Journal of Ayurvedic and Herbal Medicine 2022; 8(4): 228-235

Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2022; 8(4): 228-235
Received: 24-11-2022
Accepted: 10-12-2022

www.ayurvedjournal.com
DOI: 10.31254/jahm.2022.8403

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HPTLC Fingerprint of an ayurvedic combination- Panchavalkala

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ABSTRACT

Background: Panchavalkala is one of the most versatile ayurvedic combination of plants known for being used to reduce infections, inflammations and in wound healing and having a wide spectrum of medicinal uses. It has been formulated into various formulations like- Panchavalkala choorna, Lepa, Kashaya, kwatha, ointment, gel hand wash, decoction etc. It is It includes a group of five drugs- *Ficus benghalensis* L, *Ficus racemosa* L., *Ficus religiosa* L., *Ficus lacor* Buch. Ham, *Thespesia populnea*. WHO guidelines emphasises the need for modern and sophisticated modern instrumental method like HPTLC to standardize herbal materials. **Aim and objective:** To establish fingerprint profile of Panchavalkala combination and its individual drugs using HPTLC technique. **Method:** TLC method was developed for aqueous extract of individual drugs of Panchavalkala and Panchavalkala combination using solvent system- Toulene: ethyl acetate: methanol: formic acid (4:4:1:0.1). HPTLC fingerprinting was developed by scanning the chromatogram at 254, 366, 280 nm and at 250nm post derivatization using vanillin sulphuric acid. **Results:** The chromatograms were analyzed for number of peaks and common peaks in aqueous extract of individual drugs and Panchavalkala combination. HPTLC fingerprinting of all the individual extracts was established to identify them. The presence of common peaks found in individual extracts was confirmed in Panchavalkala combination, indicating that the developed HPTLC method can be successfully used to identify and standardize the combination. **Conclusion:** It can be concluded that HPTLC fingerprint analysis of Panchavalkala combination and its individual drugs can be used for Standardisation.

Keywords: Standardization, *Ficus benghalensis* L, *Ficus racemosa* L., *Ficus religiosa* L., *Ficus lacor* Buch. Ham, *Thespesia populnea*.

INTRODUCTION

India has enormous biodiversity. In ancient texts, approximately 1500 plants with medicinal uses are mentioned, and approximately 800 plants have been used in traditional medicine[1]. Many medicinal plants, which have been used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) and have been proposed for their fascinating multilevel activities. Some medicinal plants used in Ayurvedic preparations for their therapeutic action have been thoroughly investigated, while others remain unexplored[2]. One such distinctive Ayurvedic combination is Panchavalkala.

Panchavalkala is the combination of barks of five different plants viz. *Ficus bengalensis* Linn, *Ficus glomerata* Roxb, *Ficus religiosa* Linn, *Thespesia populnea* Soland. ex Correa, *Ficus lecor* Buch. Ham[3]. Panchavalkala is reported in the standard text as an effective combination in dealing with sepsis to reduce infections. Panchavalkala has also been reported to be used against inflammation, to clear ulcers, dress woundswounds, as a douche in leukorrhea and other vaginal diseases[4].

Standardization of plant materials is vital today. Several pharmacopeias with monographs on plant materials describe the physicochemical parameters. The WHO has emphasized the importance of ensuring the quality of medicinal plant products through the use of modern controlled techniques and the application of appropriate standards[5]. Chromatographic fingerprinting has been given as a method of identification and characterization to ensure consistent quality of herbal preparation[6].

HPTLC is a modern sophisticated TLC Analysis that provides better resolution and can estimate active constituents with reasonable certainty in less time. Methods based on high-performance thin-layer chromatography (HPTLC) could be a good alternative, as they are being investigated as an important tool in routine drug analysis. The ability of HPTLC to analyze multiple samples simultaneously while using a small amount of mobile phase is a significant advantage. This saves time and money on analysis. Furthermore, it reduces exposure risks and significantly reduces toxic organic effluent disposal issues, reducing the possibility of environmental pollution. HPTLC also allows for the detection of chromatograms with the same or different parameters on multiple occasions[7].

The present study proposed to develop HPTLC fingerprint of Panchavalkala and individual drugs.

