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HPTLC finger print profile of n-hexane extract of R*auwolfia tetraphylla* Linn

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ABSTRACT

The objective of the present study is to evaluate the phytochemical composition and HPTLC finger print profile of medicinally useful plant *R. tetraphylla* L. (Apocyanaceae) leaf n-hexane extract. The CAMAG HPTLC system was used for the finger print profiling using the mobile phase toluene: ethyl acetate: acetic acid (55:45:1 v/v). The profile showed that the leaf extract of *R. tetraphylla* exhibited several peaks with different Rf values when visualized. At 254nm and 366 nm .At 254 nm a total 14 peaks were observed and at 366nm 10 peaks were observed. The HPTLC profile of *R. tetraphylla* leaf n-hexane extract is used to identify the number of chemical components and their concentration; this in turn helps to identify the chemical constituents in medicinal products and also in identification of adulterants in medicinal products mainly herbal medicine.

Keywords: *Rauwolfia tetraphylla*, HPTLC, Fingerprint, Apocyanaceae, CAMAG

INTRODUCTION

Herbal drugs obtained from medicinal plants which in turn synthesize complex organic constitutes with frequently unknown biologically 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 circumstances of the plant, parts of the plant used, preparation of the plant for use, extraction method, volume of extract used in the final preparation and numerous 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. Synthetic drugs causes 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 to Ayurveda and additional traditional system of medicine [3]. Phytochemical analysis of plants used in folklore 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 physico-chemical characters. Hence, the current methods describing the identification and quantification of active constituents in the plant material may be functional for proper standardization of herbs and its formulations [4,5]. Finger printing can be used to identify the plant, determine active ingredients or markers, and detect impurities or contaminants such as herbicides [6]. High performance thin layer chromatography (HPTLC) is frequently used as an alternative to HPLC for the quantification of plant products because of its accuracy, simplicity, cost-effectiveness and rapidity[7]. HPTLC methods are faster, reproducible and reliable. Integrating HPTLC with digital scanning profiling gives accurate quantifiable analysis and Rf 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[8]. 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[9]. 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 serve as a instrument for authentication, identification and quality control of herbal drugs [10]. *Rauwolfia tetraphylla* (Family: Apocyanaceae) belongs to genus Rauwolfia that consists of around one thousand species, five of which are inhabitant to India [11]. *R. tetraphylla* is a woody shrub, tender parts of this plant are puberulous and grow up to 1½ m in height, leaves are four at every node, elliptic and ovate. Inflorescence develops in axillary, 5-7 flowered corymbs. Flowers are yellowish white or white, fruit is a drupe and seeds are ovoid [12]. About 30 indole alkaloids are reported in Rauwolfia and reserpine holds the first place among them. Other regularly reported alkaloids are reserpitine, ajmalicine, sarpagine, rescinnamine and yohimbine[13]. Reserpine is a potent alkaloid that depresses the lowers blood pressure and central nervous system. The leaf extract of *R. tetraphylla* is intended for the treatment of cholera, fever and eye disease. It is also used as antihypertensive, also in intestinal disorders, diarrhea and dysentery[14]. The leaves are crushed and applied over snakebite site[15]. Fruits of this plant are used to cure spleen disorders[15]. *R.tetraphylla* is economically significant because of the presence of alkaloids, which are localized in the roots[16]. The roots are functional in the treatment of hypertension, cardiovascular diseases and also as a sedative agent. The extract of the root is precious for intestinal problems. Roots support to stimulate uterine contraction in case of complicated delivery[17]. *R. tetraphylla* is becoming critically endangered due to its wide indiscriminate collection from wild, poor seed germination and lack of sufficient commercial plantation[18]. This in turn might lead to adulteration in the herbal medicinal products containing *R. tetraphylla*. Hence, a detailed study of HPTLC profiling of *R. tetraphylla* is necessary. So far the stem of *R. tetraphylla* was undertaken for HPTLC studies [19], but no reports are found on the HPTLC profile of the Leaf. Hence, in the present investigation we report the HPTLC profile of leaf n-hexane extract because of many bio important properties.

