was done followed by HPTLC studies. N-butanol: methanol: water 3:1:1 was used as mobile phase for the separation of steroids. The steroid fingerprint of leaf exhibited 7 peaks in neutral, 3 peaks in acidic and 7 peaks in basic methanolic fractions, while that of stem exhibited 8 in neutral, 6 each in acidic and basic methanolic extracts. The fingerprint of root revealed the presence of 9 peaks in neutral, 6 in acidic and 9 in basic methanolic fractions. The study revealed diverse forms of steroids in large number in the root, stem and leaf of *Achyranthes aspera* Linn. It can be concluded that HPTLC fingerprint analysis of root, stem and leaf extract of *Achyranthes aspera* Linn. can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker. The results also indicate that the plant parts are a good source of steroids.

Key words: *Achyranthes aspera* Linn. Root, stem, Leaf, Phytochemical Screening, HPTLC Fingerprinting, steroids.

INTRODUCTION:

Steroids are terpenoid lipids characterized by the sterane or steroid nucleus: a carbon skeleton with four fused rings, generally arranged in a 6-6-6-5 fashion. Steroids vary by the functional groups attached to these rings and the oxidation state of the rings. The specificity of their different biological actions is due to the various groups attached to a common nucleus. When alcohol groups (OH) are attached, steroids should properly be called sterols (e.g., cortisol), whereas ketone groups (C=O) make them sterones (e.g., aldosterone). Steroids comprise a large group of substances that mediate a very varied set of biological responses. They are important hormones in animals and plants.

Achyranthes aspera Linn. is an indigenous medicinal plant of Asia, South America and Africa. It is found throughout tropical India as a common weed in fields and wasteland [1] belonging to the family Amaranthaceae. The plant is known for various medicinal properties and used widely for the treatment of different diseases in human. In the recent time, *Achyranthes aspera* Linn. reported to have array of medicinal compounds and medicinal properties. The plant is astringent, digestive, diuretic, laxative, purgative and stomachic. The juice of the plant is used in the treatment of boil, diarrhoea, dysentery, haemorrhoids, rheumatic pains, itches and skin eruptions. The ash from the burnt plant, often mixed with mustard oil and a pinch of salt, and is used as a tooth powder for cleaning teeth. It is believed to relieve pyorrhoea and tooth ache. The leaf is emetic and a decoction is used in the treatment of diarrhoea and dysentery. A paste of the leaves is applied in the treatment of rabies, nervous disorders, hysteria, insect and snake bite [2]. *Achyranthes aspera* Linn. reported to possess wound healing activity, immune stimulatory properties, larvicidal activity, antibacterial activity and antifungal activity. Roots of *Achyranthes aspera* Linn. reported to possess antioxidant activity and anti-inflammatory properties. The main limitation in the use of traditional remedies is the lack of standardization of raw material, manufacturing process and the final product. A biomarker on the other hand is a group of chemical compounds which are in addition to being unique for that plant material alsocorrelates with biological efficacy. So the need arises to lay standards by which the right material could be selected and incorporated into the formulation. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images.[3-6]. The present study was intended to resolve the chemical profile and flavonoids constituents present in the stem, leaves, root of *Achyranthes aspera* Linn., which will be useful for the proper identification of commercial samples.

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MATERIALS AND METHODS:

Collection of plant material

Whole plant parts of *Achyranthus aspera* Linn. were collected in the month of August- September 2013 from natural habitats in Vasai region of Thane district. The plants were authenticated at Blatter's herbarium; St. Xavier's College, Mumbai and the specimens voucher were deposited in the St. Xavier's College Herbarium for further reference. The accession number for *Achyranthes aspera* L. is 62490.

Preparation and Extraction of Plant Material

After confirmation of its botanical identity the leaf, stem and roots were subjected for preliminary phytochemical studies and HPTLC finger print studies.

