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Research Article

HPTLC Fingerprint Analysis of Leaf Extract of Herbal Plant (*Thunbergia laurifolia)*

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ABSTRACT

Herbal medicinal plant *Thunbergia laurifolia*, was collected, powdered and extracted using methanol. The extract was used for studying the phytochemical compounds. Further HPTLC was performed on the extract using CAMAG HPTLC system to study the phytoconstituents. The CAMAG HPTLC system was equipped with Linomat 5 sample applicator, cabin with ultra violet light and winCATS Planar Chromatography software. The samples were analysed in the CAMAG HPTLC system at different ultra violet wavelengths- 254nm, 366nm and white light- 540nm. The results obtained showed different peaks and Rf values at 2.0 µl, 6.0 µl and 4.0 µl. The highest concentration of phytoconstituents recorded were 64.58% at 254nm, 27.06% at 366nm and 40.14% at 540 nm. The results of the HPTLC fingerprinting analysis of the methanolic leaf extract of *Thunbergia laurifolia* revealed the presence of 8 to 11 polyvalent phytoconstituents.

Keywords: *Thunbergia laurifolia*, HPTLC, CAMAG, winCATS.

INTRODUCTION

Blue trumpet vine or laurel clock is a native India plant which is known as *Thunbergia laurifolia* (Thunbergiaceae) and grown as an ornamental plant. The plant is a fast growing vine and a weed plant in few countries. The plant leaves are dark green, heart shaped with pointed tip and serrated margin tips[1-2]. The size of the leaves ranges from 20cm in length, 16 cm in width and a petiole with 6 cm in length. The shape of the flowers looks like the trumpet so the flower is known as blue trumpet. The petals are purplish blue with a yellow shade in the centre and the size of the flower ranges from 8cm in length and 6 to 8 cm in width[2].The flowers bloom early in the morning throughout the year and they are not scented. The plants are propagated by stem and root cuttings. In traditional Thai medicine, the leaves are used as an antidote for poisons, drugs and for drug addictions[3,4]. Local herbal markets in Thailand produces and markets herbal teas and capsules known as Rang Jeud in Thailand. The plant leaves were reported to have anti-diabetic, anti-inflammatory, anti-bacterial and antipyretic properties[5,6,7]. HPTLC fingerprint profiling is the most significant method used for routine screening of plant extract and herbal drug analysis. The major advantage of HPTLC is its ability to analyse several samples using a small quantity of mobile phase. HPTLC is performed to determine the specific activity of the phytoconstituents of the plant extract[8]. Phytochemical and biomedical analyses were carried out in HPTLC to quantify herbal drug, quantify active ingredient, fingerprint formulations and check adulterations[9,10,11]. In the present study, HPTLC was performed on methanolic extract of *Thunbergia laurifolia* to identify the phytoconstituents of the extract for further studies.

MATERIALS AND METHODS

Collection of samples

Thunbergia laurifolia leaves were collected from Ernakulum, Kerala. Collected leaves were washed thoroughly using double distilled water to remove the dust and debris. The leaves were allowed to shade dry for 3-5days. The dried leaf samples were blended into fine powder and stored in air tight containers until use.

Extraction of sample for testing

Sample powder of 10gms were weighed and added to a beaker containing 100 ml of methanol in the ratio of 1:10 (powder: solvent). The mixture was mixed in an electric blender and transferred into a clean conical flask. The flask was placed in the rotary incubation chamber at 28 ºc for 24 hrs for extraction. The mixture was filtered through Whatmann filter paper and collected in a petridish. The filtrate was allowed to condense by placing the petridish on magnetic stirrer with heat at 45ºc. The condensed crude extract was used for the analysis.

HPTLC Profiling (High Performance Thin Layer Chromatography)

Sample Preparation and application

HPTLC profiling was carried out following the method of Reich E and Schibli A [12]. Sample was prepared by

