Research Journal of Pharmacognosy and Phytochemistry. 6(1): January-March, 2014, 22-29

ISSN 0975-2331 (Print)
0975-4385 (Online)

www.anvpublication.org

RESEARCH ARTICLE

Development of Finger Print Profiles for *Andrograrphis echioides* Nees. and *Andrographis paniculata* Nees.

Shakila R.*, Elankani P., Jegajothipandian S.
Siddha Central Research Institute (Central Council for Research in Siddha) Arignar Anna Hospital Campus, Arumbakkam, Chennai-600 106, Tamil Nadu, India.
*Corresponding Author E-mail: **shakilasiva@gmail.com**

ABSTRACT:
Andrographis echiodes Nees and *Andrographis paniculata* Nees are two species of the Genera Andrographis of the Acanthaceae family. The whole plant of *A. echiodes* and *A. paniculata* find the place in the Indian system of medicine. The TLC and HPTLC finger print of different extracts were developed. The TLC and HPTLC of hexane, chloroform, ethyl acetate and ethyl alcohol extracts were carried out and compared. The TLC photo documentation at UV 254 nm, 366 nm and post derivatization with vanillin-sulphuric acid reagent revealed that the two species are composed of different type of compounds and the HPTLC finger print also confirmed the dissimilarity between the species. These photo documentation and finger prints can be used as a reference documents for the identification as well as quality control of the two drugs in their different forms.

KEYWORDS: Gopuram Thangi, Nilavembu, Bhunimba, Echioidinin, Andrographolide.

1. INTRODUCTION:
A. echioides (L.) Nees is known as Gopuram Thangi in Tamil. *A. paniculata* Nees. is known as Nilavembu in Tamil and Bhunimba in Sanskrit. Both *A. echioides* and *A. paniculata* are annual herbs. They are found in South India and used for curing fevers[1,2]. Echioidinin,[3,4,5] dihydroechioidinin, echioidinin, echioidin, skullcapflavone I 2'-O-methyl ether, skullcapflavone I 2'-O-glucoside,[5] androechin, echioidinin 5-O-glucoside,[6] 2'-oxygenated flavonoids and phenyl glycosides[7] were reported from *A. echioides*; andrographolide, neoandrographolide,[8,9] ninandrographolide,[10] isoandrographolide, andropanolide[11] and many other andrographolide were reported from *A. paniculata*. Andrographolide, the bitter principle present in *A. paniculata* was proven as a potent antipyretic compound. This active principle is not present in *A. echioides* but is used as an antipyretic. Authors aim to compare the two species by developing TLC photo documentation and high performance thin layer chromatographic finger printing.

2. MATERIALS AND METHODS:
2.1. Plant material
The whole plants were collected from Mettur, Tamil Nadu in the month of May and authenticated from Botanical Survey of India, Coimbatore. The specimen samples were deposited in the Institute.

Received on 13.10.2013 Modified on 30.11.2013
Accepted on 15.12.2013

The samples were shade dried, powdered and stored in air tight containers for the study.

2.2. Chemicals
The solvents hexane, chloroform, ethyl acetate, ethyl alcohol, methanol used for the study were of AR grade. Vanillin (1 g) dissolved in ethanol sulphuric acid mixture with the ratio of 95:5, v/v was used as the visualizing agent.

2.2. Sample preparation
The coarsely powdered drug was weighed accurately about 4 g each and extracted successively with hexane, chloroform, ethyl acetate and ethyl alcohol using Soxhlet extractor. The extracts were concentrated by distillation and made up to 10 ml in standard flasks with respective solvents.

2.3. TLC plate
Aluminium plate precoated with silica gel $60F_{254}$ (Merck) of 0.2 mm thickness was used for application and development.

2.4. Solvent system
Thin layer chromatographic plates with good separation of compounds were achieved after trial with different solvent systems. For the hexane extract, the solvent system of Toluene: Ethyl acetate (4.5: 0.5, v/v) was found to be suitable; similarly for chloroform extract Toluene: Ethyl

acetate (5: 1, v/v), for ethyl acetate and ethyl alcohol extracts Ethyl acetate: Methanol (10 : 1, v/v) was chosen as suitable solvent system.

2.5. Instrument

The twin trough chamber (CAMAG) was used for developing the TLC plate. With the aid of Linomat IV (CAMAG, Muttenz, Switzerland) applicator 10 mm bands of extracts with a distance of 10 mm were applied on 5 x10 cm plate. CAMAG TLC scanner 030618 attached with WINCATS software were used for finger print analysis under UV 254 nm. CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm; and in visible lights after dipping in vanillin-sulphuric acid reagent followed by heating in an air circulated oven till the development of coloured spots.

3. RESULTS:

The thin layer chromatography (TLC) pattern of hexane extract of *A. echioides* (AEH) and that of *A. paniculata* (APH) are shown in Fig. 1a,b,c. The high performance thin layer chromatography (HPTLC) finger printing of AEH and APH are shown in Fig. 2 and 3; the 3D densitometric chromatogram of both extracts is shown in Fig. 4. The R_f values and area of peaks of hexane extract detected while scanning at UV 254 nm are shown in Table 1.