The leaf, stem and roots of *Achyranthus aspera* Linn. were separated, washed thoroughly in distilled water and cut into small pieces. They were shade dried at room temperature. Dried pieces were then uniformly grinded separately using mechanical grinder to make fine powder. The powdered form of plant leaves, roots and stems were stored for future use. The powdered material is then used for preliminary phytochemical studies and HPTLC fingerprinting.

Phytochemical Screening

The preliminary phytochemical investigation of the leaf, stem and roots of *Achyranthus aspera* Linn. was carried out.

Test for Steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and green bluish color for steroids[7]. The extracts were subjected to preliminary phytochemical investigation for detection of steroids. The results are presented in Table 1.

HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Wagner **[8]** *et al.* Harborne **[9],** and Eike Reich **[10]**

Sample Preparation

Air dried plant powder was extracted with pet ether (40-60^{o}C). Hot methanol was added to the green coloured extract which was obtained. This was then evaporated to reduce the volume. The steroids spots were separated using solvent mixture. n – Butanol : Methanol : water (3:1:1) and methanolic H_2SO_4 as spray reagent [8]. Methanolic acidic, basic and neutral extracts were prepared for sample application. All the solvents used for HPTLC analysis was obtained from MERCK.

Developing Solvent System

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using N-butanol: methanol: water 3:1:1 as the mobile phase. [8][10].

Sample Application

Chromatograph was performed on 20x10 cm aluminium packed TLC plate coated with 0.2 mm layer of silica gel 60F254 ((E. Merck Ltd, Darmstadt, Germany) stored in a dessicator. 15 µl aliquots of each of the extracts was applied on 8 mm wide band by Hamilton microsyringe (Switzerland), with the nitrogen flow providing a delivery speed of 150 nl/s. The syringe was mounted on a Linomat V applicator attached to CAMAG HPTLC system and was programmed through WIN CATS software. Spotting was performed at 25±2^{o}C ascending development of the plate with elution distance of 80 mm (distance to the lower edge was 10 mm).

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 20 x 10 cm saturated with solvent vapours of N-butanol: methanol: water 3:1:1 for 20 minutes. The linear ascending development was carried out and 20 mL of mobile phase was used per chromatography run.

Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid reagent as spray reagent and dried at 100^{o}C on CAMAG plate heater for 3 min.

Photo documentation

The plate was kept in photodocumentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 540 nm and visible light. The Rf values and finger print data were recorded by WIN CATS software

Densitometric scanning

Finally, the plate was fixed in scanner stage and scanning was done at 540 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

RESULTS AND DISCUSSION:

Phytochemical Screening

The phytochemical test on methanolic extracts of *Achyranthus aspera* Linn. leaf, stem and roots showed the presence of steroids. (Table 1).

Table1: Steroidal screening of methanolic extracts of different parts of *Achyranthus aspera* Linn.

Sr. No.	Secondary metabolites	Methanolic Extracts		
		Root	Stem	Leaf
1	Steroids	++	+	+

HPTLC finger printing of *Achyranthus aspera* Linn.

The methanolic extract of stem, leaves and root of *Achyranthes aspera* Linn. showed the presence of different types of steroids with different Rf values with range 0.18 to 0.83 (Table 2 - 4). In general more degree of steroidal diversity has been observed in root when compared to the stem and leaf. Maximum number of steroids has been observed in root followed by stem and leaf. Among the 13 different steroids of leaf, 6 steroids with Rf values 0.39, 0.44, 0.45, 0.48, 0.60 and 0.80. are unique to leaf only (Table 2). Seventeen different types of steroids have been observed in stem of *Achyranthes aspera* Linn. Among these different steroids of stem, the eight steroids with Rf values 0.17, 0.19, 0.24, 0.25, 0.35, 0.51, 0.56 and 0.69 are unique to the stem and they are not present in leaves and roots of *Achyranthes aspera* Linn. The 14 steroids with Rf values 0.18, 0.22, 0.20, 0.25, 0.26, 0.41, 0.47, 0.49, 0.52, 0.67, 0.75, 0.76, 0.83 and 0.84 are showed their unique presence only in the root of *Achyranthes aspera* Linn. These are among the twenty different steroids found in the root. The steroid with the Rf value 0.53 is present in leaf, stem and root of the plant. The steroids with the Rf values 0.48, 0.53, 0.74, 0.81 are found in leaves and stem of the plant. The steroids with the Rf values 0.81, 0.37, 0.53, 0.66, 0.81, 0.82 are expressed jointly in root and stem of *Achyranthes aspera* Linn. The steroids with the Rf values 0.36, 0.53, 0.76, 0.81 are expressed jointly in root and leaf of *Achyranthes aspera* Linn.

