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Comparative HPTLC fingerprint profile of three types of Kodiveli used in Siddha

Research Article

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Abstract

Kodiveli is an important Siddha drug used in the formulations such as *Kodiveli Podi*, *Kodiveli Ennai*, *Kodiveli Thylam*, *Chithramoola Kuligai* and *Kodiveli Kudineer*. The HPTLC is a simple method to differentiate closely related species. HPTLC profiling of ethanolic extracts of roots of three species of *Plumbago* (*Plumbago zeylanica* L. (*Venkodiveli*), *P.indica* L., (*Senkodiveli*) and *P. auriculata* Lam. (*Karunkodiveli*)) has been carried out using *Toluene: Ethyl acetate: Methanol* (5:1.5:0.1) as solvent system on Silica gel 60 F_{254}-coated aluminium sheets. The preliminary qualitative phytochemical screening found the same combination of phytoconstituents while the HPTLC finger prints have given more refined picture in respect of the number of compounds present and plumbagin concentration among the three selected species of *Plumbago*. The chromatogram peak at R_f. 0.76 found common and indicating highest concentration in all the three species and it may be of the bioactive compound plumbagin. The number of peaks and their attributes were found to be different among the roots of the selected species. This result will be highly useful for the precise identification and discrimination of the authentic root materials of *P. zeylanica, P. Indica* and *P. Auriculata* from substitutes and adulterants used in the raw drug market.

Key Words: Fingerprinting, HPTLC, Kodiveli, Plumbagin, Plumbago.

Introduction

The genus *Plumbag*o is the largest genus of the family Plumbaginaceae with twenty four species (1,2). It contains a highly potent biologically active compound known as plumbagin which possess wide spectrum of therapeutic and pharmacological activities such as anticancer, antifungal, anti-inflammatory, antibacterial, antifertility, antimalarial, antidiabetic and antioxidant properties (4-7). The plumbagin has also been reported to have stimulant action on the intestine, nervous system and heart (2). Plumbagin is a 5-hydroxy-2-methyl-1,4-naphthoquinone-($C_{11}H_8O_3$), a naturally yellow colored compound. Though plumbagin presence have been reported in the roots, stems and leaves of various *Plumbago* species, the roost predominantly posess high concentration of plumbagin, especially in root bark of *Plumbago indica* L. (6-8).

In addition to plumbagin, many other potentially bioactive compounds have been reported in different species of *Plumbago*. Many studies in *Plumbago auriculata* Lam. have revealed the presence of *α*-amyrin, capensisone, *α*-amyrin acetate, isoshinanolene, *β*-sitosterol, diomuscinone and *β*-sitosterol-3 and*β*-glucoside (6, 9-11). The whole plant has been reported to be efficacious in treating anemia, rheumatic pain, sprains, dysmenorrheal, carbuncles, scabies, leprosy, inflammation and ulcers. The roots of *P. auriculata* has therapeutic potential such as antiatherogenic, cardiotonic and neuroprotective (12).

The root barks of *P. indica* is the best source of plumbagin among the other *Plumbago* species. *P. indica* also contain other variants of plumbagin such as 2, 3-epoxyplumbagin, plumbagic acid, 3-*O*-3'-bidroserone, plumbagic acid lactone, roseanone, droserone, elliptinone and zeylanone as major compounds (20-22). In addition, presence of some other compounds such as azaleatin, ayanin, palmitic acid, *α*–naphthoquinone, *α*–naphthylamine have also been reported (21-23). The root of *P. indica* has diuretic, germicidal, vesicant, and abortifacient activity. It is also used for the treatment of anaemia, diarrhea, leprosy, common wart and poses anti-tumour and anti-acne activities (13-15).

