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RESEARCH COMMUNICATION

Comparative HPTLC analysis of stem and leaf of *Achyranthes coynei* with *Achyranthes aspera*

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Abstract

Leaf and stem materials of *Achyranthes coynei* and *Achyranthes aspera* were used for HPTLC analysis. HPTLC plates were developed on n-hexane: ethyl acetate (5:1 v/v) solvent system. The densitometric profiles were evaluated to elucidate differences within and among the species. The R_f values and number of peaks obtained in densitrogram indicated chemical variation in the species. Although, both species had more or less equal number of peaks, their R_f values, %height and %area varied. Thus HPTLC analysis in absence of external standards, proved to be an informative tool for evaluating differences between these species and their parts.

Keywords: *Achyranthes aspera*; *Achyranthes coynei*; HPTLC

Introduction

Genus *Achyranthes* (Family: Amaranthaceae) comprises of about 15 species globally, including few medicinally and economically important species. *Achyranthes coynei* Sant. is an endemic, rare, endangered, threatened (RET) plant from India, recently been reported for its extended distribution from Karnataka (Pai *et al*., 2011). The plant is reported to possess antioxidant and antimicrobial properties (Upadhya *et al*., 2013; Ankad *et al*., 2013). On the other hand, *Achyranthes aspera* L. is a well known medicinal plant, reported as highly traded in India (Ved and Goraya, 2007). It is used in traditional medicinal systems in India to treat various disorders (Tandon, 2011; Upadhya *et al*., 2009). Additionally, identification and/or quantification of marker/ major compounds have been reported by Tondon (2011); Upadhya *et al*., (2014a); Pai and Joshi (2014) and Pai *et al*., (2014) from these species.

Various new and sophisticated analytical methods *viz*. HPTLC, HPLC, GC are being utilized for identifying compounds (Kokate *et al*., 2009; Patil *et al*., 2012; Upadhya *et al*., 2014b). Among these, HPTLC is the most popular, economical and reliable technique, used in differentiation and quality control analysis in pharmaceutical research (Hariprasad and Ramakrishnan, 2011). HPTLC technique offers better resolution of chemical constituents present in the plant extract, with reasonable accuracy in a shorter time (Sethi, 1996; Pawar *et al*., 2011). Generally, an identified compound serves as external standard for quality and quantity assurance of samples. In the absence of such reference compounds, the HPTLC fingerprints are compared with respect to number, sequence, position and colour of the separated bands (Mammen *et al*., 2011; Kamboj and Saluja, 2013). Furthermore, they are also employed in elucidating and comparing differences among species, their parts and/or herbal preparations (Kamboj and Saluja, 2013; Potawale *et al*., 2013). Such studies also provide an option of estimating differential patterns in samples.

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Thus, keeping in view of this aspect, present work was aimed towards finding out differentiation within and among leaf and stem extracts of *A. coynei* and *A. aspera* using HPTLC method.

Materials and Methods

Plant materials and chemicals

Leaf and stem materials of *A. coynei* and *A. aspera* were obtained from a single population from Madanabhavi, Belgaum district, Karnataka, India and specimens were authenticated and deposited at Herbarium, Regional Medical Research Centre, Belgaum (Voucher Number: *A. coynei*: RMRC 785; *A. aspera*: RMRC1250). All the solvents used during the study were of HPLC grade (Fischer Scientific, Mumbai, India).

Extraction

The plant materials (leaf and stem) of *A. coynei* and *A. aspera*, obtained from wild, were dried at room temperature and finely powdered. Previously employed method of Upadhya *et al*., (2014a) for total triterpenoid was used for extraction. A 5 g of powder was extracted with 25 mL (50% v/v) aqueous methanol by heating (2-3 min). To this, distilled water (75 mL) and concentrated H_2SO_4 (10 mL) was added with shaking. The mixture was refluxed for 5-6 hours over water bath at 95±2°C. The contents were cooled, filtered and transferred to separating funnel. To the above mixture chloroform (25 mL) was added and the layer was allowed to separate and the process was repeated twice. The chloroform layer obtained was washed using distilled water to get rid of acid. The acid free chloroform layer was dried to obtain residue. The residue was dissolved in methanol and the same was used for HPTLC analysis.

HPTLC analysis

Instrument and chromatographic conditions

A CAMAG high performance thin layer chromatography (HPTLC) system was used for detection and separation. Analysis was performed on a pre-coated TLC silica gel G60 F_{254} plates (MERCK, Germany). Sample bands (6 mm) were applied using CAMAG Automated TLC Sampler (ATS-4) equipped with 25-µL syringe operated with settings: band length 6 mm, application rate 150 µL/s, distance from the bottom of the plate (Y) 10 mm, distance from edge of plate (X) 10 mm, distance between bands were auto set (6 mm). The plates were developed to a distance of 80 mm with hexane: ethyl acetate (5:1, v/v) as mobile phase in a CAMAG twin trough glass chamber previously saturated with the mobile phase at room temperature for 20 min.

