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HPTLC FINGER PRINTING OF SOME ETHNOMEDICINALLY IMPORTANT *WRIGHTIA* SPECIES

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ABSTRACT

The paper presents HPTLC finger printing studies on two ethnomedicinally important wrightia species, viz., *Wrightia tinctoria* and *Wrightia arborea*. The high performance thin layer chromatographic finger print parameters have been developed for methanolic lead extracts to fix standards. At shorter (254 nm) and longer (366 nm) wavelength, the resolution was better for these extracts and hence, these wavelengths can be taken for obtaining optimum HPTLC finger printing for this medicinal plant.

Key words: *Wrightia tinctoria*, *Wrightia arborea*, HPTLC finger printing, Chromatography

INTRODUCTION

Standardization of ayurvedic drugs and plant materials is the need of the day. Several pharmacopoeia containing monographs on plant materials describe only the physio- chemical parameters. Hence, modern methods describing the identification and quantification of active compounds in the plant material may be useful for proper standardization of herbals and their formulation. Also, the world health organization (WHO) assembly has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards[1,2.]

Thin layer chromatography is employed to get TLC profile of various extracts and can be used in standardizing the raw drugs as well as the herbal formualtions[3]. Ordinary TLC plates do not give better resolution particularly, when the medicine involves the use of several herbs and quantification of markers by conventional solvent extraction followed by

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colorimetry is laborious and expensive. HPTLC offers better resolution and estimation of active principles with reasonable accuracy in a shorter time[4].

Various *Wrightia* species in India are known to possess ethnomedicinal and folklore claims[5]. They also have medicinal properties viz., astringent, stomachic, tonic, febrifuge and used as uterine sedative (*Wrightia tinctoria*)[6]. It relieves tooth ache, when chewed and believed to be used as antidiarrhoeal; bark is useful in menstrual and renal complaints. Stem bark and root bark are believed to be useful in snake bite and scorpion – stings (*Wrightia arborea*)[7].

Wrightia arborea (Dennst.) Mabb. (Family Apocynaceae) is a small deciduous tree and is upto 14 m in height abunding in yellow milky juice, young branches and smooth bark with yellowish grey colour, pubescent branchelets, which occurs at hills[8,] at 800 – 1400 m^2. The plant *Wrightia tinctoria* (Roxb) R. Br., (Family Apocynaceae) is a densely, foliaceous, deciduous tree, with white milky juice, often slender cord- like branches and leaves opposite. It grows in plains and slopes of the hills.[6]

In view of the immense medicinal value of these species, two ethnomedicinally important species viz, *Wrightia arborea* (Demmst.) Mabb and *Wrightia tinctoria* (Roxb) R. Br. are taken up for HPTLC studies.

On our continued study on this topic herein; we report HPTLC finger printing studies of methanolic leaf extracts of *Wrightia* species and the integration spectrum, which will be useful as standard parameter.

EXPERIMENTAL

Materials and methods

Plant material

Fresh leaves of *Wrightia arborea* and *Wrightia tinctoria* were collected from Shevaroy Hills at Salem district and was identified and authenticated (BSI/SC/5/23/08-09/ Tech; BSI/SC/5/23/08-09/Tech-741) by Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

Preparation of extracts

The leaves of *Wrightia arborea* and *Wrightia tinctoria* were collected and dried in shade separately. Then they were powdered and extracted individually with methanol in Soxhlet extractor[9-11].

HPTLC analysis[12,13]

HPTLC was performed on 20 x 10 cm (L X B) aluminum sheets precoated with silica gel 60 F_{254} (E. Merck) plates 0.2 mm thickness. Camag twin trough chamber was saturated with the developing solvent. The sample (5 μL) were applied as bands on the plates using Camag linomate 5 sample applicator. The width of the applied band was 6.0 mm and the space between the bands was fixed as 13.0 mm. The speed of application was maintained at 150 nL/sec. The plate was developed in chloroform : methanol mixture (95 : 5) in twin trough chambers.