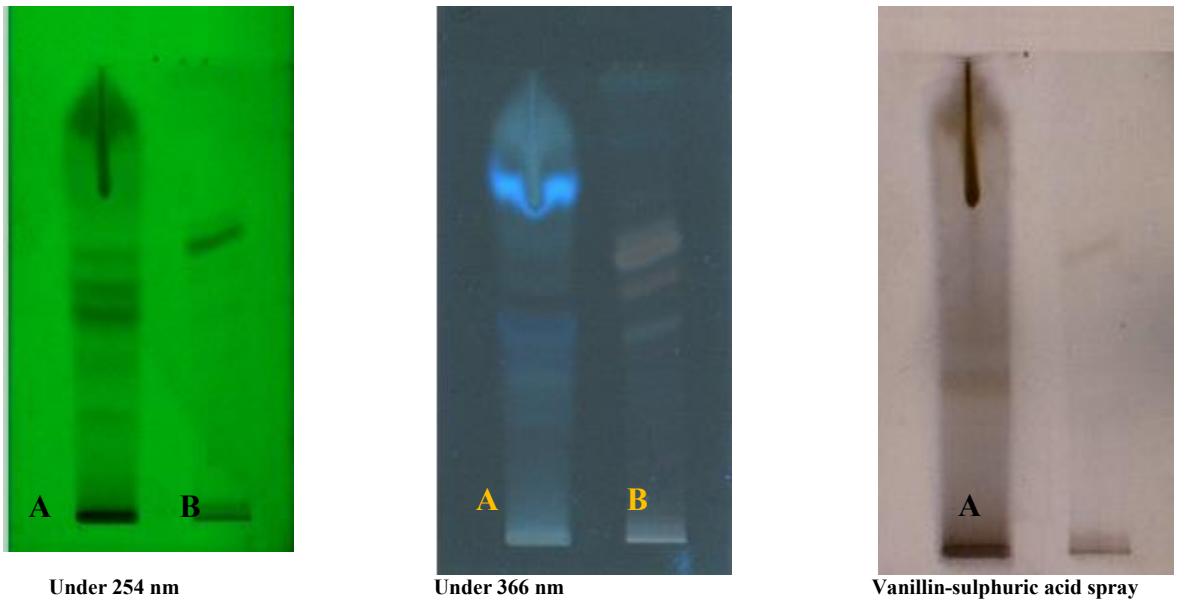
The TLC pattern of chloroform extract of *A. echioides* (AEC) and that of *A. paniculata* (APC) are shown in Fig. 5a,b,c. The HPTLC finger printing of AEC and APC are shown in Fig. 6 and 7; the 3D densitometric chromatogram of both extracts is shown in Fig. 8. The R_f values and area of peaks of chloroform extracts detected when scanned at UV 254 nm are shown in Table 2.

The TLC pattern of ethyl acetate extract of *A. echioides* (AEEA) and that of *A. paniculata* (APEA) are shown in Fig. 9a,b,c. The HPTLC finger printing of AEEA and APEA are shown in Fig. 10 and 11; the 3D densitometric chromatogram of both extracts is shown in Fig. 12. The R_f values and area of peaks of ethyl acetate extracts detected when scanned at UV 254 nm are shown in Table 3.

The TLC pattern of ethyl alcohol extract of *A. echioides* (AEE) and that of *A. paniculata* (APE) are shown in Fig. 13a,b,c. The HPTLC finger printing of AEE and APE are shown in Fig. 14 and 15; the 3D densitometric chromatogram of both extracts is shown in Fig. 16. The R_f values and area of peaks of chloroform extracts detected when scanned at UV 254 nm are shown in Table 4.

4. DISCUSSION:

TLC pattern (Fig. 1a,b,c) of AEH at UV 254 nm showed 6 spots at R_f 0.23, 0.35, 0.45, 0.50, 0.58, 0.92 (all green) and that of APH showed only two spots at R_f 0.45 and 0.58 (both green). At 366 nm, AEH showed 6 spots at R_f 0.26, 0.35, 0.43, 0.53, 0.63 and 0.75 (all pale blue); APH showed 6 spots at 0.43 (pale blue), 0.53 (bluish brown), 0.62 (bluish brown), 0.73 and 0.85 (both bluish brown). After derivatization, AEH showed 3 spots at R_f 0.35 and 0.47 (both brown).



Under 254 nm **Under 366 nm** **Vanillin-sulphuric acid spray**

Figure 1. TLC photo documentation of hexane extracts of A. *A. paniculata*; B. *A. echioides* whole plants

HPTLC finger print of AEH (Fig. 2) and APH (Fig. 3) showed 14 peaks. The R_f and percentage area of peaks are shown in Table 1. The major peaks of AEH appeared at 0.77 (32.63 %), 0.40 (16.23 %), 0.66 (12.40 %), 0.45 (11.26 %). The percentage area of peaks at R_f 0.52 and 0.86 were 8.75 % and 6.19 % respectively. The individual contribution of other spots ranged from 0.13 to 2.32 %. The major peaks of APH appeared at 0.56 (32.43 %) and 0.89 (13.21 %). The other peaks appearing at R_f 0.80 (8.71 %), 0.40 (6.89 %), 0.13 (6.62 %), 0.50 (6.62 %), 0.14 (5.98 %) were individually contributing >5% to the total area of the separated peaks. The percentage area of all other peaks <5 % only. Though both the extracts showed 14 peaks, the major peaks of AEH and APH are entirely different and R_f of other peaks are also different which is evident from the 3D chromatogram (Fig. 4).