Table 1 Densitogram attributes of HPTLC run of leaf and stem extract of *A. coynei* and *A. aspera*

Plant	*A. coynei*										*A. aspera*									
Parts	*Leaf*					*Stem*					*Leaf*					*Stem*				
Pk	R_f	Ht AU	Ht %	A AU	A %	R_f	Ht AU	Ht %	A AU	A %	R_f	Ht AU	Ht %	A AU	A %	R_f	Ht AU	Ht %	A AU	A %
1	-0.01	743.9	61.22	28631.6	63.95	-0.01	710.2	44.89	25464.6	49.15	-0.01	739.2	57.40	25434.7	68.98	-0.02	632.6	28.35	12273.7	25.69
2	0.10	59.6	4.91	1279.6	2.86	0.04	170.8	10.79	3180.0	6.14	0.08	65.1	5.06	821.3	2.23	-0.01	680.6	30.50	14952.2	31.30
3	0.14	60.7	4.99	1812.3	4.05	0.07	117.7	7.44	2055.9	3.97	0.13	54.0	4.19	1021.1	2.77	0.05	237.9	10.66	4942.5	10.35
4	0.22	72.9	6.00	2635.2	5.89	0.12	79.0	4.99	3077.0	5.94	0.16	35.3	2.74	903.3	2.45	0.07	167.7	7.51	1936.2	4.05
5	0.24	65.9	5.42	2175.1	4.86	0.16	54.0	3.41	693.5	1.34	0.23	54.3	4.22	1729.3	4.69	0.09	179.4	8.04	3952.7	8.27
6	0.31	20.6	1.69	375.4	0.84	0.21	63.0	3.98	1966.2	3.80	0.29	27.8	2.16	372.7	1.01	0.17	47.5	2.13	710.6	1.49
7	0.42	14.0	1.16	97.0	0.22	0.25	76.7	4.85	2667.4	5.15	0.33	17.9	1.39	339.9	0.92	0.21	39.4	1.77	917.1	1.92
8	0.45	17.6	1.45	222.3	0.50	0.32	17.2	1.09	295.4	0.57	0.39	22.2	1.72	652.1	1.77	0.24	31.3	1.40	726.6	1.52
9	0.48	20.1	1.66	626.6	1.40	0.48	97.4	6.15	3479.0	6.72	0.42	23.8	1.85	412.5	1.12	0.28	12.3	0.55	174.2	0.36
10	0.61	19.1	1.57	290.9	0.65	0.56	21.7	1.37	513.3	0.99	0.46	26.5	2.06	442.2	1.20	0.32	18.5	0.83	321.7	0.67
11	0.66	12.8	1.06	191.4	0.43	0.58	19.6	1.24	280.7	0.54	0.53	27.3	2.12	354.6	0.96	0.37	19.5	0.87	475.7	1.00
12	0.71	16.7	1.38	406.4	0.91	0.63	23.9	1.51	555.4	1.07	0.56	27.3	2.12	401.9	1.09	0.42	24.9	1.12	397.1	0.83
13	0.86	91.1	7.49	6025.0	13.46	0.69	27.4	1.73	724.4	1.40	0.79	42.7	3.32	898.1	2.44	0.48	58.2	2.61	2282.4	4.78
14						0.73	15.8	1.00	226.2	0.44	0.87	48.7	3.78	1258.8	3.41	0.57	17.9	0.80	403.4	0.84
15						0.86	87.9	5.56	6628.3	12.79	0.89	46.6	3.62	1118.7	3.03	0.86	63.7	2.86	3304.6	6.92
16											0.96	29.0	2.25	711.5	1.93					

Pk – Peak; R_f – Retention factor; Ht – Height; AU – Area Under Curve; A - Area; the highlighted values in above table indicate application position of extracts on HPTLC plate, not considered for the analysis

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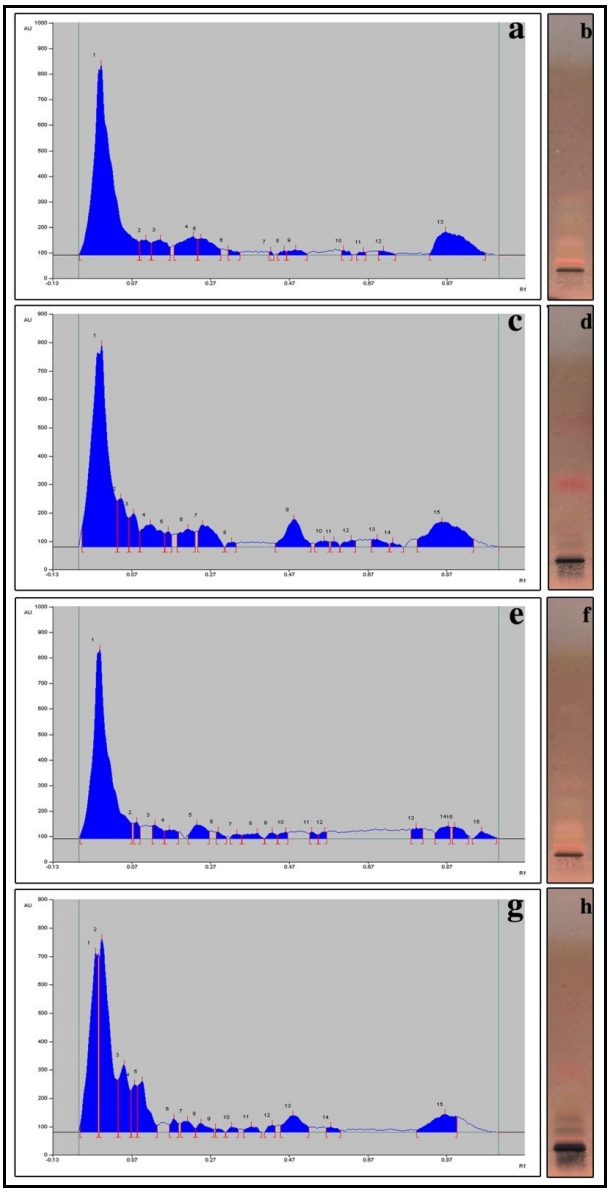