MATERIALS AND METHODS

Collection of the plant material

The fresh leaves of *R. tetraphylla* were collected in nursery of medicinal plants near namada chilume, Tumkur and were authenticated at the Department of Botany, Tumkur University, Tumkur, Karnataka, India. The leaves were washed thoroughly two to three times with running tap water and once with sterile distilled water and immediately sprayed with alcohol. The leaf material was then dehydrated under shade. After complete aeration, the samples leaf was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in an appropriate 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. Weighed amount (250 gm) of coarsely powdered leaf material was extracted with n-hexane. Extraction was carried out

nearly 18 hr. (appr.45 cycles). Extraction continued until the solvent became colorless. The extract obtained were further concentrated by evaporating solvent using Buchie type evaporator under reduced pressure and controlled temperature of 40-50 °C. Finally, upon evaporation, green colour paste form of extract was obtained with 9.58 gm of yield. The obtained extracts was dried under vacuum, packed and stored in refrigerator until further use.

HPTLC fingerprinting profile

The HPTLC fingerprint profile of the leaf extract of *R.tetraphylla* was carried out using Camag HPTLC system (Muttenz, Switzerland) operational with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner3, winCATS software and Hamilton (Reno, Nevada, USA). A constant application rate of 20 µl of sample was applied on 8mm wide band using Camag Linomat-V automated applicator with the nitrogen flow providing a dosage speed of 150nl/s from syringe on Pre-coated silica gel aluminum plates 60 F254 10 x 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 toluene: ethyl acetate: acetic acid (55:45:1 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, dervitization reagent i.e. Anisaldhyde-Sulphuric acid reagent was sprayed onto the plate and again 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 creation of orange coloured spots corresponding to the constituents of *R. tetraphylla* CAMAG leaf extract was observed. The plates were scanned within 10 min, using densitometric TLC scanner III with win CATS software in the remission mode at 254 and 366 nm. The peaks were detected and their Rf values and peak areas were calculated.