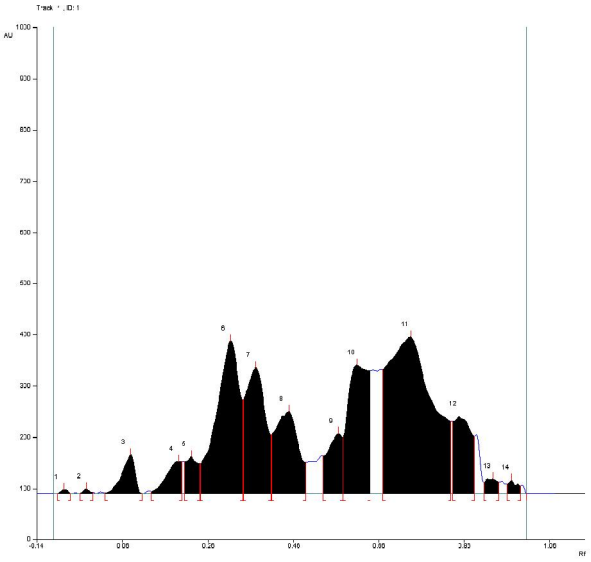


Figure 2. HPTLC densitometric chromatogram of hexane extract of *A. echiodes*

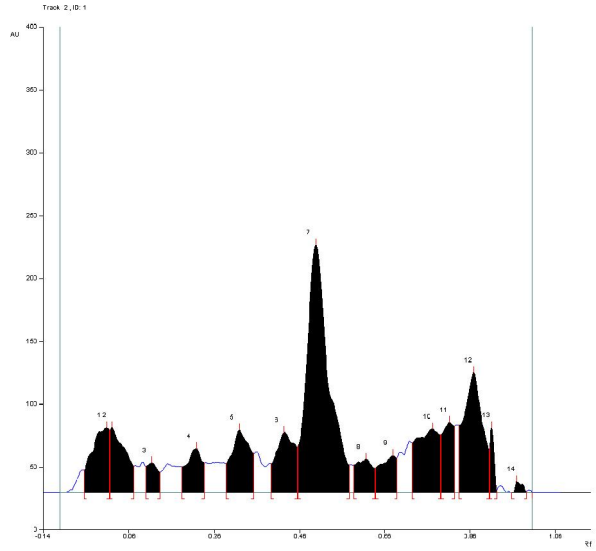


Figure 3. HPTLC densitometric chromatogram of hexane extract of *A. paniculata*

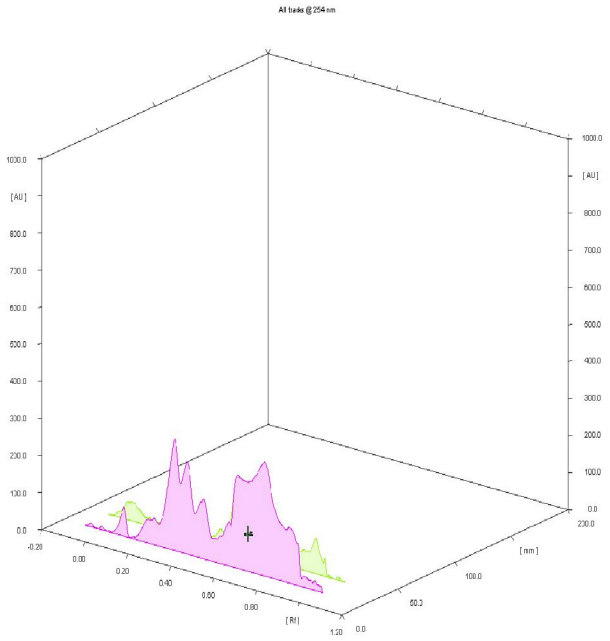


Figure 4. HPTLC densitometric 3D chromatogram of hexane extract of *A. echiodes* and *A. paniculata*

Table 1. R_f value and percentage area of peaks of hexane extracts at UV 254 nm

Sl.No	AEH		APH	
	R_f	% Area of peak	R_f	% Area of peak
1.	0.06	0.13	0.13	6.62
2.	0.10	0.14	0.14	5.98
3.	0.19	2.32	0.22	1.92
4.	0.29	2.29	0.31	4.05
5.	0.32	2.09	0.40	6.89
6.	0.40	16.23	0.50	6.62
7.	0.45	11.26	0.56	32.43
8.	0.52	8.75	0.66	3.36
9.	0.62	4.13	0.72	3.55
10.	0.66	12.40	0.80	8.71
11.	0.77	32.63	0.83	4.56
12.	0.86	6.19	0.89	13.21
13.	0.94	0.86	0.92	1.65
14.	0.97	0.57	0.97	0.65

TLC (Fig. 5) of AEC at UV 254 nm showed 5 spots at R_f 0.43, 0.52, 0.64, 0.71 and 0.79 (all green); APC showed two spots at R_f 0.07 and 0.79 (both green). At 366 nm, AEC showed 8 spots at R_f 0.07 (pale pink), 0.19 (pink), 0.33 (pink), 0.43 (brown), 0.57 (pink), 0.66 (pale blue), 0.72 (pink), 0.79 (brown); APC showed 7 spots with R_f 0.07 (pink), 0.17 (pink), 0.31 (pink), 0.39 (pink), 0.66 (blue), 0.72 (pink) and 0.79 (brown). The spot at R_f 0.43 of AEC is not seen in APC. After derivatization, AEC showed 6 spots at R_f 0.07 (purple), 0.16 (grey), 0.48 (violet), 0.64 (yellowish brown), 0.72 (purple) and 0.81 (violet); APC showed 4 spots at R_f 0.07 (violet), 0.48 (violet). 0.64 (violet) and 0.79 (violet). The spots at R_f 0.64 of both APH and APC are not same compounds.