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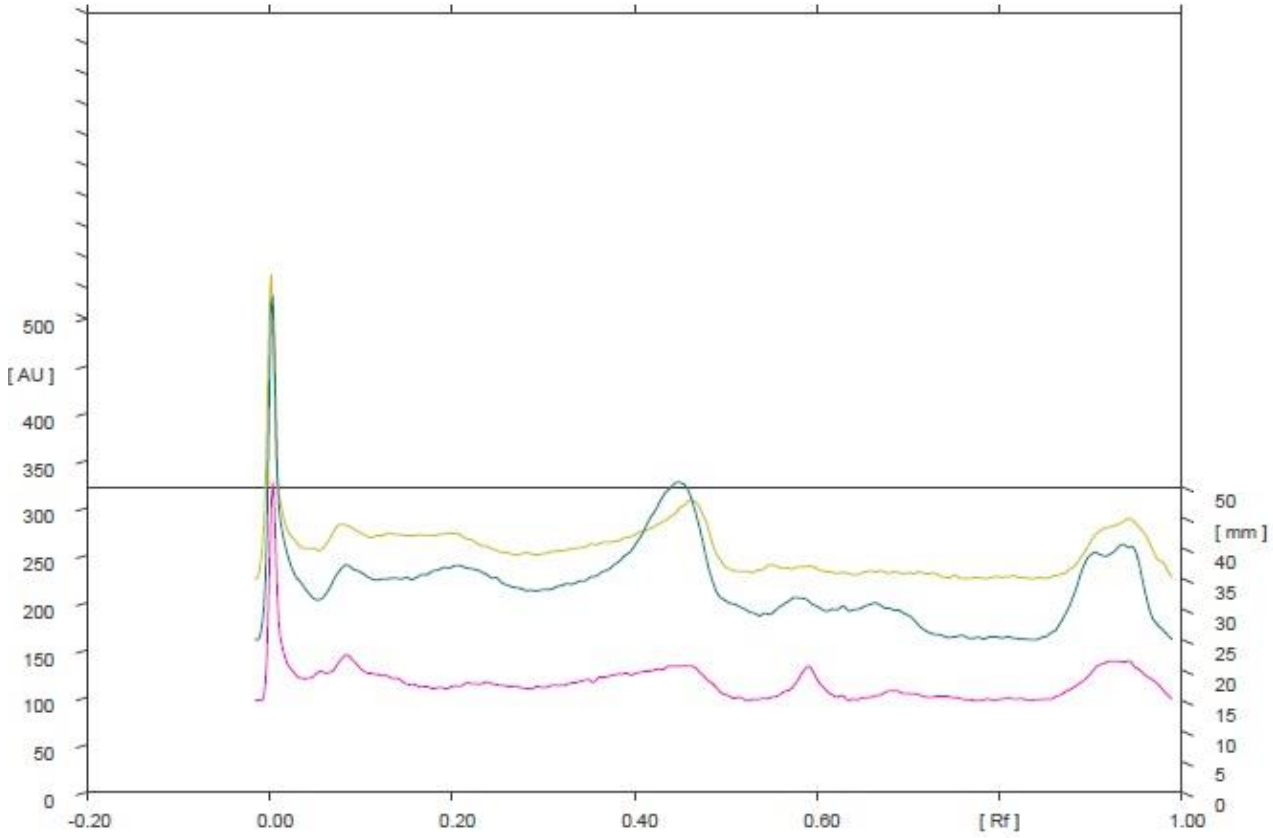

Figure1: Chromatogram of methanolic extract of *Thunbergia laurifolia* at UV- 254nm

Table 1: Rf Values of the methanolic extract of *Thunbergia laurifolia* at UV- 254nm

TRACK	PEAK	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1	1	-0.01	0.4	0	230	**64.58**	0.04	23	1956.9	33.66
	2	0.04	23.1	0.08	48.3	13.57	0.14	23.5	1942	33.4
	3	0.55	2.3	0.59	36.2	10.16	0.62	4.1	726.9	12.5
	4	0.85	1.3	0.92	41.7	11.69	0.93	41.1	1188.3	20.44
2	1	-0.01	2.1	0	362.9	**37.75**	0.05	41.9	4161.8	13.93
	2	0.05	42.1	0.08	79	8.21	0.12	62.9	2621.1	8.77
	3	0.17	68.2	0.21	78.1	8.12	0.28	51.4	4756.8	15.92
	4	0.35	66.3	0.45	166.2	17.28	0.54	25	10501.4	35.15
	5	0.55	26.7	0.58	44.3	4.61	0.61	29.8	1478.8	4.95
	6	0.64	31.7	0.66	38.5	4.01	0.74	1.9	1501.8	5.03
	7	0.85	2.7	0.9	92	9.57	0.92	88.2	2146.9	7.19
	8	0.92	88.4	0.94	100.5	10.45	0.99	0.8	2703.5	9.05
3	1	-0.01	1.8	0	320.9	**56.09**	0.04	31.8	2961.5	21.48
	2	0.05	29.5	0.08	57.2	10	0.11	43.1	1708.9	12.39
	3	0.18	45.3	0.2	47.9	8.37	0.25	27.4	1883	13.66
	4	0.37	38.2	0.46	82.7	14.46	0.51	7.6	4432.6	32.15
	5	0.85	0.2	0.94	63.4	11.08	0.99	1.7	2801	20.32

weighing 100 mg of methanolic extract of *Thunbergia laurifolia* and dissolved in 1 ml of methanol.