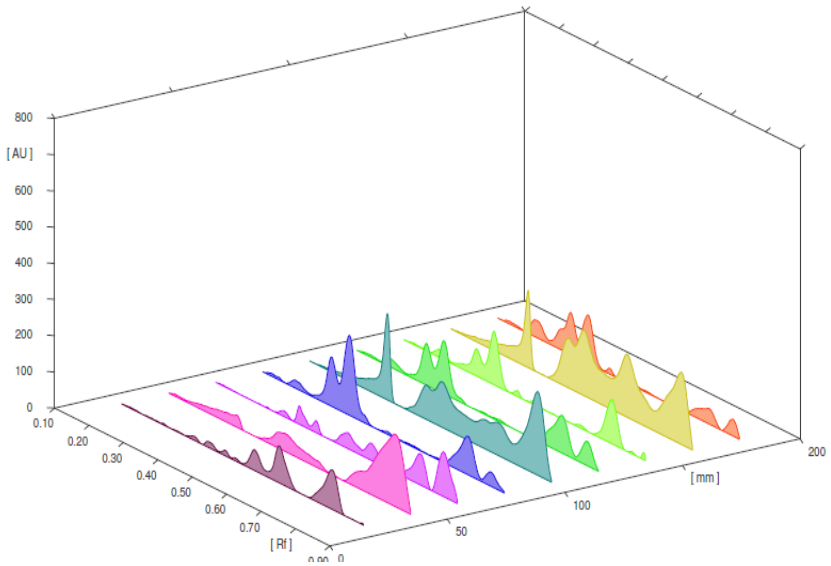


Figure 1: Steroids all tracks chromatogram of the methanolic extracts of *Achyranthes aspera* Linn Leaf, stem and root.

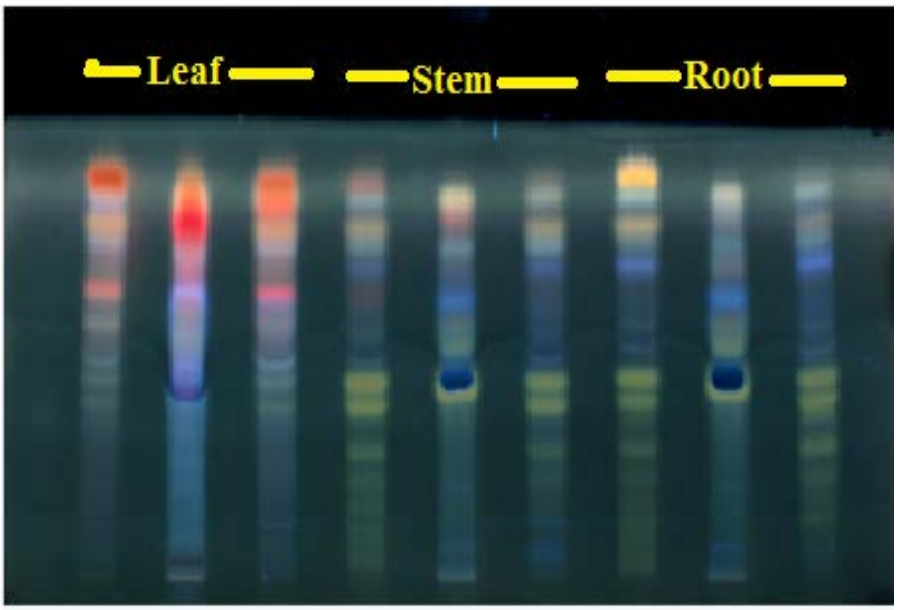


Plate 1: HPTLC Profile of Steroids in methanolic extracts of leaf, stem and root of *Achyranthes aspera* Linn. at 540 nm after derivatization.