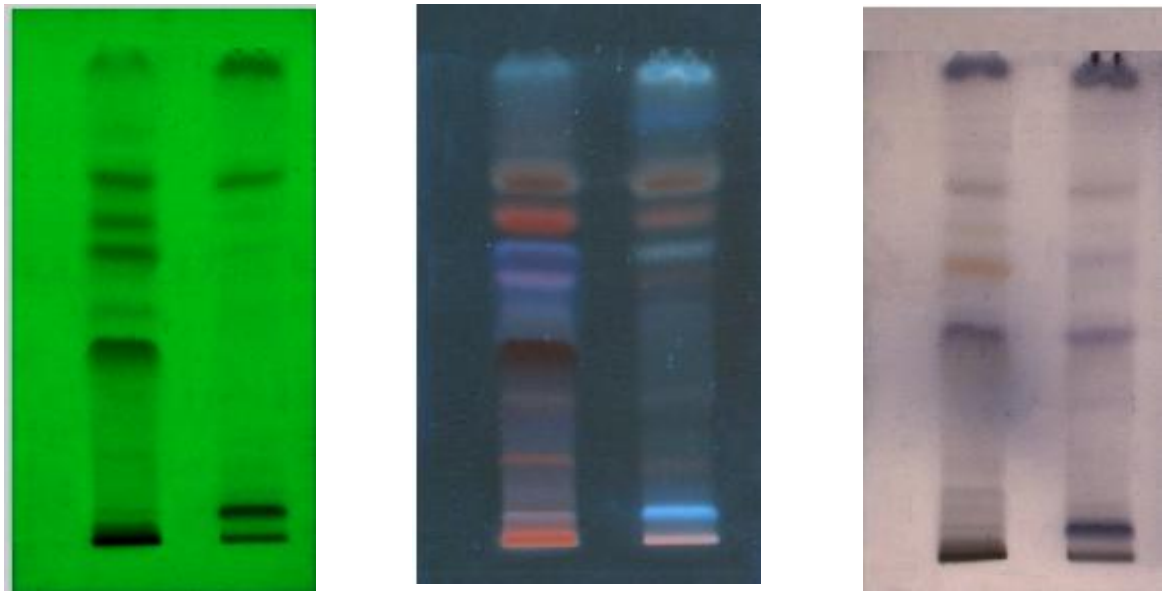


Figure 5. TLC photo documentation of chloroform extracts of A. *A. paniculata*; B. *A. echioides* whole plants

The HPTLC of AEC (Fig. 6) showed 15 peaks in which the peak at R_f 0.40 (33.61 %), 0.58 (20.77 %), 0.73 (14.39 %), 0.65 (14.15) and 0.47 (6.08 %) were the major. All other peaks were minor. The HPTLC of APC (Fig. 7) showed 21 peaks out of which only 3 peaks at R_f 0.06 (30.79 %), 0.73 (21.48%) and 0.94 (16.76%) were major. The peak at R_f 0.58 contributed 5.29 % to the total area. All other peaks were minor with less than 5 % of total area. Both extracts showed peak at R_f 0.7 with different peak area. The R_f and percentage area of all the peaks are shown in Table 2. The dissimilarity of both extracts were seen through the 3D chromatogram (Fig. 8).

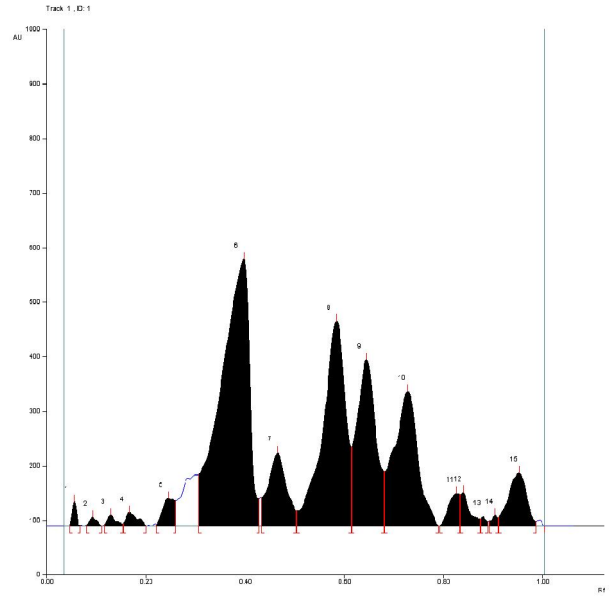


Figure 6. HPTLC densitometric chromatogram of chloroform extract of *A. echiodes*

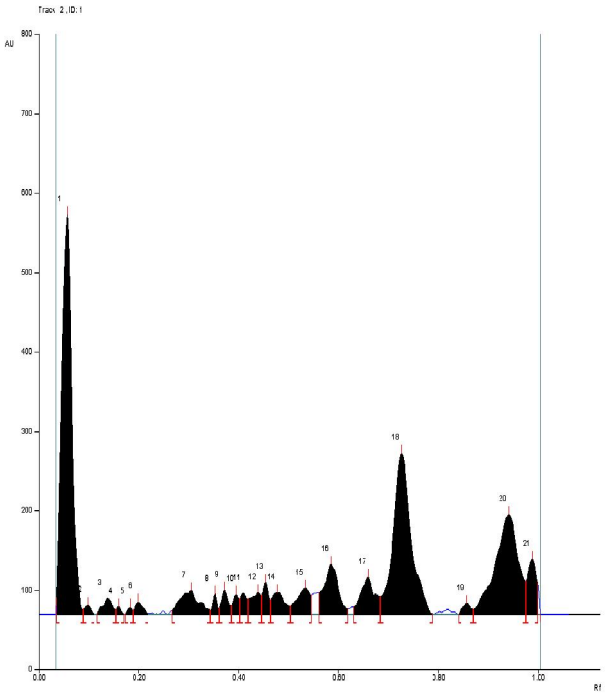


Figure 7. HPTLC densitometric chromatogram of chloroform extract of *A. paniculata*