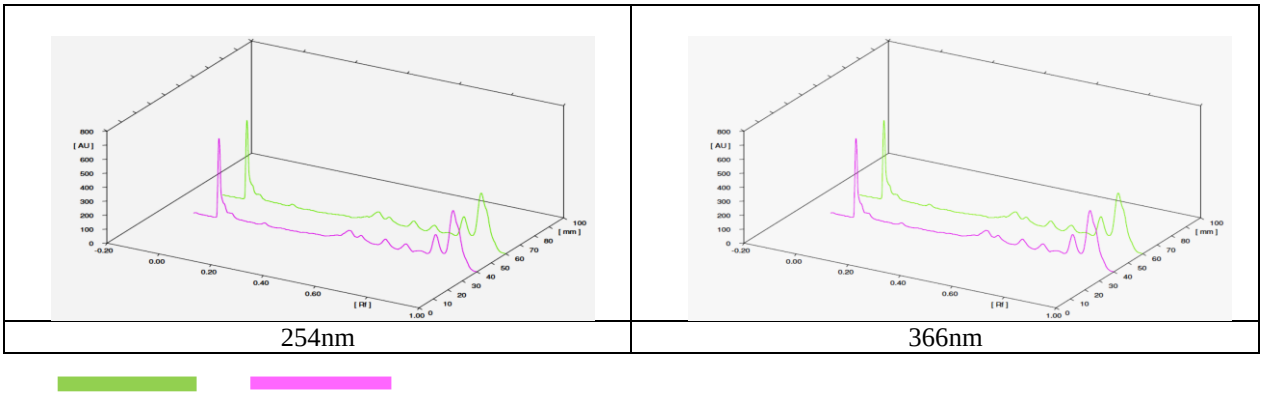
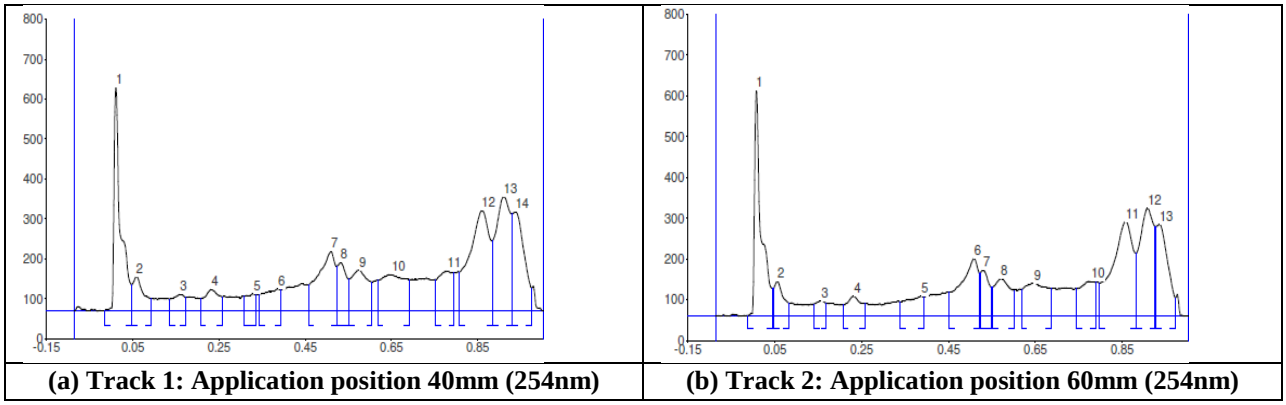
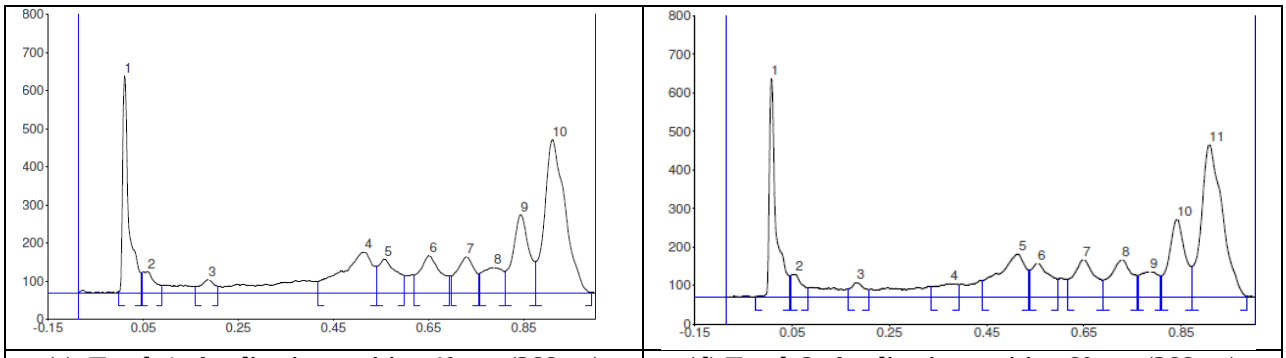


Figure 1: Chromatogram of leaf n-hexane extract of *R. tetraphylla* (254nm and 366nm) at both 40 and 60 mm application positions



(a) Track 1: Application position 40mm (254nm) **(b) Track 2: Application position 60mm (254nm)**



(c) Track 1: Application position 40mm (366nm) | **(d) Track 2: Application position 60mm (366nm)**

Figure 2: HPTLC densitogram of *R.tetraphylla* leaf n-hexane extract at 20µl application position 40mm and 60mm at 254 nm (a) and (b) and at 366 nm (c) and (d)

Table 1: Peak list and Rf values of the densitogram of 20µl n-hexane extract of *R. tetraphylla*, at application position 40 mm at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	2.5	0.01	560.6	25.54	0.04	66.6	7770.6	12.78
2	0.04	66.9	0.06	87.2	3.97	0.09	31.9	1989.3	3.27
3	0.13	30.2	0.16	42.0	1.91	0.17	33.7	1092.3	1.80
4	0.20	31.3	0.23	54.8	2.49	0.26	35.9	1731.7	2.85
5	0.31	37.1	0.33	43.4	1.98	0.33	41.4	924.1	1.52
6	0.34	42.2	0.38	55.7	2.54	0.39	54.3	1959.9	3.22
7	0.46	66.9	0.51	149.2	6.79	0.52	112.4	5518.4	9.08
8	0.52	113.0	0.53	122.0	5.56	0.55	80.6	2313.3	3.81
9	0.55	80.9	0.57	102.5	4.67	0.60	73.1	3710.0	6.10
10	0.62	77.0	0.65	91.5	4.17	0.69	79.5	4976.7	8.19
11	0.75	79.4	0.78	100.2	4.56	0.79	95.7	3261.2	5.36
12	0.81	97.8	0.86	251.7	11.46	0.88	175.9	10692.3	17.59
13	0.88	176.9	0.91	286.4	13.04	0.93	243.2	8832.9	14.53
14	0.93	243.9	0.94	248.1	11.30	0.97	57.6	6014.9	9.89