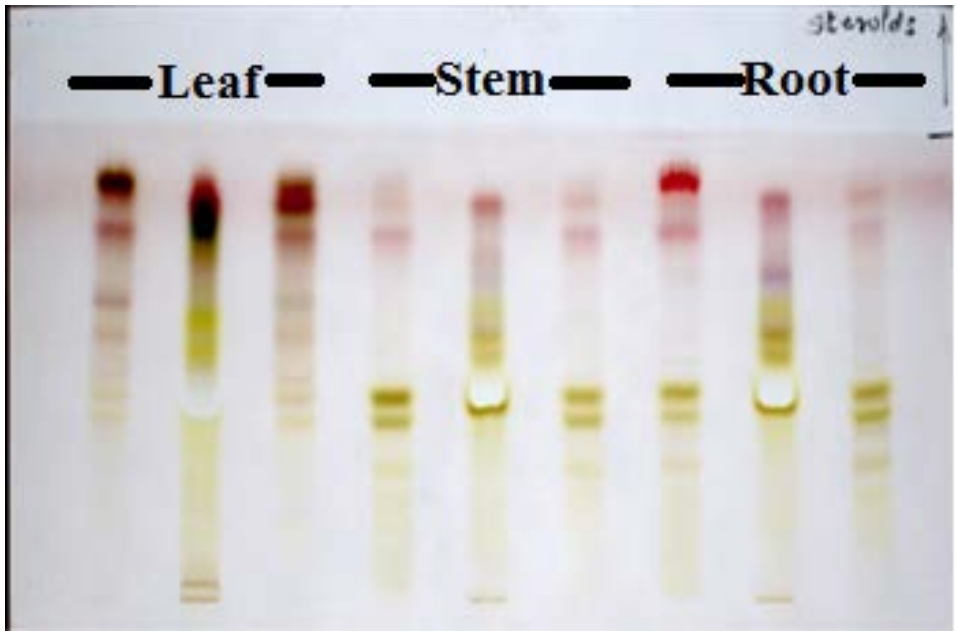
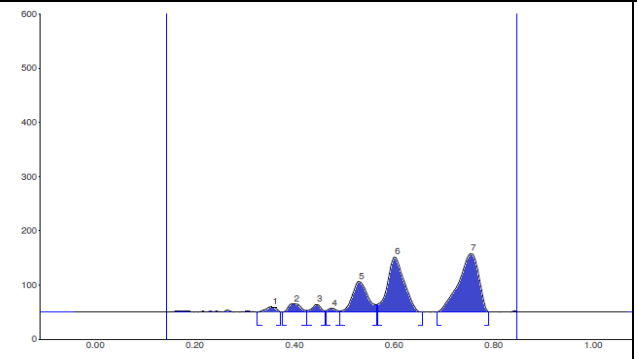
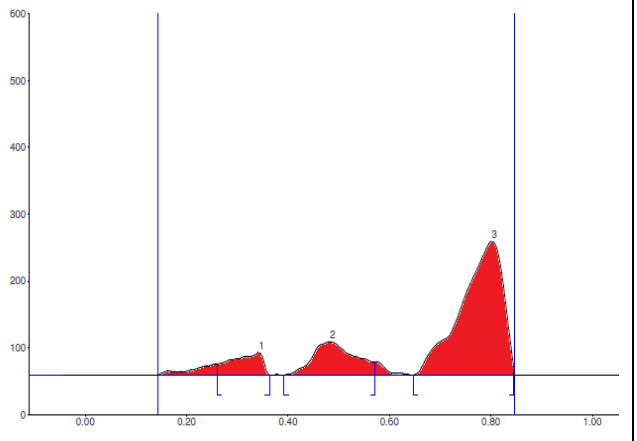


Plate 2: HPTLC Profile of Steroids in methanolic extracts of leaf, stem and root of *Achyranthes aspera* Linn. in visible light after derivatization.