MATERIALS AND METHODS

Materials

The barks of *Ficus bengalensis Linn, Ficus racemosa Roxb*, *Ficus religiosa Linn*, *Thespesia populenoides L* and *Ficus lacor Buch Ham* which are constituents of ayurvedic formulation Panchavalkala were collected from Indus Herbs, Basavanagudi , Bengaluru, Karnataka. The barks were authenticated at Central Ayurveda Research Institute, Bengaluru. The specimen samples were deposited in the Institute.

Preparation of extracts

Fresh barks were dried in a Hot Air oven at 40°C for 3 days , powdered, and sieved to pass through #10 mesh. All the powders were stored in air tight containers for further use. 200 g of powdered bark materials were subjected to refluxation using 800 ml of water as a solvent for 12 hrs. All five barks were extracted individually. The crude extracts were then subjected to rotary evaporator at 70°C to get a semisolid extract.

Preparation of Panchavalkala

1g of each of the dried extract of the five barks was, weighed and mixed in a china dish to prepare a homogenous mixture and stored in the refrigerator for further use.

HPTLC Fingerprinting of individual extracts:

Sample preparation

100 mg of each extracts was mixed in 10 ml of Methanol (10mg/ml). To dissolve the extract, they were ultrasonicated for 1 hour and then filtered through Whatman filter paper qualitative 1. The filtrate was then used for the study.

Solvent system: Toluene: ethyl acetate: methanol: formic acid (4:4:1:0.1).

Derivatizing agent: Vanillin sulphuric acid.

Procedure:

Individual extracts were applied (5µl each) on TLC Plate Silica gel 60F 254 in form of ba and of 8 mm thickness using LINOMAT 5. The plates were then developed in a Twin trough chamber previously saturated with mobile phase – Toluene: ethyl acetate: methanol: formic acid (4:4:1:0.1) for 20 mins. The plates were allowed to develop to a distance of 70 mm and air-dried for 15 mins. The developed plates were visualized in visualizing chamber and scanned in CAMAG TLC Scanner under 254 and 366 nm. With help of CAMAG WinCATS software -the chromatograms were recorded , number of peaks and their Rf values were noted. The plate was subjected a to multiwavelength scan to determine wavelength giving maximum number of peaks. The plate was derivatizedatised using vanillin sulphuric acid and dried in oven at 110°C for 5 mins. The plate was placed in TLC Scanner and scanned from 200-600 nm. The wavelength giving maximum number of peaks is selected ana the number of peaks at that wavelength was noted.

HPTLC Fingerprinting of Panchavalkala:

Sample preparation

10 mg/ml solution was prepared in methanol. The solution was subjected to ultrasonication for 1 hour and filtered through the Whatman filter paper qualitative 1.

Solvent system: Toluene: ethyl acetate: methanol: formic acid (4:4:1:0.1).

Derivatizing agent: Vanillin sulphuric acid.

Procedure:

Panchavalkala was applied (5µl each) on TLC Plate Silica gel 60F 254 in form of baa nd of 8 mm thickness using LINOMAT 5. The plates were then developed in Twin trough chamber previously saturated with mobile phase – Toluene: ethyl acetate: methanol: formic acid (4:4:1:0.1) for 20 mins. The plates were allowed to develop to a distance of 70 mm and air-dried for 15 mins. The developed plates were visualizedd in visualizing chamber and scanned in CAMAG TLC Scanner under 254 and 366nm With help of CAMAG WinCATS software. The chromatograms were recorded, number of peaks and their Rf values were noted at 254,366,280 nm and after derivatization with vanillin sulphuric acid at 250nm.

RESULTS

The present work attempted to prepare HPTC fingerprints of aqueous extracts of individual drugs and the Panchavalkala combination. The TLC plate of extracts at 366nm and post derivatisation at 250nm is shown in Figure 1.