Table 2: Peak list and Rf values of the densitogram of 20µl n-hexane extract of *R. tetraphylla*, at application position 60 mm at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	1.8	0.00	554.2	27.55	0.04	67.3	7802.8	14.34
2	0.04	68.1	0.05	86.0	4.28	0.08	33.3	1707.0	3.14
3	0.14	30.0	0.15	37.8	1.88	0.17	32.0	794.5	1.46
4	0.20	27.4	0.23	49.2	2.45	0.26	30.9	1518.6	2.79
5	0.34	35.4	0.38	48.5	2.41	0.39	47.8	1801.9	3.31
6	0.45	59.3	0.51	141.9	7.06	0.52	105.8	5317.1	9.77
7	0.52	105.8	0.53	13.4	5.64	0.55	70.8	2107.1	3.87
8	0.55	71.0	0.57	91.8	4.56	0.60	63.6	3142.0	5.77
9	0.62	65.4	0.64	80.5	4.00	0.68	68.3	3998.9	7.35
10	0.74	67.5	0.78	85.8	4.26	0.79	82.8	2762.0	5.08
11	0.80	81.5	0.86	232.3	11.55	0.88	154.7	10095.9	18.55
12	0.88	154.8	0.91	264.1	13.13	0.92	220.0	7780.8	14.30
13	0.93	220.1	0.93	225.7	11.22	0.97	44.9	5591.1	10.27

Table 3: Peak list and Rf values of the densitogram of 20 µl n-hexane extract of *R. tetraphylla*, at application position 40 mm at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	3.0	0.01	569.7	33.05	0.04	52.7	6841.9	13.61
2	0.04	52.9	0.06	55.8	3.24	0.09	19.8	1353.6	2.69
3	0.16	16.3	0.18	34.7	2.01	0.21	17.3	939.9	1.87
4	0.42	30.6	0.51	107.1	6.21	0.54	69.2	6366.7	12.67
5	0.54	69.5	0.56	89.0	5.16	0.60	46.1	3027.9	6.02
6	0.62	46.6	0.65	98.0	5.68	0.69	44.1	3836.7	7.63
7	0.69	43.9	0.73	95.2	5.52	0.75	50.3	3233.6	6.43
8	0.76	50.9	0.79	66.6	3.86	0.81	56.7	2627.9	5.23
9	0.81	57.2	0.84	205.5	11.92	0.87	81.8	6286.9	12.51
10	0.87	82.0	0.91	402.3	23.34	0.99	0.9	15741.3	31.32

Table 4: Peak list and Rf values of the densitogram of 20 µl n-hexane extract of *R. tetraphylla*, at application position 60 mm at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	1.2	0.00	569.2	32.20	0.04	55.7	7205.9	13.98
2	0.04	56.1	0.05	59.9	3.39	0.08	24.7	1230.1	2.39
3	0.16	22.1	0.18	37.8	2.14	0.21	19.9	964.3	1.87
4	0.33	27.3	0.37	35.5	2.01	0.39	32.8	1468.2	2.85
5	0.44	43.3	0.51	112.6	6.37	0.53	70.8	5710.9	11.08
6	0.54	70.8	0.55	89.6	5.07	0.59	47.8	3159.4	6.13
7	0.61	47.1	0.65	98.0	5.54	0.69	45.1	3881.5	7.53
8	0.69	45.0	0.73	98.4	5.57	0.76	56.5	3854.8	7.48
9	0.76	57.0	0.78	66.7	3.77	0.81	55.9	2358.1	4.57
10	0.81	56.0	0.84	203.0	11.49	0.87	79.7	6114.5	11.86
11	0.87	80.4	0.91	397.0	22.46	0.98	2.7	15603.7	30.27