Table 2: Densitogram and corresponding Rf values of methanolic extracts of Leaf of *Achyranthes aspera* Linn. in different neutral, acidic and basic fraction.

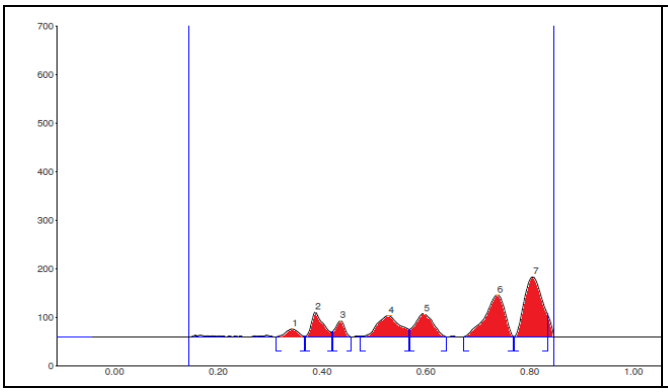
Leaf Neutral	Leaf Neutral
	

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.33	0.1	0.36	10.8	3.39	0.37	0.3	193.8	2.15
2	0.38	0.0	0.40	16.0	5.05	0.43	3.0	332.9	3.69
3	0.43	3.2	0.45	14.8	4.68	0.46	2.6	219.7	2.44
4	0.46	2.7	0.48	7.7	2.44	0.49	2.1	106.8	1.18
5	0.49	2.2	0.53	56.9	17.95	0.57	14.5	1508.5	16.72
6	0.57	14.8	0.60	102.2	32.22	0.66	0.4	2929.1	32.48
7	0.69	0.6	0.76	108.7	34.26	0.79	0.4	3728.9	41.34

Leaf Acidic	Leaf Acidic
	

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.26	16.1	0.34	33.4	11.81	0.36	0.0	1698.4	8.99
2	0.39	0.0	0.48	49.6	17.53	0.57	19.0	3454.7	18.29
3	0.65	0.0	0.80	199.9	70.66	0.85	8.1	13740.1	72.73

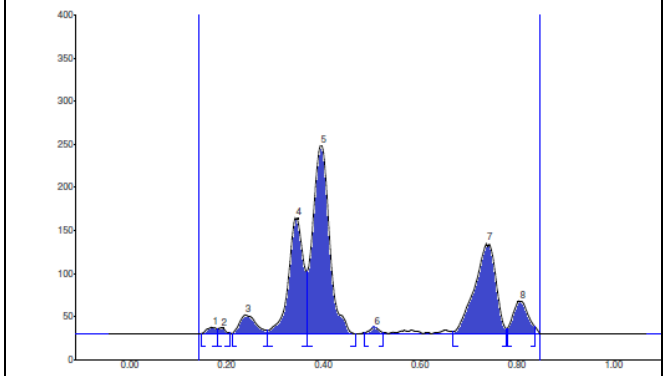
Leaf Basic	Leaf Basic



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.31	0.0	0.34	15.9	3.99	0.37	0.7	308.7	2.85
2	0.37	0.8	0.39	50.8	12.70	0.42	9.8	929.5	8.57
3	0.42	10.4	0.44	33.1	8.27	0.46	0.0	470.5	4.34
4	0.47	0.5	0.53	43.6	10.91	0.57	15.6	1562.3	14.40
5	0.57	15.9	0.60	47.4	11.86	0.64	0.2	1340.0	12.35
6	0.68	0.0	0.74	85.6	21.41	0.77	0.8	2715.5	25.03
7	0.77	0.2	0.81	123.5	30.87	0.84	46.8	3521.1	32.46

Table 3: Densitogram and corresponding Rf values of methanolic extracts of Stem of *Achyranthes aspera* Linn. in different neutral, acidic and basic fraction.