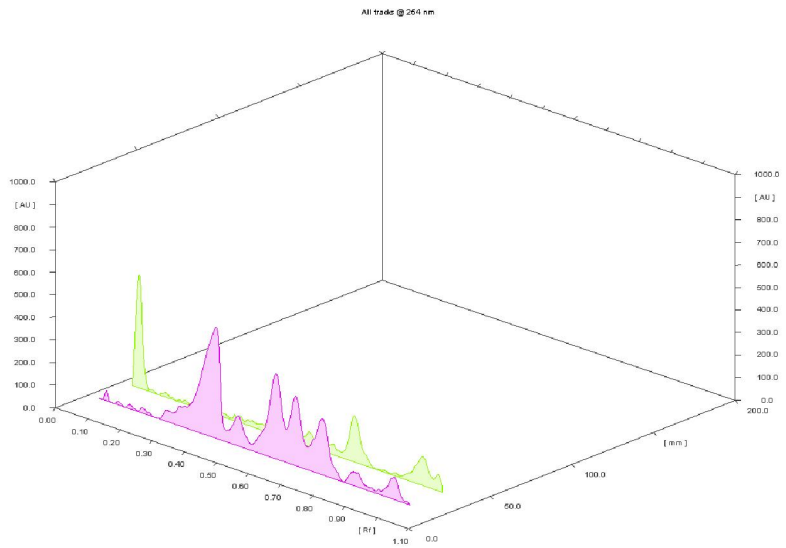


Figure 8. HPTLC densitometric 3D chromatogram of chloroform extract of *A. echiodes* and *A. paniculata*

Table 2. R_f value and percentage area of peaks of chloroform extracts at UV 254 nm

Sl.No	AEC		APC	
	R_f	% Area of peak	R_f	% Area of peak
1.	0.06	0.47	0.06	30.79
2.	0.09	0.27	0.10	0.40
3.	0.13	0.42	0.14	1.17
4.	0.17	0.67	0.16	0.25
5.	0.25	1.50	0.18	0.24
6.	0.40	33.61	0.20	0.72
7.	0.47	6.08	0.30	3.37
8.	0.58	20.77	0.35	0.73
9.	0.65	14.15	0.37	1.17
10.	0.73	14.39	0.40	0.83
11.	0.83	1.54	0.41	1.04
12.	0.84	1.37	0.44	1.56
13.	0.88	0.22	0.45	1.41
14.	0.90	0.29	0.48	2.03
15.	0.95	4.25	0.53	2.61
16.			0.58	5.29
17.			0.66	3.92
18.			0.73	21.48
19.			0.86	0.57
20.			0.94	16.76
21.			0.99	3.56

TLC (Fig. 9) of AEEA at UV 254 nm showed 5 spots at R_f 0.22, 0.38, 0.57, 0.69 and 0.83 (all green); APEA showed a spot at R_f 0.83 (green); in addition to this spots, other spots of AEEA are also seen in APEA in very low concentration. At 366 nm, AEEA showed 6 spots at R_f 0.26 (creamy blue), 0.33 (pale blue), 0.38 (pale blue), 0.53 (brown), 0.60 (pale blue) and 0.79 (brown)); APEA showed 5 spots with R_f 0.24 (creamy blue), 0.33 (pale blue), 0.52 (pale blue), 0.67 (pale blue) and 0.87 (blue). The spot at R_f 0.79 of AEC and APC are not one and same compound. After derivatization, AEEA showed 5 spots at R_f 0.10 (brown), 0.24 (brown), 0.34 (brown), 0.53 (brown) and 0.66 (brown). APEA showed 3 spots at R_f 0.10 (grey), 0.21 (grey) and 0.79 (violet).

The HPTLC of AEEA (Fig. 10) showed 7 peaks at R_f values 0.54 (25.85 %), 0.90 (24.68 %), 0.23 (16.64 %), 0.76 (10.87 %), 0.85 (8.72 %), 0.33 (8.23 %) and 0.64 (5.01 %). The HPTLC of APEA (Fig. 11) showed 11 peaks at R_f values 0.78 (25.69 %), 0.93 (23.07 %), 0.89 (18.23 %) and 0.84 (11.06 %). The peak at R_f value 0.34 contributed to 5.39 % and other peaks were minor. The R_f values and the area of all peaks are shown in Table 3. The 3D chromatogram (Fig. 12) also showed that the HPTLC pattern of both extracts was differing from each other

Table 3. R_f value and percentage area of peaks of ethyl acetate extracts at UV 254 nm

Sl.No	AEEA		APEA	
	R_f	% Area of peak	R_f	% Area of peak
1.	0.23	16.64	0.13	3.17
2.	0.33	8.23	0.18	2.49
3.	0.54	25.85	0.24	4.75
4.	0.64	5.01	0.34	5.39
5.	0.76	10.87	0.47	2.58
6.	0.85	8.72	0.49	3.19
7.	0.90	24.68	0.57	0.37
8.			0.78	25.69
9.			0.84	11.06
10.			0.89	18.23
11.			0.93	23.07

TLC (Fig. 13) of AEE at UV 254 nm showed 4 spots at R_f 0.26, 0.38, 0.52 and 0.86 (all green); APE showed a spot at R_f 0.78 (green). At 366 nm, AEE showed 2 spots at R_f 0.26 (creamy blue) and 0.38 (pale blue); APE showed a spot at R_f 0.34 (creamy blue). After derivatization, AEE showed 3 spots at R_f 0.09 (brown), 0.26 (brown) and 0.53 (brown). APE also showed 3 spots at R_f 0.09 (grey), 0.24 (grey) and 0.78 (grey).