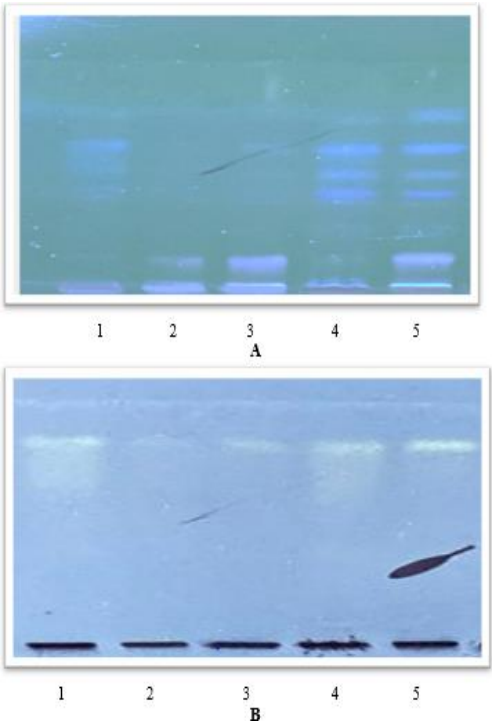


Figure 1: HPTLC Profile of individual extracts at A- 366nm and B- 250nm post derivatisation. Where - Track 1 –Extract of *Ficus racemose,* Track 2 – Extract of *Ficus religiosa,* Track 3- Extract of *Ficus benghalensis.* Track 4- Extract of *Ficus lacor.* Track 5- Extract of *Thespesia populnea*.

The analysis of chromatograms at 254nm and 366nm indicated the number of peaks in all the extracts as tabulated in Table 1.

The wavelength giving a maximum number of peaks was found to be 280nm. The number of peaks at this wavelength is given in Table 1. The chromatograms, 3D display under UV 280nm is shown in Figure 2.

The analysis of the plate after derivatization indicated 250nm to a give maximum number of peaks. The peaks at 250nm and 280nm are also tabulated in Table 1. The chromatograms, 3D display under UV 250nm is shown in Figure 2, 3 and 4.

The maximum number of peaks in all the extracts were seen post derivatisation at 250nm. *Ficus racemosa* extract showed highest number of peaks in 280nm. The number of peaks decreased from 6 to 4 as wavelength changed from 254nm to 366nm and showed only 5 peaks in 250nm. *Ficus religiosa* showed highest number of peaks in 280nm. The number of peaks at 254 and 280nm was 7 while at 366nm it reduced to 5. *Ficus benghalensis* showed highest number of peaks in 280nm. The number of peaks at 254nm was 7 while at 366nm and 280nm it reduced to 4 and 6 respectively. *Ficus lacor* showed highest number of peaks in 280nm. The number of peaks at 254nm and 280nm the number of peaks were 6 while at 366nm it reduced to 2. *Thespesia populnea* extract showed highest number of peaks in 280nm. The number of peaks at 254nm and 280nm was 4 and 5 respectively but at 366nm it was reduced to 3.

The chromatograms were analyzed to note the common Rf values in all the extracts at different wavelengths. All the extracts were found to exhibit peak with Rf value 0.82-0.86 common at 254nm and 280nm. All the tracks showed a common peak with Rf value 0.64 at 366nm. The common peaks at Rf value 0.82-0.86 and 0.61-0.65 were observed in all the extracts post derivatization at 250nm.

The presence of these peaks was selected as a criteria for standardization of Panchavalkala combination. The HPTLC fingerprinting of the combination was established using the selected wavelengths- 254, 366, 280 and 250nm.

HPTLC Fingerprinting of Panchavalkala:

The TLC Plate of the Panchavalkala combination at 366nm and post derivatization at 250nm is shown in Figure 5.

The number of peaks at 254, 366nm is tabulated in Table 2. The number of peaks at 280nm wavelength are given in Table 1. The densitograms, 3D display under UV 280nm is shown in Figure 6.

The plate after derivatization was scanned at 250nm. The number of peaks was tabulated in Table 2. The densitograms, 3D display under UV 250nm is shown in Figure 7.

Maximum number of peaks i.e 12 were seen post derivatization at 250nm. Panchavalkala had 8 peaks at 254nm and 280nm while it had 6 peaks at 366nm.

The results of HPTLC analysis of individual extracts showed the presence of peak with Rf value of 0.82-0.86 common at 254nm and 280nm, 0.64nm at 366nm, 0.82-0.86 and 0.61-0.65 at 250nm. Presence of these peaks was selected for standardization of combination. The analysis of chromatograms of combinations confirmed the presence of common peaks as shown in Table-3.