The roots of *P. zeylanica* contains naphthoquinones such as plumbagin, biplumbagin(chitranon), chloroplumbagin, maritinone, elliptinone, lapachol etc. and coumarins like 5-methoxyseselin, seselin, suberosin, xanthyletin, xanthoxyletin, plumbic acid, along with other compounds such as plumbazeylanone, droserone, isozeylinone and zeylinone (16). Roots of *P. zeylanica* possess different bioactive properties such as stomachic, carminative, astringent to bowel, anthelmintic and used

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to cure bronchitis, itching, leucoderma, inflammation, intestinal troubles like dysentery, piles, disease of liver, ascites etc (17-18)

Siddha medicine is one of the oldest traditional systems of Indian medicine mainly followed in south India. The leaves and roots of *Plumbago* species (*Kodiveli*) are used as ingredients of many Siddha and Ayurveda formulations. *P. zeylanica* (Tamil names: *Venkodiveli*, *Venchitramoolam* and *Venkodimoolam*), *P. indica*, (Tamil names: *Senkodiveli*, *Sivappuchitramoolam* and *Chenkodimooli*) and *P. auriculata* (Tamil names: *Karunkodiveli*, *Karuppuchitramoolam* and *Nilachitramoolam*) are the three species of *Plumbago* used in Siddha system of medicine (29). The major Siddha formulations using *Plumbago* species are *Kodiveli Podi*, *Kodiveli Ennai*, *Kodiveli Thylam*, *Chithramoola Kuligai* and *Kodiveli Kudineer*. The accurate identification and assurance of the quality and authenticity of the plant part used in the respective formulations is paramount important as the therapeutic and pharmacological properties of each ingredient may vary depending on the formulations.

Figure 1: Pictures of dried root samples and flowers of (a) *P. zeylanica* (b) *P. indica* and (C) *P. auriculata*

The dried roots of *P. zeylanica*, *P. indica* and *P. auriculata* available in the raw drugs market are mostly indistinguishable and difficult to recognize in between them as well as from the adulterant and substitutes by a direct morphological analysis (Fig.1). The present study aimed to find a quality standard for the root materials of *P. zeylanica*, *P. indica* and *P. auriculata* through a comparative qualitative phytochemical screening and HPTLC fingerprint profiling in order to authenticate the respective raw drug samples in the market.

Materials and Methods

Root of *Plumbago zeylanica* L. (*Venkodiveli*), *Plumbago indica* L. (*Senkodiveli*) and *Plumbago auriculata* Lam.(*Karunkodiveli*) were collected from Siddha Medicinal Plants Garden, Mettur Dam and authenticated by the Research Officer (Botany), SMPG, Mettur Dam. Samples were washed with water, shade dried and powdered. The powdered samples were used for further analyses.

Extraction

Ethanolic extraction of roots of *P. zeylanica* L., *P. indica* L., and *P. auriculata* Lam.were taken by refluxing the materials at $60^0$for 10 min.

Preliminary Phytochemical Analysis

The ethanolic extracts of all samples were subjected to qualitative phytochemical screening to analyze the presence of various phytoconstituents (19).

HPTLC Fingerprinting

The ethanolic extracts of the root samples were subjected to HPTLC profiling at Siddha Regional Research Institute (CCRS), Thiruvananthapuram. A number of solvent systems were tried but the optimum separations of constituents and maximum resolution were achieved using the solvent system: *Toluene: Ethyl acetate: Methanol* (5:1.5:0.1) hence it was selected.The sample volume applied as track 1 with 5 µl and track 2 with 10 µl. The samples, track 1and track 2 were applied with a band width of 8mm each on pre-saturated silica gel 60 F_{254}-coated aluminium sheets through CAMAG Hamilton syringe using Automatic TLC Sampler 4 (ATS4). The plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm). The developed chromatogram was air dried and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm, 366 nm and followed by scanning at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with *win CATS* software.

Post chromatographic derivatization of the plate was carried out using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till

the colour of the bands appear. The plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

Results and Discussion

The preliminary phytochemical screening (Table 1) of ethanol extracts of dried root samples of *P. zeylanica* L., *P. indica* L., and *P. auriculata* Lam. revealed the presence of saponins, tannins, terpenoids, phenols, quinones, glycosides and alkaloids in all the three species while the steroides, coumarins, carbohydrates, liginans, flavanoides and proteins were absent in all three samples. As the preliminary phytochemical screening gave same results for *P. zeylanica, P. indica*and *P. auriculata* HPTLC profiling was carried out for getting more precise differences.