Fig 1. HPTLC densitogram profile and plate image of a and b: *A. coynei* – Leaf extract; c and d: *A. coynei* - Leaf extract; e and f: *A. aspera* – Stem extract; g and h: *A. aspera* – Stem extract

After the run, plates were dried in air current using drier and derivatised using anisaldehyde – sulphuric acid reagent followed by heating for 5 min at 110±2°C. Visualization of bands on plate in white light was recorded using CAMAG TLC visualizer. TLC plates were scanned at 540 nm and the data for the peaks were generated.

Results and Discussion

HPTLC analysis

During the present study a comparative analysis was carried out on two species of genus *Achyranthes viz. A. coynei* and *A. aspera* using HPTLC technique. Better band separation was obtained using hexane: ethyl acetate (5:1 v/v) as solvent system during the procedure. HPTLC plates were scanned at 540 nm in white lamp using automatic detector mode in CAMAG TLC scanner. The scan start position (Y axis) at 5.0 mm and scan end position (Y axis) at 85.0 mm with a slit dimension of 6.00 x 0.45 mm at a scan speed of 20 mm/ sec for each track was employed. The data resolution was set at 100 µm/ step. The densitogram and image for each track of *A. coynei* (leaf and stem) and *A. aspera* (leaf and stem) are presented in Fig. 1 a-h. The results (*viz.* peak number, R_f value, height, % height, area and % area) for the HPTLC run of both the species (leaves and stem) are presented in Table 1. It may be noted that the negative R_f values in the Table 1 indicate application position.

Comparison within the species

Variations in densitogram of leaf and stem extract of *A. coynei* and *A. aspera* were observed. *Achyranthes coynei* stem extracts showed 5 peaks different from that of leaf extracts while, in leaf extracts 3 peaks were different from stem extracts (Table 1). In contrary, 7 peaks observed in *A. aspera* leaf extracts were absent in stem extracts in comparison to 5 peaks not matching with leaf extracts. In general, stem extract was diverse in chemical nature over leaf extract.

Comparison of leaf extracts of A. coynei *with* A. aspera

Leaf extract of *A. coynei* showed 13 peaks whereas16 peaks were observed in leaf extract of *A. aspera*. This indicates the difference in the banding pattern and variations in the chemical constituents of both the species. Peak number seven in *A. coynei* and nine in *A. aspera* showed similar R_f value (0.42) indicating chemically identical constituents. Interestingly, height and area of the peak was higher *A. aspera* (Table 1, Fig. 1 a, c). Furthermore, other peaks showed different R_f values among the leaves of both species. Peak 13 in *A. coynei* had highest area and height at R_f 0.86 compared to the other species.

Comparison of stem extracts of A. coynei *with* A. aspera

A total of 15 peaks were observed in stem extracts of both the species. Peak with negative R_f values were not considered for analysis. Three peaks at R_f 0.21, 0.48 and 0.86 were coinciding in both the species of *Achyranthes* under study. However, these peaks in *A. coynei* were nearly double the area of those in *A. aspera*, indicating higher amount of those similar chemical constituents (Table 1, Fig. 1 e, g). Other peaks with different R_f

values among the stem extracts of both species indicated variation in chemical nature.

Results of the present investigation indicate variations among parts and species. HPTLC analysis provided adequate information in differentiating these medicinal plants based on R_f values and number of peaks. Similar observations on HPTLC analysis have been worked and assessed by Hariprasad & Ramakrishnan (2011) in *Rumex vesicarius*, Sethi (1996) in pharmaceutical formulations and Mammen *et al*., (2011) in seasonal, geographical variation in *Aerva lanata*. Although, studies on specific triterpenoids *viz.,* betulinic acid and oleanolic acid are reported in both the plant species using HPLC method (Pai *et al*., 2014; Upadhya *et al*., 2014a). However, these fingerprint patterns can be used to determine the chemical variations in and within the species.

Conclusion

A comparative account of leaf and stem extracts of *A. coynei* with that of *A. aspera* depicted large variation. HPTLC analysis reveals clear differentiation in leaves and stems, both within and among the species. Further, detailed qualitative and quantitative investigations are required for identification of these chemical entities.

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