Table 1: Data showing number of Peaks in extracts at different wavelengths

Track number	Extracts	254nm	366nm	280nm	250nm
1	*Ficus racemose*	6	4	11	5
2	*Ficus religiosa*	7	5	7	11
3	*Ficus benghalensis*	7	4	6	10
4	*Ficus lacor*	6	2	6	16
5	*Thespesia populnea*	4	3	5	14

Table 2: Data showing number of peaks in combination at different wavelength

Sample	254nm	366nm	280nm	250nm
Panchavalkala	8	6	8	12

Table 3: Common Rf values in extracts and combination at different wavelength

Sample	254nm	366nm	280nm	250nm
Panchavalkala	0.81	0.64	0.82	0.63, 0.85

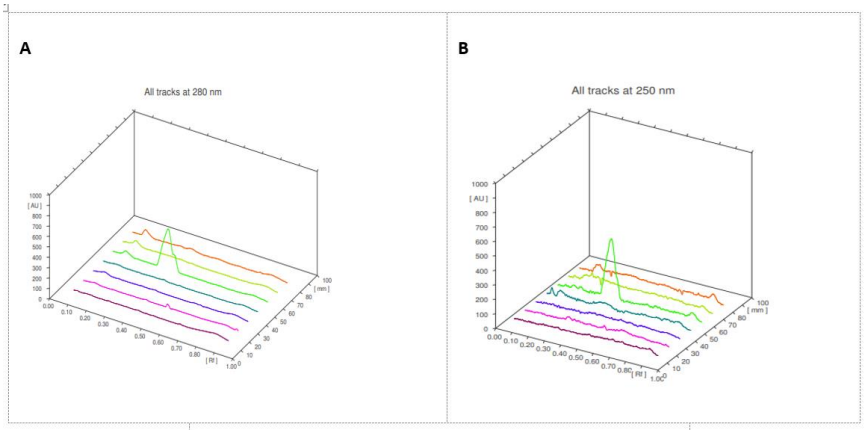
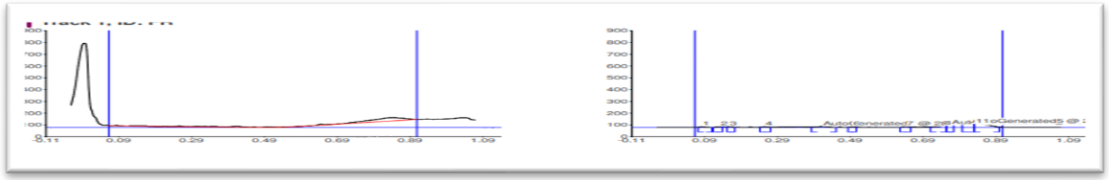
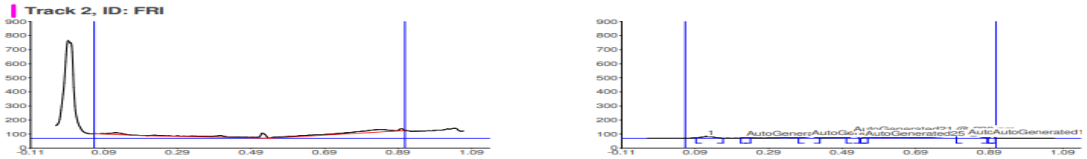


Figure 2: Chromatogram of all the extracts at A- 280nm and B- 250nm



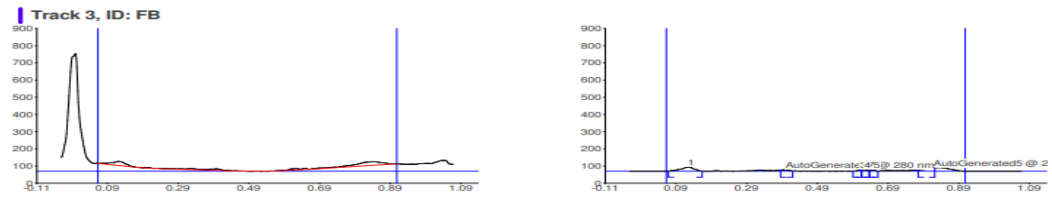
eak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	1.8	0.08	6.7	5.09	0.11	0.0	66.6	2.57	unknown *
2	0.11	0.0	0.13	5.6	4.24	0.14	5.3	75.8	2.92	unknown *
3	0.15	5.5	0.16	7.1	5.37	0.17	0.8	74.7	2.87	unknown *
4	0.24	1.5	0.26	6.8	5.18	0.27	1.6	82.9	3.19	unknown *
5	0.38	2.6	0.42	8.5	6.45	0.45	0.0	202.6	7.80	AutoGenerated7
6	0.48	1.4	0.50	5.1	3.83	0.51	1.9	50.9	1.96	unknown *
7	0.63	0.4	0.65	9.0	6.84	0.66	1.8	103.1	3.97	unknown *
8	0.71	3.3	0.75	11.0	8.34	0.75	10.7	241.2	9.29	unknown *
9	0.76	10.8	0.77	16.0	12.10	0.78	15.0	209.1	8.05	AutoGenerated27
10	0.80	19.9	0.82	28.1	21.28	0.83	27.7	587.1	22.60	AutoGenerated5
11	0.83	27.7	0.84	28.2	21.29	0.90	3.5	904.0	34.80	unknown *