Table 1: Phytochemical screening of ethanol extract of roots of *P. zeylanica, P. indica* and *P. auriculata*

Phyto-constituents	*P.zeylanica*	*P.indica*	*P.auriculata*
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Phenols	+	+	+
Steroids	-	-	-
Quinones	+	+	+
Coumarins	-	-	-
Glycosides	+	+	+
Carbohydrates	-	-	-
Alkaloids	+	+	+
Lignans	-	-	-
Flavanoids	-	-	-
Proteins	-	-	-

**Present: (+), Absent: (-)*

The HPTLC was performed on silica gel 60 F_{254}-coated aluminium sheets with *Toluene: Ethyl acetate: Methanol* (5:1.5:0.1) solvent system due to the optimum separation with maximum resolution in it. HPTLC fingerprint profile and corresponding chromatograms scanned at UV 254 nm, UV 366 nm and 575 nm are illustrated in Fig. 1, 2 and 3. The chromatogram scanned at UV 254 nm showed 6, 7 and 5 peaks for track1 and 7, 9 and 6 peaks for track 2 of *P. zeylanica, P. indica,* and *P. auriculata* respectively (Fig.2). Similarly, scanning at UV 366 nm revealed the presence of 4, 10 and 7 peaks for track1 and 9, 11 and 6 peaks for track 2 (Fig. 3) and the chromatogram of 575 nm showed 7, 6 and 6 peaks for track 1 and 8, 8 and 7 peaks for track 2 (Fig.4) of *P. zeylanica, P. indica,* and *P. auriculata* respectively. The number of peak represents the various phytoconstituents in the samples and the peak area represents the concentration of the corresponding compounds. It was analysed that fingerprint profile and chromatogram of scanning at 254 nm revealed significant details for the discrimination of the bio-active compounds compared to that of 366 nm and 575 nm. The chromatogram data (Number of peaks, R_f value and peak percentage of the compounds) generated upon scanning at 254 nm, 366 nm and 575 nm of track 1 and track 2 of all samples are illustrated in Table 2 and 3 respectively. The peaks detected sharply at R_f 0.44, 0.76 and 0.85 at 254 nm, R_f 0.65 at 366 nm and R_f 0.77 and 0.90 at 575 nm found to be common in both track 1 and track 2 of all the three species while many other peaks detected were found to be varying among the species with respect to the R_f value and other attributes (Fig. 2 and Table 2 & 3). Among them the peak at R_f 0.76 at 254 nm having the largest peak area percentage (62.75 %, 61.75 % and 59.60 % for track 1 and 61.60 %, 58.13 % and 65.44 % for track 2 of *P. zeylanica, P. indica,* and *P. auriculata* respectively) in all the samples among all scanned wavelengths indicating the highest concentration of the compound. It has been inferred from the earlier studies that the R_f value of plumbagin may vary depending on the solvent used for the extraction and mobile phase selected for chromatography (Table 4) on silica gel 60 F_{254} precoated aluminium plates. The plumbagin was spotted at R_f 0.84 in the HPTLC screening of chloroform extracts of *P. zeylanica* root on *Toluene: Ethyl acetate* (3:1) system at 254 nm (24), Rf 0.91 in the methanolic extract of *P. Indica* roots on *Toluene: Formic acid* (9.9: 1) at 272 nm (23), Rf 0.67 in the ethanolic extract of roots of *P. indica* and *P. zeylanica* on *Hexane: Ethyl acetate* (8:2) at 265 nm (25), R_f 0.85 in the methanolic extract of root bark of *P. zeylanica* on *Toluene : Ethyl acetate* (6:4) at 366 nm (26) and at R_f 0.44 in the acetone extract of root, stem and leaves of *P. Indica, P. zeylanica* and *P. auriculata* on *Petroleum ether: Ethyl acetate* (94:6) at 254 nm (27). So, in this study result, the peaks at R_f 0.76 at 254 nm is found to be stable and common among *P. zeylanica, P. indica,* and *P. auriculata* and possess highest peak area percentage. This range of R_f value may be representing plumbagin as it is the major bioactive compound reported common to all these three species. Similarly the varying number of peaks detected at diverse ranges of R_f values (Fig. 2-4) may be representing the corresponding number of phytoconstituents specific to each species as the *P. zeylanica, P. indica,* and *P. auriculata* were reported to have wide range of compounds which impart their pharmacological properties and therapeutic potentials along with the plumbagin. The accurate identification, precise purification, optimal quantification and molecular level structural elucidation of the compound presented in HPTLC fingerprint profile of this study may be unravelled by further analysis through column chromatography, GCSM, FTIR and NMR spectroscopy as they have been used in the minute level comparison of closely related species (28).