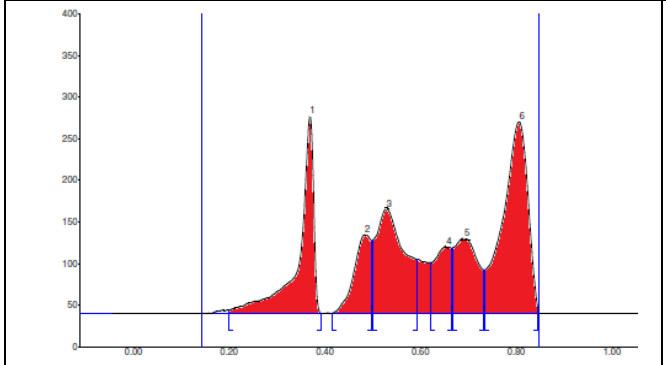
Stem Neutral



Stem Neutral

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.15	0.0	0.17	7.8	1.44	0.18	5.7	120.9	0.77
2	0.18	5.8	0.19	6.8	1.25	0.21	0.9	83.2	0.53
3	0.21	0.1	0.24	21.9	4.03	0.28	3.5	618.0	3.95
4	0.29	3.6	0.34	135.8	24.99	0.37	72.1	3489.5	22.29
5	0.37	73.0	0.40	219.6	40.41	0.47	0.0	6330.7	40.44
6	0.49	0.7	0.51	7.4	1.36	0.52	1.1	116.7	0.75
7	0.67	2.4	0.74	105.7	19.46	0.78	5.7	3906.8	24.95
8	0.78	6.3	0.81	38.4	7.06	0.84	8.2	990.6	6.33

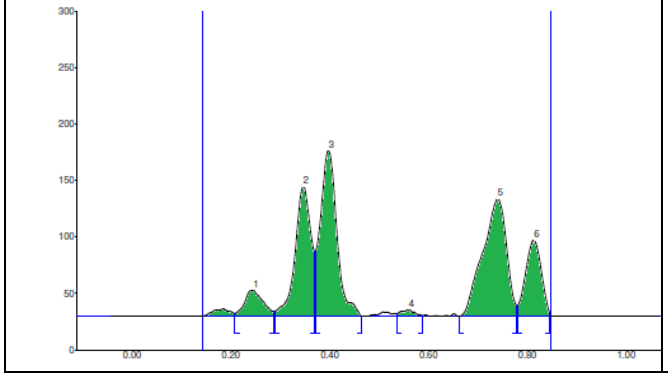
Stem Acidic



Stem Acidic

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.20	4.3	0.37	237.7	27.62	0.39	0.0	6071.6	19.30
2	0.42	0.1	0.48	94.9	11.03	0.50	87.5	2855.3	9.08
3	0.50	87.9	0.53	125.7	14.61	0.59	64.9	6289.2	19.99
4	0.62	61.5	0.66	80.8	9.39	0.67	78.9	2415.9	7.68
5	0.67	78.9	0.69	90.8	10.55	0.73	52.2	3768.7	11.98
6	0.74	52.4	0.81	230.6	26.80	0.85	4.8	10057.8	31.97