The developed plate was dried and then scanned using D_2 (Deuterium) lamp at 254 nm ad 366 nm using Camag TLC scanner 3 equipped with Win CATS Version 3 software.

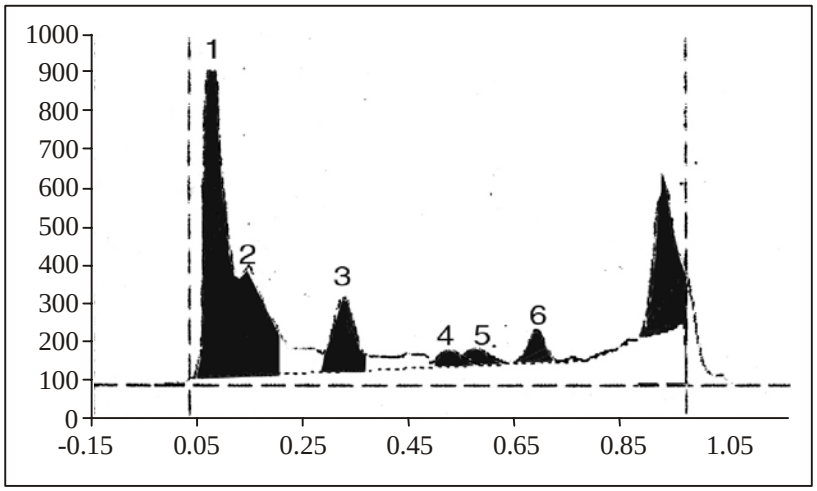
RESULTS AND DISCUSSION

The HPTLC spectrum of methanol leaf extract of *Wrightia arborea* and *Wrightia tinctoria* are shown in Figs. 1-6. In the spectrum of Wrightia tinctoria methanolic extract at 254, 366 and 465 nm[14] after spray using phosphomolybdic acid) about 44.48%, 60.50% and 31.97% of the compound remained at the point of application and the remaining compounds were resolved into 6, 4 and 6 peaks, respectively. The peak at R_f 0.94, 0.15 and 0.93, respectively, showed the presence of 19.50%, 20.79% and 19.32%, while the other peaks were ranging from 1.56-16.22%, 4.08-10.52% and 5.42-18.51% (Figs. 1-3).

The spectrum of *Wrightia arborea* methanolic extract at 254, 366 and 465 nm after spray using phosphomolybdic acid showed that about 58.43%, 12.46% and 25.05% of the compound remained at the point of application and the remaining compounds were resolved into 11, 5 and 6 peaks, respectively. The peak at R_f 0.13, 0.08 and 0.47, respectively showed the presence of 14.59%, 22.73% and 27.6%, while the other peaks were ranging from 1.05% - 6.50%, 12.61%-2.30% and 4.71% - 14.57% (Fig. 4-6).

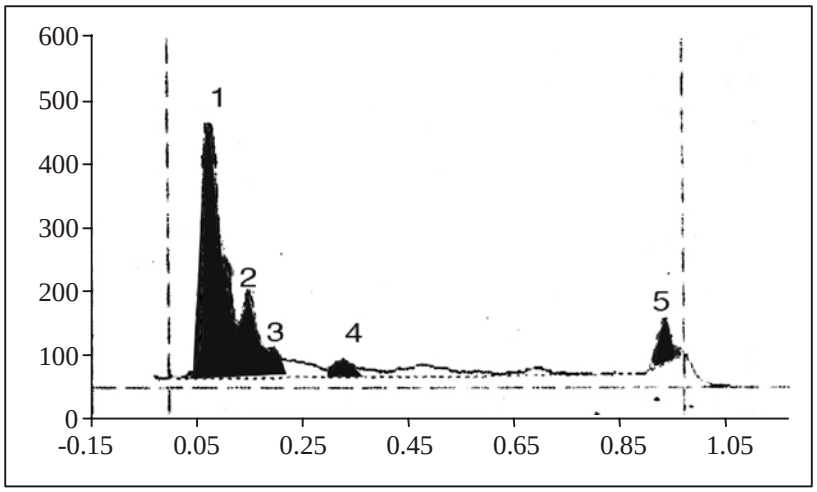
The methanolic leaf extracts of both; *Wrightia tinotoria* and *Wrightia arborea*, gave good finger prints with many peaks at 254 nm (Figs. 1 and 4) while at 366 nm, these did not had many peaks (Figs. 2 and 5). The finger prints of *Wrightia tinctoria* and *Wrightia arborea* showed no similar compound at 254 nm where as the peak with R_f 0.15 and 0.93 was found to be similar at 366 nm and 465 (Figs. 3 and 6).