Table 2: HPTLC peak table of *Track 1* (5µl) scanned at 254 nm, 366 nm and 575 nm

Sample	*P. zeylanica*			*P. indica*			*P. auriculata*		
UV Scan(λ)	Peak	Max R_f.	Area %	Peak	Max R_f.	Area %	Peak	Max R_f.	Area %
254 nm	1	0.01	5.60	1	0.01	7.51	1	0.17	2.13
	2	0.19	2.04	2	0.19	9.26	2	0.43	10.31
	3	0.44	8.60	3	0.44	12.09	3	0.57	19.17
	4	0.56	13.05	4	0.57	6.34	4	0.75	59.60
	5	0.76	62.75	5	0.76	61.75	5	0.84	8.79
	6	0.85	7.96	6	0.85	2.45			
				7	0.90	0.60			
366 nm	1	0.02	9.50	1	0.02	10.64	1	0.01	8.96
	2	0.43	19.47	2	0.08	7.79	2	0.07	10.93
	3	0.65	35.15	3	0.10	7.80	3	0.42	18.89
	4	0.84	35.88	4	0.15	9.20	4	0.62	10.48
				5	0.22	4.52	5	0.64	10.78
				6	0.44	26.79	6	0.69	11.83
				7	0.65	10.28	7	0.81	28.12
				8	0.69	6.88			
				9	0.83	11.30			
				10	0.90	4.80			
575 nm	1	0.03	0.69	1	0.01	3.74	1	0.03	0.53
	2	0.12	1.09	2	0.03	0.52	2	0.12	7.53
	3	0.32	5.00	3	0.44	35.93	3	0.47	13.84
	4	0.43	12.81	4	0.52	23.56	4	0.52	27.11
	5	0.52	31.05	5	0.77	13.72	5	0.78	11.99
	6	0.77	13.91	6	0.90	22.52	6	0.90	39.00
	7	0.90	35.45						

Table 3: HPTLC peak table of *Track 2*(10 µl) scanned at 254 nm, 366 nm and 575 nm.