Stem Basic

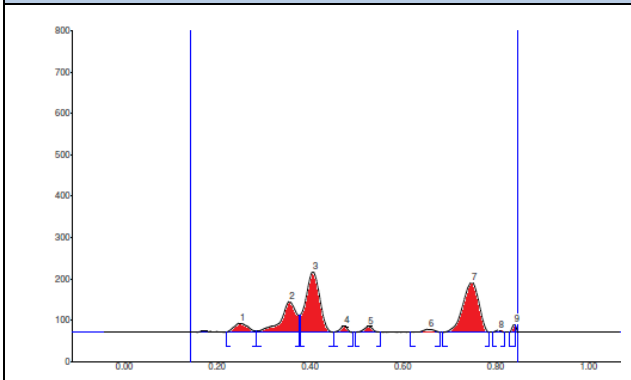


Stem Basic

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.21	2.4	0.25	23.1	4.98	0.29	4.0	717.2	5.11
2	0.29	4.4	0.35	115.4	24.91	0.37	57.1	3096.9	22.06
3	0.37	57.7	0.40	146.9	31.71	0.47	0.0	4115.6	29.32
4	0.54	2.3	0.56	6.1	1.31	0.59	0.4	130.3	0.93
5	0.66	0.2	0.74	104.4	22.54	0.78	9.2	4182.2	29.80
6	0.78	9.3	0.82	67.4	14.55	0.85	2.2	1793.7	12.78

Table 4: Densitogram and corresponding Rf values of methanolic extracts of Root of *Achyranthes aspera* Linn. in different neutral, acidic and basic fraction.

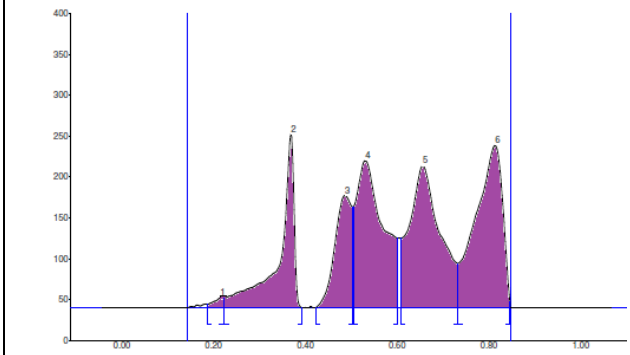
Root Neutral



Root Neutral

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.22	0.1	0.25	22.8	5.26	0.28	3.1	583.4	5.43
2	0.29	3.3	0.36	75.2	17.34	0.38	41.3	2106.7	19.60
3	0.38	41.8	0.41	147.2	33.96	0.45	0.8	3719.1	34.61
4	0.45	0.9	0.47	16.4	3.78	0.49	0.2	210.2	1.96
5	0.50	0.2	0.53	15.1	3.48	0.55	1.2	245.0	2.28
6	0.62	0.4	0.66	8.4	1.94	0.68	0.4	172.4	1.60
7	0.69	0.1	0.75	122.7	28.30	0.79	0.2	3542.0	32.96
8	0.79	0.2	0.81	5.9	1.36	0.82	0.0	66.0	0.61
9	0.83	0.1	0.84	19.8	4.57	0.84	19.4	102.1	0.95

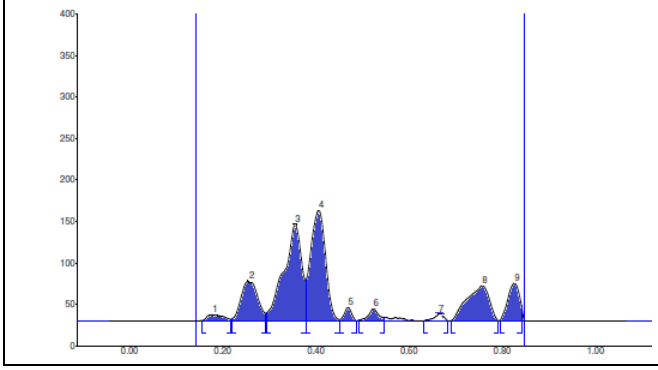
Root Acidic



Root Acidic

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.19	4.1	0.22	11.9	1.30	0.22	11.3	221.2	0.57
2	0.22	11.4	0.37	212.9	23.25	0.39	0.0	5675.4	14.74
3	0.42	0.2	0.49	137.6	15.03	0.50	123.3	4209.2	10.93
4	0.51	123.5	0.53	180.3	19.69	0.60	85.3	8942.9	23.22
5	0.61	85.0	0.66	174.0	19.00	0.73	54.3	9751.0	25.32
6	0.73	54.3	0.82	198.8	21.72	0.85	7.7	9710.1	25.21