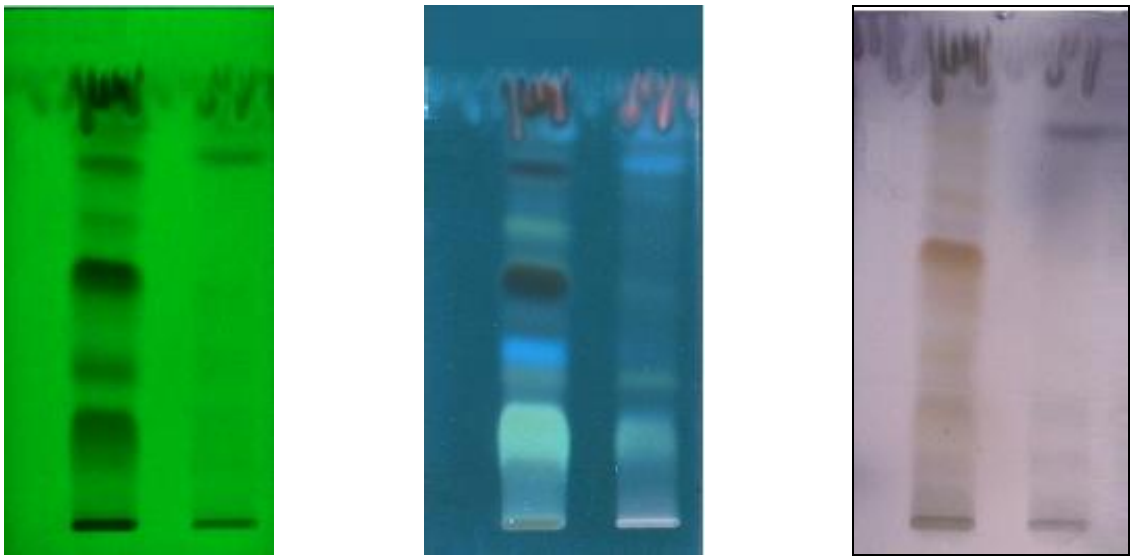


Figure 9. TLC photo documentation of ethyl acetate extracts of *A. paniculata*; B. *A. echioides* whole plants

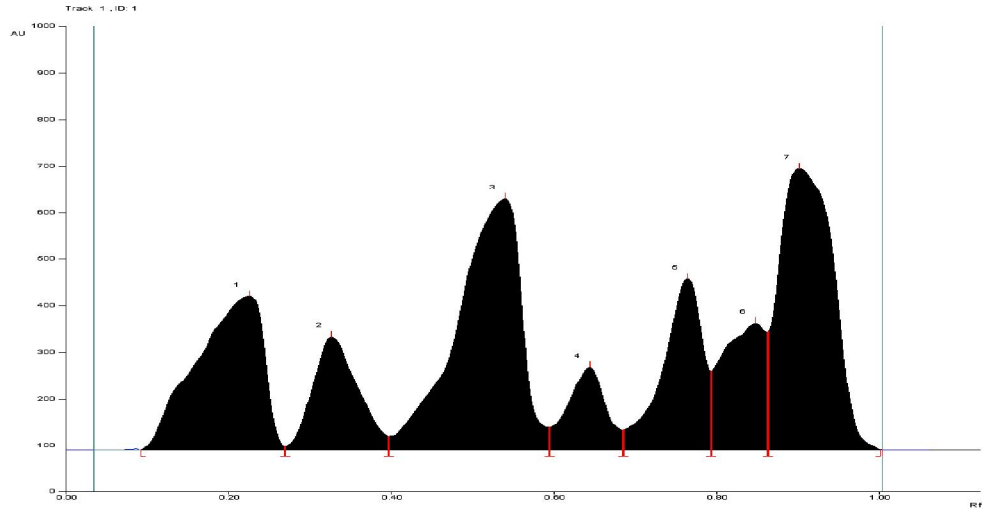


Figure 10. HPTLC densitometric chromatogram of ethyl acetate extract of *A. echiodes*

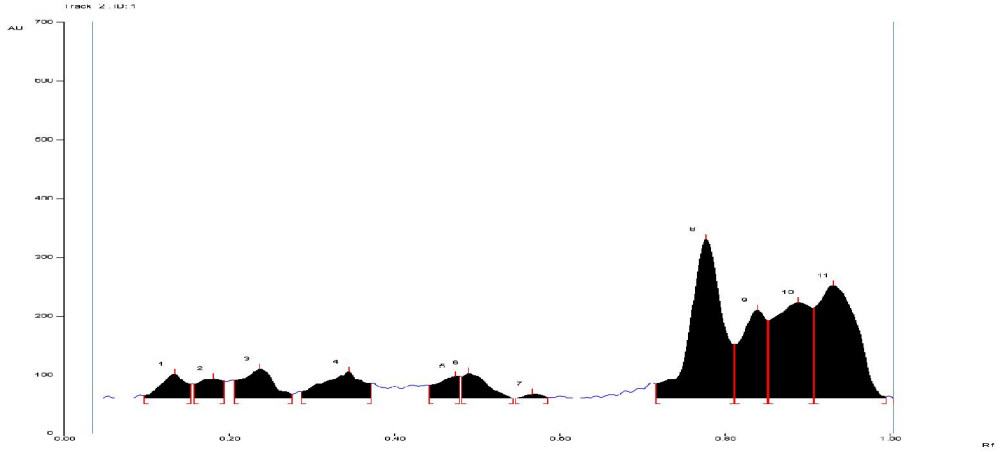


Figure 11. HPTLC densitometric chromatogram of ethyl acetate extract of *A. paniculata*