Sample	*P. zeylanica*			*P. indica*			*P. auriculata*		
UV Scan(λ)	Peak	Max R_f.	Area %	Peak	Max R_f.	Area %	Peak	Max R_f.	Area %
254 nm	1	0.01	7.37	1	0.01	4.97	1	0.01	3.51
	2	0.18	1.83	2	0.08	0.60	2	0.18	1.90
	3	0.44	8.74	3	0.12	0.25	3	0.43	7.84
	4	0.54	8.83	4	0.20	10.26	4	0.55	15.05
	5	0.56	7.34	5	0.44	14.13	5	0.76	65.44
		0.76	61.60	6	0.58	3.94	6	0.85	6.25
	6			7	0.65	4.47			
	7	0.85	4.29	8	0.76	58.13			
				9	0.85	3.24			
366 nm	1	0.02	9.90	1	0.03	7.95	1	0.02	10.54
	2	0.10	12.31	2	0.08	7.05	2	0.07	9.39
	3	0.29	10.00	3	0.10	6.51	3	0.17	10.27
	4	0.32	7.06	4	0.14	8.12	4	0.43	16.67
	5	0.35	3.88	5	0.27	7.09	5	0.65	32.84
	6	0.43	16.19	6	0.32	7.38	6	0.82	20.28
	7	0.49	7.17	7	0.44	27.44			
	8	0.65	19.92	8	0.50	5.86			
		0.83		9	0.65	8.68			
	9		13.56	10	0.69	3.75			
				11	0.83	10.16			
575 nm	1	0.01	5.63	1	0.01	5.94	1	0.03	0.72
	2	0.03	0.85	2	0.04	0.37	2	0.12	6.19
	3	0.12	0.96	3	0.16	0.47	3	0.32	4.15
	4	0.32	5.49	4	0.44	39.15	4	0.44	11.34
	5	0.44	10.32	5	0.52	17.32	5	0.52	29.65
	6	0.52	30.02	6	0.68	0.85	6	0.77	14.52
	7	0.77	17.23	7	0.77	14.36	7	0.90	33.43
	8	0.91	29.49	8	0.90	21.55			

Table 4: HPTLC R_f values of plumbagin in different extracts with different solvent system used on Silica gel 60 F_{254}.

Sl.No.	Extraction solvent	Mobile Phase	UV Scan(λ)	R_f Value	Reference
1	Methanol	Toluene: Formic acid (9.9: 1)	272 nm	0.91	[23]
2	Chloroform	Toluene : Ethyl acetate (3:1)	254 nm	0.84	[24]
3	Ethanol	Hexane: Ethyl acetate (8:2)	265nm	0.67	[25]
4	Methanol	Toluene : Ethyl acetate (6:4)	366 nm	0.85	[26]
5	Acetone	Petroleum ether: Ethyl acetate (94:6)	254 nm	0.44	[27]