Root Basic



Root Basic

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.16	0.5	0.18	7.5	1.74	0.22	1.9	217.9	1.78
2	0.22	2.1	0.26	47.8	11.09	0.29	9.3	1453.5	11.88
3	0.29	9.4	0.36	115.9	26.90	0.38	49.0	3755.7	30.69
4	0.38	49.9	0.41	133.3	30.94	0.45	1.5	3580.5	29.26
5	0.45	1.5	0.47	16.2	3.76	0.49	0.1	205.9	1.68
6	0.49	0.6	0.52	14.7	3.42	0.55	3.5	261.5	2.14
7	0.63	0.0	0.67	7.2	1.67	0.68	0.2	134.0	1.09
8	0.69	0.2	0.76	42.6	9.88	0.79	0.2	1698.0	13.87
9	0.80	0.5	0.83	45.7	10.60	0.84	18.1	931.3	7.61

CONCLUSION:

In general more degree of steroid diversity was found in root of *Achyranthes aspera* Linn. as compared to its stem and least diversity is found in its leaf. The maximum number of steroid has been observed in root followed by stem. The fingerprints so developed are useful in confirming the identity and purity of the medicinal plant raw material. The HPTLC fingerprint developed may serve as a supplement chromatographic data and the information thus generated may be explored further as a tool for standardization[11,12]. It can be concluded that HPTLC fingerprint analysis of root, stem and leaf extract of *Achyranthes aspera* Linn. can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker. The data obtained in the present work will be helpful in determining the known and unknown steroidal compounds of the plant and also provide the chemical basis for the wide use of this plant as therapeutic agent for treating various diseases. Isolation and identification of these compounds can lead to synthesis of new drug

REFERENCE:

1. Palo Alto, Article, 'Mastermind steroids found in plants'. Carnegie institution for science, Department of plant Biology, Aticle: November30, 2010

2. Krishnamurthi A. The Wealth of India. Vol. I. A publication and information directorate. New-Delhi: Council of Scientific and Industrial Research; 2003, p. 92.
3. Londonkar R, Chinnappa R, Abhay K, Potential antibacterial and antifungal activity of *Achyranthes aspera* L Racent research in Science and technology 2011,3(4): 53-57.
4. Manikandan A, Victor Arokia Doss A. Evaluation of biochemical contents, Nutritional value, trace elements, SDS-PAGE and HPTLC profiling in the leaves of Ruellia tuberosa L. and Dipteracanthus patulus (Jacq.) J Chem Pharm Res 2010; 2(3): 295-303.
5. Ramya V, Dheena Dhayalan V, Umamaheswari S. In vitro studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC. J Chem Pharm Res 2010; 2(6):86-91.
6. Patil AG, Koli SP, Patil DA, Chnadra N. Phamacognostical standardization and HPTLC finger print of Crataeva tapia Linn. SSP. Odora (Jacob.) Almeida leaves. Int J Pharm Biosci 2010; 1(2):1-14.
7. Siddiqui, A.A., Ali, M. Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, pp 126-131. 1997.
8. Wagner H, Baldt S, Zgainski EM. Plant drug analaysis. Berlin:Springer; 1996.
9. Harborne JB. Phytochemcial methods. 3rd ed. London: Chapman and Hall; 1998.
10. Eike Reich, Anne Schibli, High-Prformance Thin-Layer Chromatography for the Analysis of medicinal Plants, Theime Medical Publishers,Inc. New York, 2006:175-192.
11. K. Srilatha Srinivas, A. Saraf Aparna ; High Performance Thin Layer Chromatographic Determination of Chrysin in Oroxylum Indicum Vent. from Different Geographical Regions of India ; E-Journal of Chemistry 2012, 9(1), 313-317
12. Priya Alphonso, Aparna Saraf; Chemical profile studies on the secondary metabolites of medicinally important plant Zanthoxylum rhetsa (Roxb.) DC using HPTLC; Asian Pacific Journal of Tropical Biomedicine (2012) S1293-S1298.