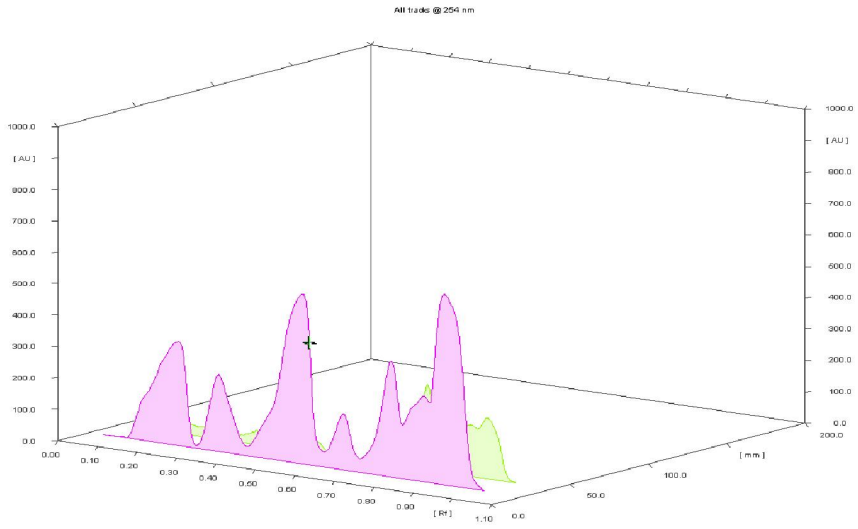
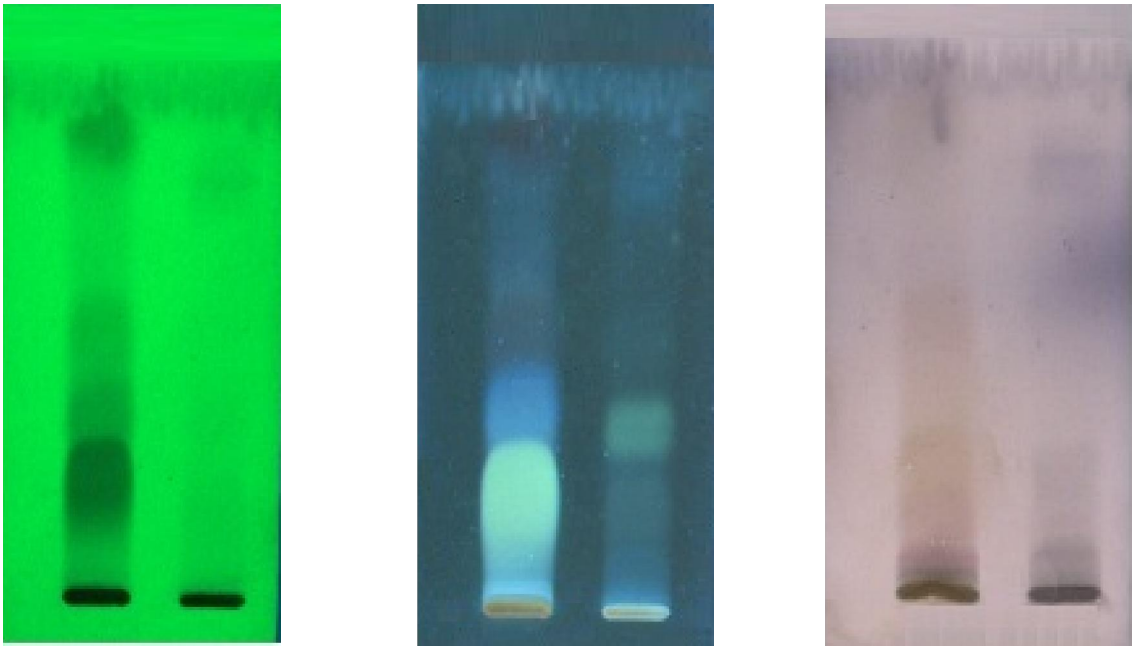


Figure 12. HPTLC densitometric 3D chromatogram of ethyl acetate extract of *A. echiodes* and *A. paniculata*

The HPTLC finger print of AEE (Fig. 14) showed 12 peaks in which the peak at R_f 0.73 was the major of all the peaks. The other major peaks were 0.31 (16.23 %), 0.61 (12.40 %), 0.37 (11.26 %), 0.45 (8.75 %), 0.85 (6.19 %) and 0.56 (4.13 %). Other peaks were minor. The HPTLC finger print of AEP (Fig. 15) showed 13 peaks of which the peak at R_f 0.50 (32.43 %) and 0.87 (13.21 %) were the major peaks. The peaks at R_f value 0.77 (8.71 %), 0.32 (6.89 %), 0.42 (6.62 %) and 0.02 (5.98 %) were individually contributing more than 5 % to the total peak area and other peaks were minors. The R_f value and the peak area are shown in Table 4. The 3D densitometric chromatogram (Fig. 16) showed the difference between the two extracts.



Under 254 nm **Under 366 nm** **Vanillin-sulphuric acid spray**

Figure 13. TLC photo documentation of ethanol extracts of A. *A. paniculata*; B. *A. echioides* whole plants