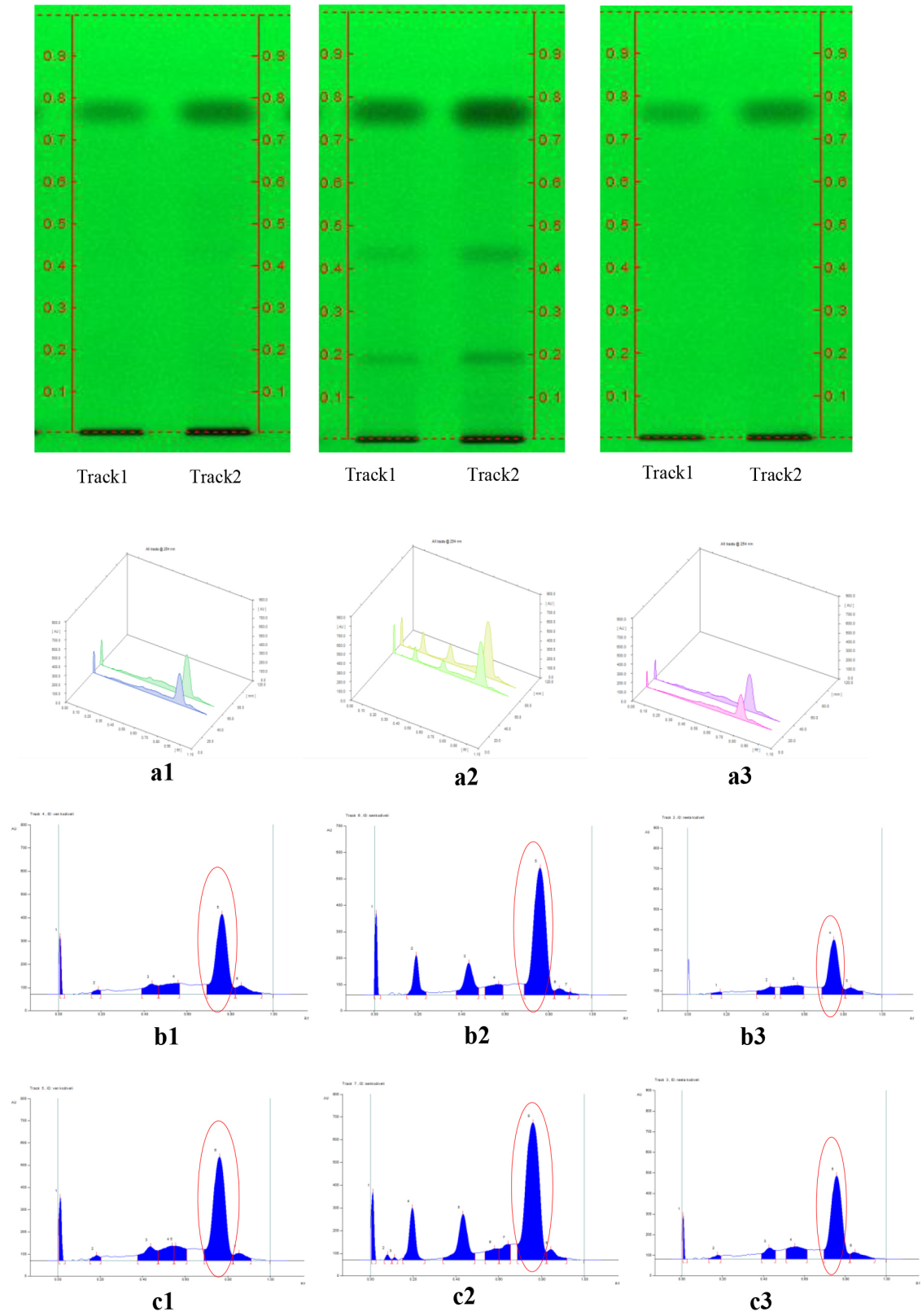

FIGURE 2. HPTLC finger print and Chromatogram peaks of *Plumbago* roots under UV 254 nm. **a1, b1** and **c1:** HPTLC finger print; **a2**, **b2** and **c2:** 3D overlay of Chromatogram peak of all tracks; a3, b3 and c3: 2D overlay of Chromatogram peaks of track 1; **a3, b3** and **c3**: 2D overlay of Chromatogram peaks of track 2 of *P.zeylanica, P.indica and P.auriculata* respectively