Stationary Phase

Chromatography was performed on HPTLC aluminium sheets silica gel 60F 254 of size 5.0 x 10.0 cm.

Sample application-CAMAG Linomat 5

HPTLC was performed on CAMAG Linomat 5. The spray gas used for application was inert gas. Methanol was used as the sample solvent with a dosage speed of 150nl/s with predosage volume of 0.2 µl. The samples were applied using CAMAG LINOMAT 5 sample applicator with syringe size of 100 µl on 3 tracks. Application position Y at 8.0mm with a band length of 7.0mm.The application

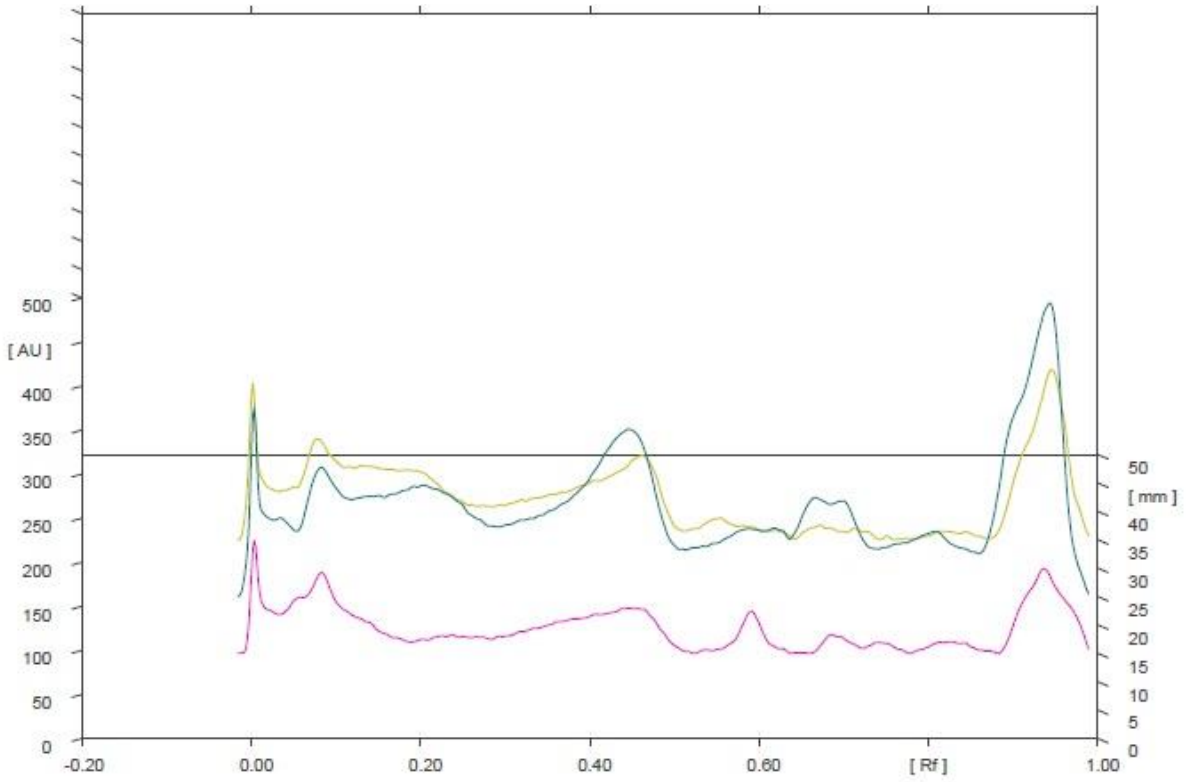

Figure2: Chromatogram of methanolic extract of *Thunbergia laurifolia* at UV- 366nm

Table 2: Rf Values of the methanolic extract of *Thunbergia laurifolia* at UV- 366nm