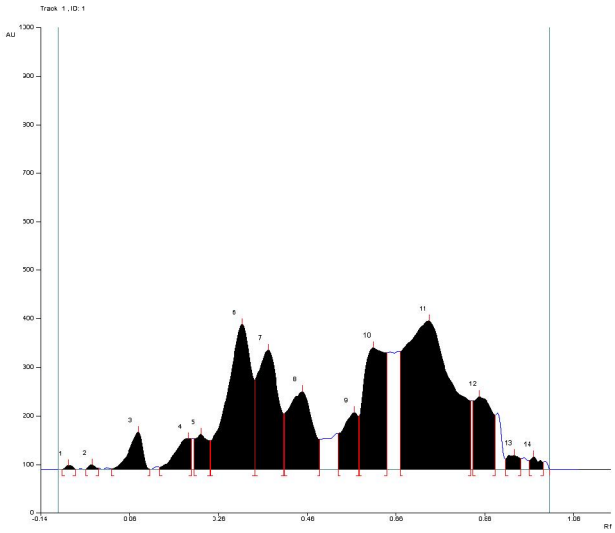


Figure 14. HPTLC densitometric chromatogram of ethanol extract of *A. echioides*

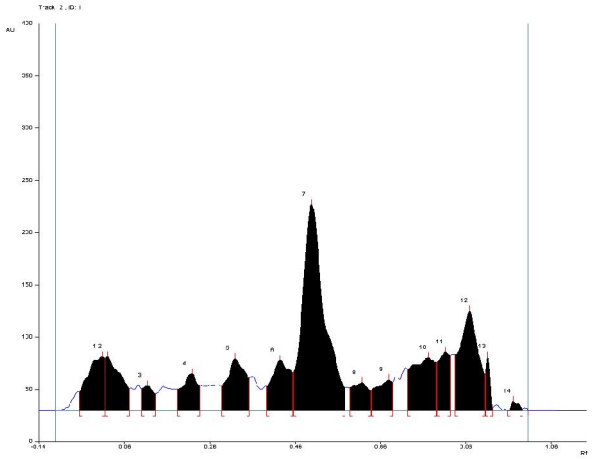


Figure 15. HPTLC densitometric chromatogram of ethyl acetate extract of *A. paniculata*

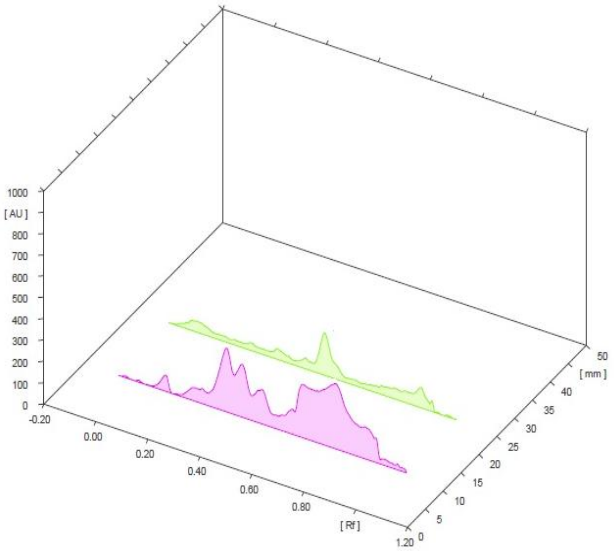


Figure 16. HPTLC densitometric 3D chromatogram of ethanol extract of *A. echiodes* and *A. paniculata*

Table 4. R_f value and percentage area of peaks of ethyl alcohol extracts at UV 254 nm

Sl.No	AEE		APE	
	R_f	% Area of peak	R_f	% Area of peak
1	0.08	2.32	0.02	5.98
2.	0.19	2.29	0.11	1.92
3.	0.22	2.09	0.22	4.05
4.	0.31	16.23	0.32	6.89
5.	0.37	11.26	0.42	6.62
6.	0.45	8.75	0.50	32.43
7.	0.56	4.13	0.61	3.36
8.	0.61	12.40	0.68	3.55
9.	0.73	32.63	0.77	8.71
10.	0.85	6.19	0.81	4.56
11.	0.93	0.86	0.87	13.21
12.	0.97	0.57	0.91	1.65
13.			0.97	0.46

CONCLUSION:

The generated TLC photo documentation patterns and the HPTLC finger print profiles of different extracts will be helpful for authentication of *A. echioides* as well as *A. paniculata* in any formulation and in powder form.

REFERENCES:

1. Kirtikar, K.R.; Basu, B.D. *Indian Medicinal Plants*; Periodical Experts Book Agency, New Delhi, India. 1975; Volume 3: 1884-1886.
2. Chopra, R.N.; Nayer, S.L.; Chopra, I.C. *Glossary of Indian Medicinal Plants*; Council of Scientific and Industrial Research: New Delhi, India. 1980; 18.
3. Govindachari TR, Parthasarathy PC, Pai BR, Subramaniam PS. Chemical examination of Andrographis echioides-I: structure and synthesis of echioidinin. Tetrahedron. 21(9); 1965: 2633-2640.
4. Govindachari TR, Parthasarathy PC, Pai BR, Subramaniam PS. Chemical examination of Andrographis echioides-II: structure and synthesis of echioidin. Tetrahedron. 21(12); 1965: 3715-3720.
5. Jayaprakasam B, Damu AG, Gunasekar D, Blond A, Bodo B. Dihydroechioidinin, a flavanone from *Andrographis echioides*. Phytochemistry. 52(5); 1999: 935-937.
6. Jayaprakasam B, Gunasekar D, Rao KV, Blond A, Bodo B. Androechin, a new chalcone glucoside from *Andrographis echioides*. J Asian Nat Prod Res. 3(1); 2001: 43-48.
7. Shen DY, Juang SH, Kuo PC, Huang GJ, Chan YY, Damu AG, Wu TS. Chemical constituents from *Andrographis echioides* and their anti-inflammatory activity. Intl J Mol Sci. 14(1); 2013: 496-514.
8. Fujito T, Fujitani R, Takeda Y, Takaishi Y, Yamada T, Kido M, Miura I. on the diterpenoids of *Andrographis* paniculata: X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. Chem Pharm Bull. 32; 1984: 2117.
9. Chen LX, Qu GX, Qiu F. Studies on diterpenoids from *Andrographis paniculata.* Zhongguo Zhong Yao Za Zhi. 31; 2006: 1594-1597.
10. Meng Z. Isolation and identification of a new glucoside-bicyclic diterpenoid lactone from *Andrographis* paniculata Nees. Nanjing Yaoxueyuan Xuebao. (1): 1982; 15-20. Chem Abstr. 99: 1983; 191697j.
11. Pramanick S, Banerjee S, Achari B, Das B, Sen AK Sr, Mukhopadhyay S, Neuman A, Prange T. Andropanolide and isoandrographolide, minor diterpenoids from Andrographis paniculata: structure and X-ray crystallographic analysis. J Nat Prod. 69: 2006; 403-405.