Thus, the TLC profile at 366 nm and 465 nm showed the presence of same R_f value as 0.15 at 366 nm and 0.93 at 465 nm, in case of *Wrightia tinctoria* and *Wrightia arborea* methanolic extracts of leaves.



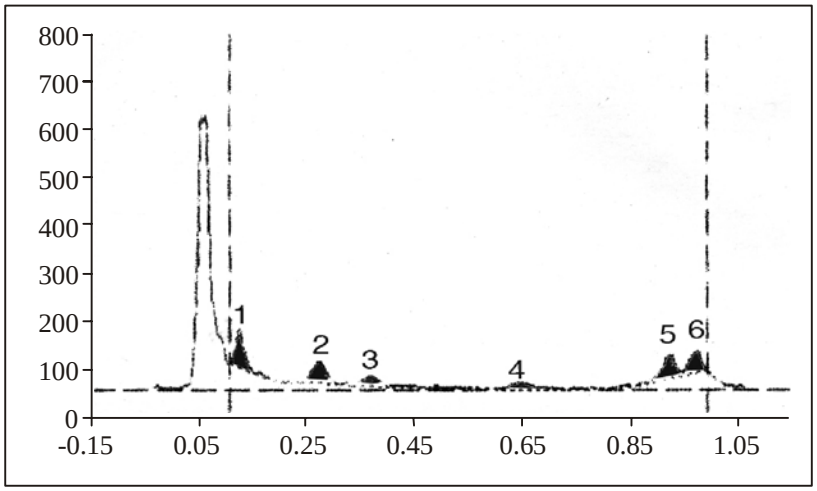
Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.05	0.8	0.08	797.8	44.48	0.13	257.8	26088.8	43.53
2	0.13	258.3	0.15	290.9	16.22	0.24	63.7	12766.9	21.30
3	0.29	51.5	0.33	194.5	10.85	0.40	33.6	7433.7	12.40
4	0.50	23.8	0.52	28.0	1.56	0.54	14.4	681.4	1.14
5	0.54	14.4	0.58	48.1	2.68	0.65	0.7	1743.6	2.91
6	0.65	0.9	0.70	84.5	4.71	0.74	0.9	2285.8	3.81
7	0.89	0.1	0.94	349.8	19.50	0.97	62.8	8935.8	14.91

Fig. 1: HPTLC Finger printing of methanol extract of *Wrightia tinctoria* leaf at 254 nm