TRACK	PEAK	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1	1	-0.01	0.4	0	128.3	**27**	0.03	43.6	1523.4	9.86
	2	0.03	43.8	0.08	91.4	19.8	0.17	17.7	4313.2	27.92
	3	0.34	28.5	0.45	51.3	11.12	0.51	2.2	3969.4	25.7
	4	0.56	5.2	0.59	47.8	10.36	0.62	5.1	945.7	6.12
	5	0.66	0.1	0.69	21.2	4.6	0.72	5.5	475.8	3.08
	6	0.72	5.9	0.74	12.3	2.66	0.78	0.1	263.5	1.71
	7	0.78	0.4	0.82	13.4	2.9	0.87	2.4	454.7	2094
	8	0.88	0.5	0.94	95.9	20.76	0.99	15.2	3500.7	22.66
2	1	-0.01	0.8	0	214.2	13.83	0.03	86.9	2496.4	4.39
	2	0.03	87.2	0.03	89.2	5.76	0.05	74.1	1328.1	2.34
	3	0.05	74.6	0.08	146.6	9.46	0.12	110	4778.9	8.41
	4	0.17	116.3	0.2	126.6	8.17	0.28	79.9	7754.7	13.65
	5	0.34	92.3	0.45	189.9	12.26	0.51	53.5	13267.8	23.36
	6	0.54	56.8	0.59	77.2	4.98	0.6	74.3	2977.6	5.24
	7	0.61	74.3	0.62	77.7	5.02	0.64	65.2	1331.9	2.34
	8	0.64	65.8	0.67	112.4	7.25	0.68	104.5	2923.3	5.15
	9	0.69	104.7	0.7	108.6	7.01	0.74	54.4	2793.7	4.92
	10	0.77	60.8	0.81	73.8	4.77	0.85	50.5	3236.5	5.7
	11	0.86	48.7	0.94	333.3	**21.5**	0.99	3.2	13913.7	24.49
3	1	-0.01	1.3	0	177.3	24.83	0.03	54.7	2104.1	9.23
	2	0.05	59.1	0.08	114.5	16.03	0.11	82	3391.6	14.88
	3	0.17	78.2	0.19	79.2	11.09	0.26	39.5	3423	15.01
	4	0.36	51.1	0.46	95.7	13.39	0.51	9.8	6063	26.6
	5	0.53	12.5	0.55	24.8	3.46	0.59	14.6	708.8	3.11
	6	0.61	0.8	0.67	16.7	2.34	0.69	9.7	365.5	1.6
	7	0.7	8.2	0.71	12.8	1.79	0.74	0.6	204	0.89
	8	0.87	1.5	0.95	193.3	**27.06**	0.99	9.1	6538.8	28.68

position of the samples were at 15.0mm, 25.0mm and 35.0mm with sample volumes of 2.0 µl, 6.0 µl and 4.0 µl respectively.

Development of Chromatogram –Glass tank

The chromatogram was developed in Twin trough glass chamber 20x10 cm saturated with previously equilibrated mobile phase for 30 minutes. The mobile phase solvent position was 70.0 mm and the volume used was 10 ml. The