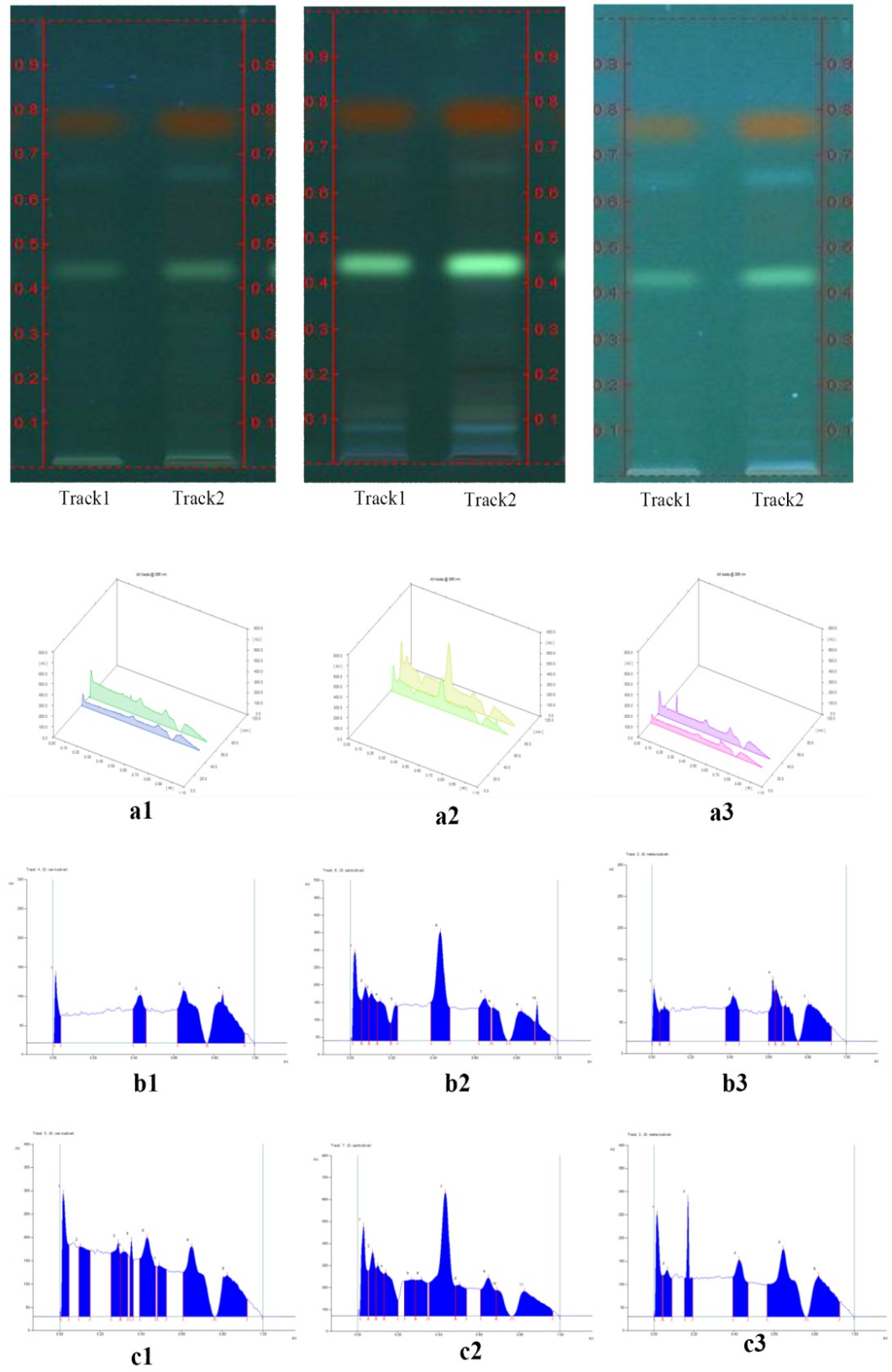

FIGURE 3. HPTLC finger print and Chromatogram peaks of *Plumbago* roots under UV 366 nm. **a1, b1** and **c1:** HPTLC finger print; **a2**, **b2** and **c2:** 3D overlay of Chromatogram peak of all tracks; a3, b3 and c3: 2D overlay of Chromatogram peaks of track 1; **a3, b3** and **c3**: 2D overlay of Chromatogram peaks of track 2 of *P. zeylanica, P. indica and P. auriculata* respectively