A



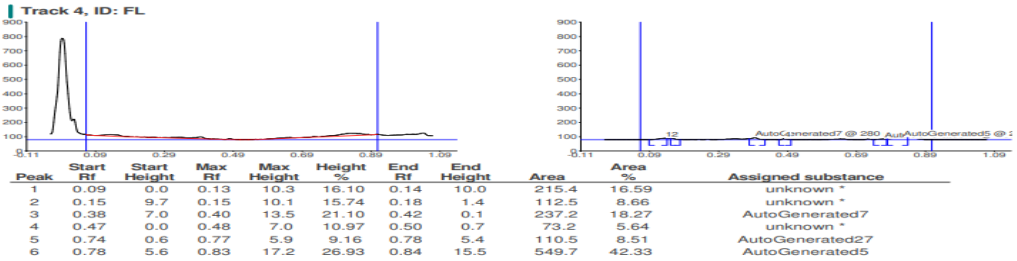
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	2.8	0.12	13.8	12.65	0.17	0.1	397.3	17.67	unknown *
2	0.21	2.8	0.23	5.2	4.71	0.24	3.3	79.0	3.51	AutoGenerated26
3	0.37	3.7	0.41	12.4	11.29	0.43	3.2	300.0	13.34	AutoGenerated7
4	0.50	4.6	0.52	36.8	33.65	0.54	0.3	436.9	19.43	AutoGenerated21
5	0.54	0.2	0.55	6.4	5.88	0.56	5.7	64.7	2.88	AutoGenerated25
6	0.80	12.7	0.83	18.6	16.99	0.89	2.9	831.9	37.00	AutoGenerated5
7	0.89	2.7	0.90	16.2	14.83	0.91	1.7	138.6	6.16	AutoGenerated10

B



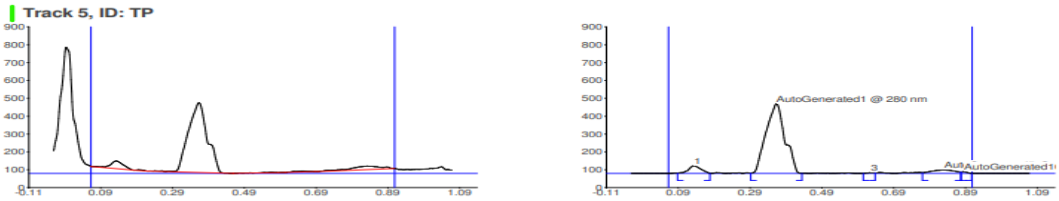
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.2	0.12	23.1	29.32	0.16	0.6	778.1	45.50	unknown *
2	0.39	6.5	0.40	9.2	11.71	0.42	1.7	166.0	9.71	AutoGenerated7
3	0.59	1.0	0.61	8.2	10.36	0.62	7.7	80.0	4.68	unknown *
4	0.62	7.8	0.62	9.6	12.13	0.64	3.9	114.8	6.72	unknown *
5	0.64	4.1	0.65	7.9	10.08	0.66	0.1	87.0	5.09	unknown *
6	0.78	6.8	0.82	20.8	26.40	0.82	20.6	484.3	28.32	AutoGenerated5