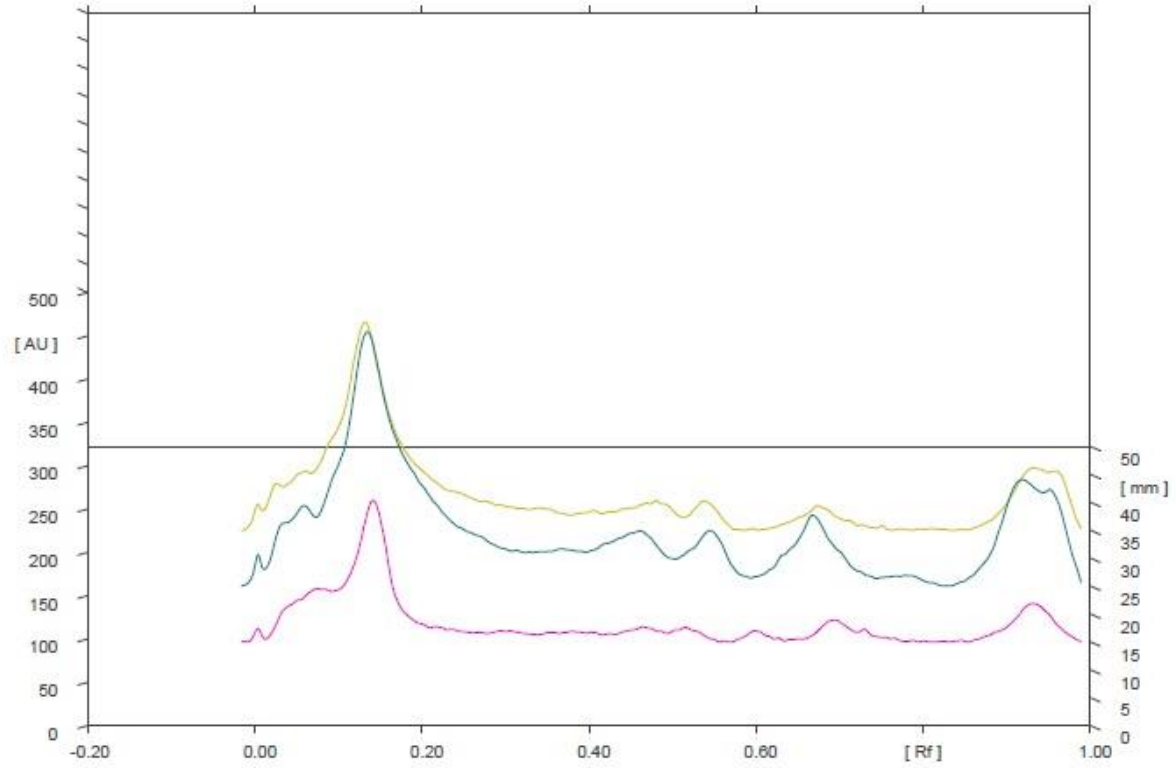

Figure3: Chromatogram of methanolic extract of *Thunbergia laurifolia* at white light- 540nm

Table 3: Rf Values of the methanolic extract of *Thunbergia laurifolia* at white light- 540nm

TRACK	PEAK	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1	1	-0.01	0.5	0	15.2	4.28	0.01	3.2	98.8	0.91
	2	0.01	3.9	0.07	61.5	17.38	0.09	58.1	2160.2	19.94
	3	0.1	50.9	0.14	163.1	**40.09**	0.21	10.2	5489.2	50.67
	4	0.49	9.6	0.51	15.8	4.75	0.55	2.8	391.5	3.61
	5	0.57	0	0.6	12.9	3.64	0.62	3.3	229.7	2.12
	6	0.65	3	0.7	25.5	7.22	0.72	11	645.9	5.96
	7	0.72	11.1	0.73	14.6	4.13	0.76	3.7	200.6	1.85
	8	0.85	0.1	0.93	44.3	12.52	0.99	0.5	1617.7	14.93
2	1	-0.01	1	0	36.2	4.13	0.01	19.2	268.1	0.73
	2	0.01	20.4	0.06	92.7	10.55	0.07	79.3	2665.4	7.23
	3	0.07	79.6	0.13	293.4	**33.41**	0.31	40.3	18639.6	50.53
	4	0.4	38.8	0.46	63.7	7.26	0.5	31.9	3208.9	8.7
	5	0.5	31	0.55	64	7.29	0.59	9.3	2160.2	5.86
	6	0.6	9.6	0.67	81.7	9.31	0.74	8.7	3413.7	9.25
	7	0.76	10.2	0.78	12.3	1.4	0.81	3.6	311	0.84
	8	0.83	0.1	0.92	122.8	13.98	0.94	107.5	4287	11.62
	9	0.95	107.9	0.95	111.3	12.67	0.98	25	1931.3	5.24
3	1	-0.01	0	0.03	53.8	8.98	0.03	50.4	840	3.9
	2	0.03	50.5	0.06	68.5	11.43	0.07	65.6	1345.6	6.25
	3	0.07	66.5	0.13	240.5	**40.14**	0.27	33.5	13142.3	61.04
	4	0.44	24.7	0.48	34.2	5.71	0.51	14.6	1199.2	5.57
	5	0.51	14.8	0.54	33.6	5.62	0.57	0.9	761.8	3.54
	6	0.61	1.1	0.67	28.1	4.69	0.72	5.2	944.3	4.39
	7	0.85	0.2	0.93	72.1	12.03	0.95	66.5	2165.6	10.06
	8	0.95	66.5	0.96	68.4	11.42	0.99	2.2	1131.8	5.26