Fig: 3a At UV 254nm

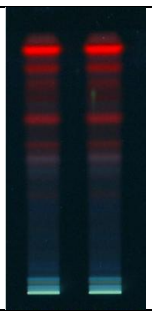


Fig 3b: At UV 366nm

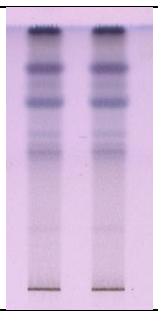


Fig 3c: After Derivatization at UV 254nm

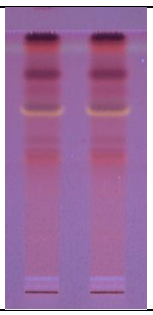


Fig 3d: After Derivatization at UV 366nm

Figure 3: The n-hexane extracts of *R. tetraphylla* were subjected to HPTLC analysis by specific solvent system toluene: ethyl acetate: acetic acid (55:45:1 v/v) and detected under UV at 254nm (a and c) and 366nm (b and d) before and after Derivatization

RESULTS AND DISCUSSION

The densitogram shown in Figure 2: Track 1: *R. tetraphylla* at application position 40 mm (254 nm) indicates that all sample constituents were separated. It is evident from Table 1 i.e. Peak list and Rf values of the densitogram of *R. tetraphylla*, 20µl n-hexane extract at 254 nm found 14 spots, respectively. The following Max Rf 0.00, 0.05, 0.15, 0.23, 0.38, 0.51, 0.53, 0.57, 0.64, 0.78, 0.86, 0.91 and 0.94 (Fig-2 a) indicating Rf values 0.86, 0.91, 0.94, 0.51, 0.65, 0.57 and 0.78 were found to be more predominant as the percentage area was more with 17.59%, 14.53 %, 9.89 %, 9.08 %, 8.19 %, 6.10 % and 5.36%, respectively.

Table 2. Peak list and Rf values of the densitogram of 20µl n-hexane extract shown in Figure: 2 b) Track 2: *R. tetraphylla* at application position 60mm (254nm) found 13 spots, respectively. The following Max Rf 0.00, 0.05, 0.15, 0.23, 0.38, 0.51, 0.53, 0.57, 0.64, 0.78, 0.86, 0.91 and 0.93 (Fig-2 b) indicating Rf values 0.86, 0.91, 0.93, 0.51, 0.64, 0.57 and 0.78 were found to be more predominant as the percentage area was more with 18.55%,14.30%, 10.27%, 9.77%,7.35%, 5.77% and 5.08%, respectively.

Table 3. Peak list and Rf values of the densitogram of 20µl n-hexane extract shown in Figure: 2 c) Track 1: *R. tetraphylla* at application position 40mm (366nm) found 10 spots, respectively. The following Max Rf 0.01, 0.06, 0.18, 0.51, 0.56, 0.65, 0.73, 0.79, 0.84, and 0.91(Fig-2 c) indicating Rf values 0.91, 0.51, 0.84, 0.73, 0.56 and 0.79 were found to be more predominant as the percentage area was more with 31.32%, 12.67%, 12.51%, 6.43, 6.02 and 5.23%, respectively.

Table 4. Peak list and Rf values of the densitogram of 20 µl n-hexane extract shown in Figure: 2 d) Track 2: *R. tetraphylla* at application position 60mm (366nm) found 11 spots respectively. The following Max Rf 0.00, 0.05, 0.18, 0.37, 0.51, 0.55, 0.65, 0.73, 0.78, 0.84 and 0.91 (Fig-2d) indicating Rf values 0.91, 0.84, 0.51, 0.65, 0.73, 0.55 and 0.78 were found to be more predominant as the percentage area was more with 30.27%, 11.86%, 11.08%, 7.53%, 7.48% , 6.13% and 4.57%, respectively. From the results we can say that the leaf n-hexane extract 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 phytoconstituents present in n-hexane 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 analyze between two phases. Among various chromatographic analytical techniques HPTLC has a firm place as a reliable method for analyzing several samples of divergent nature and composition at the same time [20]. HPTLC is a valuable tool for reliable identification, it provides chromatographic finger prints that can be visualized and stored as electronic images which can be used several times without any errors and change [21]. HPTLC analysis of the n-hexane extract of *R. tetraphylla* studied revealed the presence of major phyto constituents. Results obtained from evaluation of HPTLC fingerprint images will be helpful in the identification unknown bioactive compounds with bio-activity and ensure therapeutic efficacy.

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