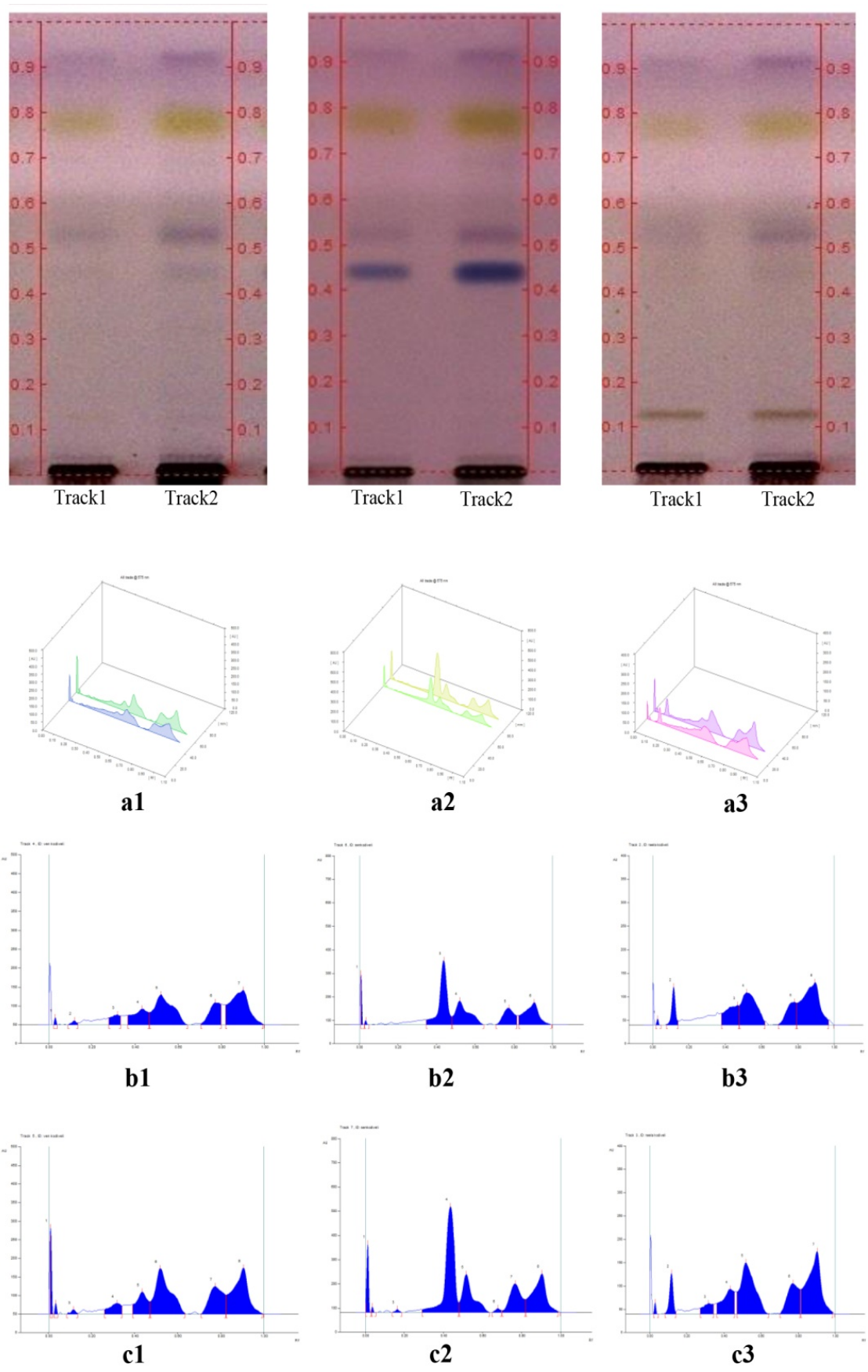

FIGURE 4. HPTLC finger print and Chromatogram peaks of *Plumbago* roots under UV 575 nm. **a1, b1** and **c1:** HPTLC finger print; **a2**, **b2** and **c2:** 3D overlay of Chromatogram peak of all tracks; a3, b3 and c3: 2D overlay of Chromatogram peaks of track 1; **a3, b3** and **c3**: 2D overlay of Chromatogram peaks of track 2 of *P. zeylanica, P. indica and P. auriculata* respectively

Conclusion

The phytochemical screening results of ethanolic extract of roots of *Plumbago zeylanica* L., *Plumbago indica* L., and *Plumbago auriculata* Lam. revealed similar combination of phyto-constituents. The HPTLC fingerprints were given more transparent details in respect of the diversity in phytoconstituents present in the root samples of each species. The chromatogram peak covered at R_f 0.76 present in all samples with highest peak area percentage may be plumbagin as it is the bio-active marker reported in all species of plumbago. The difference in the number of peaks appeared on each species on various scanning wavelengths indicating the corresponding number of phytochemicals present in them. Further sophisticated studies may be elucidated the exact details of the compounds contained in each samples. The present study results may be highly useful to distinguish the authentic samples from substitutes and adulterants.

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