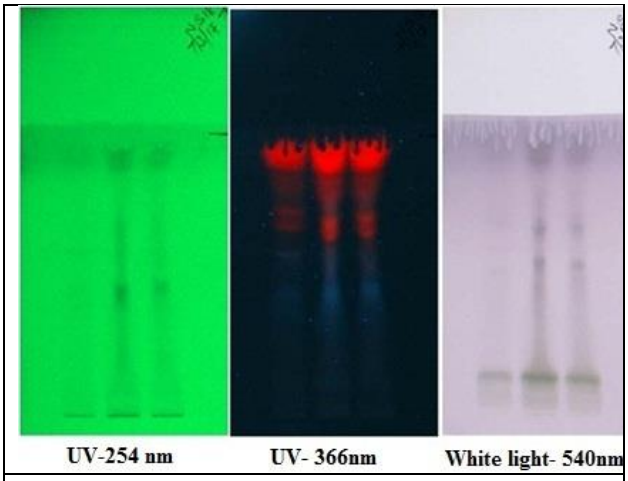

Figure 5: TLC profiling of methanolic extract of Thunbergia *laurifolia* at different wavelengths

chromatogram was dried in oven at 60°c for 5 minutes. Post chromatographic derivatization was carried out by drying in oven at 120 °c for 20 minutes.

Detection – CAMAG TLC Scanner

The sample application position was 8.0 mm and the solvent front position was 70.0mm. CAMAG TLC Scanner was used to scan the chromatogram. Light was used as the optical system with a scanning speed of 20mm/s and data resolution of the scanner was 100µm/step. The images were captured at different wavelength range of UV- 254nm, UV- 366nm and white light- 540nm. The peak values were recorded and the retention factors (Rf) and % area were calculated by the WinCats planar chromatography software. The images were visualized using CAMAG visualize.

RESULTS AND DISCUSSION

The samples were loaded in three tracks at 15.0 mm, 25.0 mm and 35.0 mm with corresponding sample volume of 2.0 µl, 6.0 µl and 4.0 µl and scanned at 254nm, 366nm and 540nm. The HTPLC results scanned at 254nm are shown in table 1 and figure 1. In track 1, 2.0 µl of the sample was added at 15.0mm position, which showed 4 peaks corresponding to various phytoconstituents. The number of polyvalent constituents in track one was 4 with ascending end Rf value ranging from 0.04 to 0.93. The highest concentration of the phytoconstituent was found to be 64.58 %. In track 2, 6.0 µl of the sample was loaded at 25mm position and 8 peaks where obtained with end Rf values ranging from 0.05 to 0.99. The highest concentration of phytoconstituent was found to be 37.75%. Similarly in track 3, 4.0 µl of the sample was added at 35.0 mm position which was separated into 5 peaks with end Rf value ranging from 0.04 to 0.99. The highest concentration of the phytoconstituent obtained was found to be 56.09 %. The samples scanned at 366nm are shown in table 2 and figure 2. In track 1, the samples were separated into 8 peaks with end Rf value ranging from 0.03 to 0.99. The highest concentration of the phytoconstituent obtained was found to be 27.80 %. In track 2, the phytoconstituents were separated into 11 peaks with end Rf value ranging from 0.03 to 0.99 and the highest concentration of phytoconstituent obtained was found to be 21.50 %. In track 3, the sample loaded was separated into 8 peaks with end Rf value ranging from 0.03 to 0.99 and the highest concentration of phytoconstituent obtained was found to be 27.06%.

The samples scanned at white light- 540 nm are shown in table 3 and figure 3. In track 1, the sample was separated into 8 peaks with end Rf value ranging from 0.01 to 0.99 with highest concentration of phytoconstituent obtained was found to be 40.09 %. In the track 2, the sample was separated into 9 peaks with end Rf value ranging from 0.01 to 0.98 with highest phytoconstituent obtained was found to be 33.41%. In track 3, the sample was separated into 8 peaks with end Rf ranging from 0.03 to 0.99 with highest concentration of phytoconstituent obtained was found to 40.14%. The chromatogram image of the phytoconstituents taken at various wavelengths UV-254 nm, UV-366 nm and white light- 540 nm are shown in Figure 5.