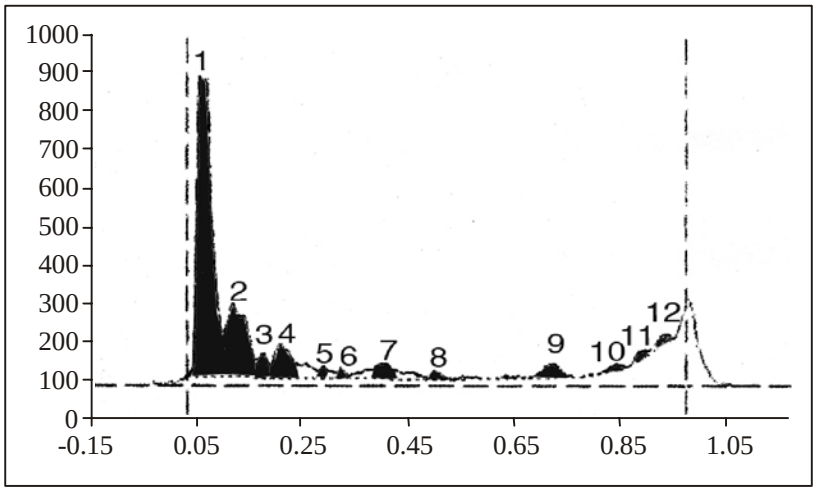
Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.04	8.4	0.08	404.3	60.50	0.13	86.8	12394.2	61.67
2	0.14	87.3	0.15	138.9	20.79	0.23	26.3	4336.4	21.58
3	0.23	26.6	0.23	27.5	4.12	0.30	12.5	976.0	4.86
4	0.30	12.6	0.33	27.3	4.08	0.36	12.6	875.8	4.36
5	0.91	0.9	0.94	70.3	10.52	0.98	1.7	1516.4	7.54

Fig. 2: HPTLC Finger printing of methanol extract of *Wrightia tinctoria* leaf at 366 nm



Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.12	1.4	0.13	78.7	31.97	0.16	1.8	1006.0	21.40
2	0.25	0.3	0.28	45.6	18.51	0.32	0.6	944.1	20.08
3	0.32	0.1	0.37	13.3	5.42	0.40	1.7	437.2	9.31
4	0.61	2.3	0.65	16.2	6.59	0.68	6.4	474.2	10.09
5	0.89	1.7	0.93	47.5	19.32	0.95	6.4	996.4	21.19
6	0.95	6.8	0.98	44.8	18.19	1.00	2.5	843.3	17.94

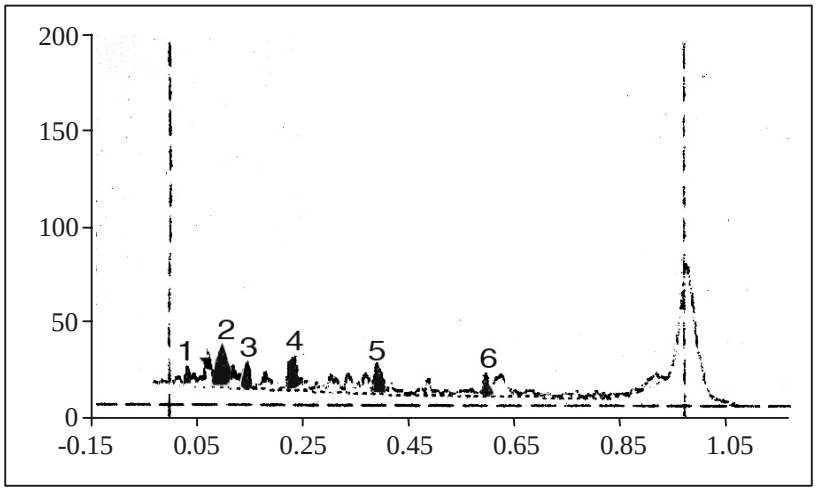
Fig. 3: HPTLC Finger printing of methanol extract of *Wrightia tinctoria* leaf at 465 nm



Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.05	13.2	0.06	785.3	58.43	0.11	111.6	18043.4	55.33
2	0.11	112.2	0.13	196.1	14.59	0.17	45.5	6074.5	18.63
3	0.17	46.4	0.19	52.3	3.89	0.20	32.1	819.0	2.51
4	0.20	33.3	0.22	87.4	6.50	0.26	31.9	2322.1	7.12
5	0.26	32.4	0.27	37.6	2.80	0.29	18.9	612.4	1.88
6	0.29	19.0	0.30	30.5	2.27	0.34	8.6	602.7	1.85
7	0.35	7.7	0.41	40.2	2.99	0.45	13.8	1625.2	4.98
8	0.45	13.9	0.46	15.8	1.18	0.50	3.0	342.0	1.05
9	0.69	3.9	0.73	37.0	2.75	0.77	0.6	1038.8	3.19
10	0.81	0.1	0.85	14.1	1.05	0.87	0.3	250.1	0.77
11	0.87	0.1	0.89	20.6	1.53	0.91	8.1	317.1	0.97
12	0.91	8.3	0.94	27.2	2.03	0.96	0.3	563.6	1.73

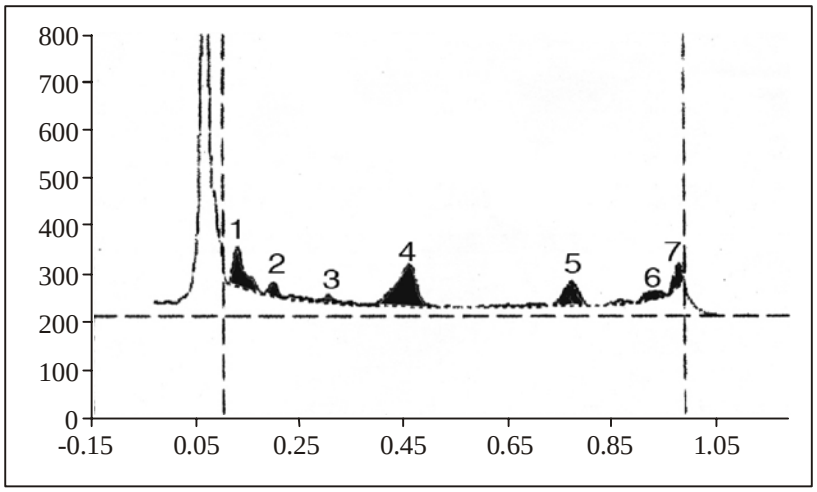
Fig. 4: HPTLC Finger printing of methanol extract of *Wrightia arborea* leaf at 254 nm

The HPTLC studies have shown that it is more versatile than the ordinary TLC methods, as the spots were well resolved. Though further work to characterize the other chemical constituents and quantitative estimation with marker compounds is also necessary and these data can also be considered along with the other values for fixing standards to these plants.



Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.03	0.6	0.04	11.0	12.46	0.05	2.3	104.2	11.37
2	0.06	2.4	0.08	20.1	22.73	0.09	5.2	208.1	22.71
3	0.14	2.5	0.15	11.1	12.61	0.16	0.2	65.6	7.16
4	0.22	1.1	0.24	17.9	20.30	0.27	0.8	271.9	29.68
5	0.39	1.7	0.40	15.9	18.05	0.42	1.7	179.6	19.61
6	0.59	1.7	0.60	12.2	13.86	0.61	4.7	86.6	9.46

Fig. 5: HPTLC Finger printing of methanol extract of *Wrightia arborea* leaf at 366 nm



Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %
1	0.12	0.8	0.14	78.5	25.05	0.18	0.1	1387.1	20.83
2	0.18	0.1	0.20	26.0	8.31	0.22	0.2	321.2	4.82
3	0.29	3.3	0.31	11.2	3.57	0.33	3.1	187.9	2.82
4	0.40	2.4	0.47	86.5	27.61	0.51	1.8	2722.2	40.88
5	0.74	2.3	0.78	50.7	16.17	0.82	1.2	1201.5	18.04
6	0.91	0.4	0.93	14.8	4.71	0.96	0.5	356.3	5.35
7	0.97	0.6	0.98	45.7	14.57	0.99	17.6	483.3	7.26

Fig. 6: HPTLC Finger printing of methanol extract of *Wrightia arborea* leaf at 465 nm

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