DISCUSSION

Methanolic extract of *Thunbergia laurifolia* was loaded into three tracks with different volumes to compare and evaluate phytoconstituents by measuring the peaks and Rf values. The extract was subjected to UV light wavelength of 254nm, 366nm and 540nm. Various peaks and Rf values at different wavelength were assessed to understand the phytoconstituents present in the methanolic extract of *Thunbergia laurifolia.* Further a comparative evaluation of the peaks and Rf values with standard has to be performed to asses and identify the compounds in *Thunbergia laurifolia.* Similar studies were carried out in methanolic extract, water extract and alcoholic extract of *Wrightia tinctoria*[13]. The methanolic extract showed the presence of indole derivates and the alcoholic and water extract showed the existence of lupeol after derivatisation. *Decalepis hamiltonii* plant roots were also studied by HPTLC using ethyl extract of the plant root[14]. The HPTLC fingerprinting profile of the plant *Thunbergia laurifolia* is considered as an important source of information to identify the phytoconstituents and to determine quality and purity of the extract for further studies.

CONCLUSION

The results indicate that the methanolic extract of the leaves contains a considerable amount of bioactive phytoconstituents. It may be concluded that results obtained from qualitative evaluation of HPTLC finger print profiles could be useful in further studies.

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REFERENCES

1. Chan EWC, Lim YY. Antioxidant activity of Thunbergia laurifolia tea. J Trop Forest Sci 2006; 18(2):130-6.

2. Putiyanan S, Chansakaow S, Phrutivorapongkul A, Charoensup W.Standard pharmacognostic characteristic of some Thai herbal medicine. Chiang Mai Univ J Nat Sci 2008; 7(2):239-55.
3. Thongsaard W, Marsden CA. A herbal medicine used in the treatment of addiction mimics the action of amphetamine on in vitro rat striatal dopamine release. Neurosci Lett 2002; 329:129-32.
4. Thongsaard W, Marsden CA, Morris P, Prior M, Shah YB. Effect of Thunbergia laurifolia, a Thai natural product used to treat drug addiction,on cerebral activity detected by functional magnetic resonance imaging in the rat. Psychopharmacology 2005; 180:752-60.
5. Kanchanapoom T, Kasai R, Yamasaki K. Iridoid glucosides from Thunbergia laurifolia. Phytochemistry 2002; 60:769-71.
6. Chivapat S, Chavalittumrong P, Attawish A, Bansiddhi J, Padungpat S.Chronic toxicity of Thunbergia laurifolia Lindl. extract. J Thai Tradit Altern Med 2009; 7(1):17-24.
7. Aritajat S, Wutteerapol S, Saenphet K. Anti-diabetic effect of Thunbergia laurifolia Linn. aqueous extract. SE Asian J Trop Med Public Health 2004; 35(2):53-8.
8. Irmgard M, Steffen W, Abraham U, Eike R. Validated HPTLC methods for the determination of salicin in Salix sp. and of harpagoside in Harpogophytum procumbens. J. Pharm. Biomed. Anal, 2008; 48(3): 587-591.
9. Priya A, Aparna S. Chemical profile studies on the secondary metabolites of medicinally important plant Zanthoxylum rhetsa (Roxb.) DC. using HPTLC. Asian Pac. J. Trop. Biomed., 2012; 293-298.
10. Bhagat RB, Kulkarni DK. Phytochemical evaluation and in vitro antimicrobial activity of Jatropha glandulifera Roxb. (Family: Euphorbiaceae). Int. J. of Pharm. & Life Sci., 2013; 4(12): 3178-3184.
11. Banu RH, Nagarajan N. TLC and HPTLC fingerprinting of leaf extracts of Wedelia chinensis (Osbeck) Merrill. J. Pharmacognosy and Phytochemistry, 2014; 2(6): 29-33.
12. Reich E and Schibli A. A Standardized approach to modern high-performance thin- layer chromatography (HPTLC). Journal of Planar Chromatography 2004; 17: 438-443.
13. Sathyanarayanan S. Selvam P, Jose A, George RM, Revikumar KG, Neytsa J. Preliminary phytochemical screening and study of antiviral activity and cytotoxicity of Wrightia tinctoria. Int. J. Chem. Sci, 2009; 7(1): 1-5.
14. Kadirvelmurugan V, Tamilvannan M, Arulkumar M, Senthilmurugan V, Thangaraju K , Dhamotharan R and Ravikumar S. Phytochemical, HPTLC Finger Print Analysis and Antimicrobial Activity of Ethyl Acetate Extract of Decalepis hamiltonii (Wight & Arn.). Journal of Academia and Industrial Research 2017; 5(9): 126-131.