C



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	0.0	0.13	10.3	16.10	0.14	10.0	215.4	16.59	unknown *
2	0.15	9.7	0.15	10.1	15.74	0.18	1.4	112.5	8.66	unknown *
3	0.38	7.0	0.40	13.5	21.10	0.42	0.1	237.2	18.27	AutoGenerated7
4	0.47	0.0	0.48	7.0	10.97	0.50	0.7	73.2	5.64	unknown *
5	0.74	0.6	0.77	5.9	9.16	0.78	5.4	110.5	8.51	AutoGenerated27
6	0.78	5.6	0.83	17.2	26.93	0.84	15.5	549.7	42.33	AutoGenerated5

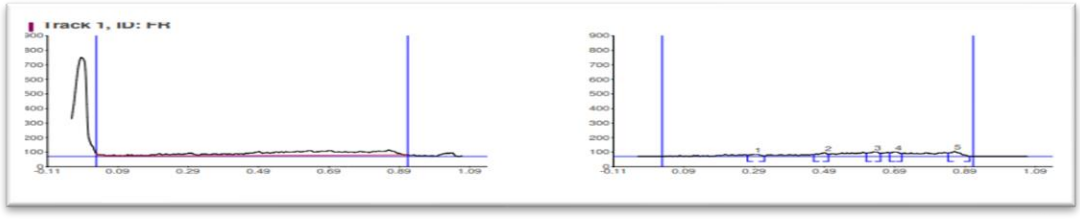
D



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	1.8	0.13	43.2	9.23	0.18	0.4	1257.5	6.35	unknown *
2	0.29	2.4	0.36	390.5	83.45	0.43	0.9	17264.1	87.20	AutoGenerated1
3	0.61	0.0	0.63	5.0	1.07	0.64	4.1	74.8	0.38	unknown *
4	0.77	4.9	0.83	19.4	4.14	0.88	8.3	1068.4	5.40	AutoGenerated5
5	0.88	8.4	0.89	9.9	2.11	0.91	0.4	132.9	0.67	AutoGenerated10

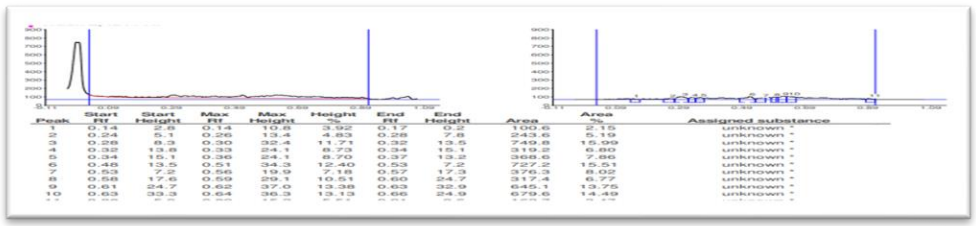
E

Figure 3: HPTLC fingerprinting of extracts at 280nm- A- extracts of *Ficus raacemosa*, B- extract of *Ficus religiosa*, C- extract of *Ficus benghalensis*, D- extract of *Ficus lacor*, E- extract of *Thespesia populnea*



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.27	10.1	0.29	17.5	12.24	0.32	3.9	432.9	12.47	unknown *
2	0.46	13.5	0.49	26.4	18.42	0.50	15.5	599.6	17.27	unknown *
3	0.61	23.5	0.63	32.5	22.71	0.65	21.3	817.5	23.55	unknown *
4	0.68	26.0	0.69	31.5	22.00	0.71	20.8	705.8	20.33	unknown *
5	0.84	25.1	0.86	35.3	24.63	0.90	1.4	915.2	26.37	unknown *

A



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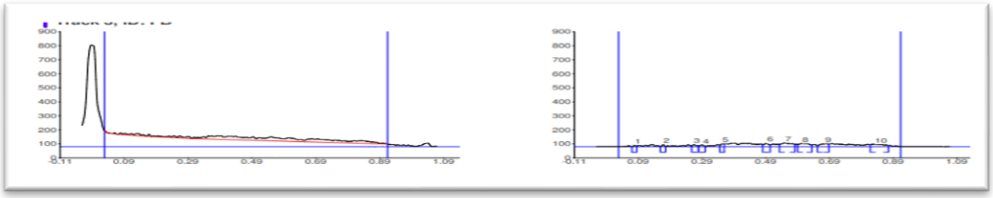
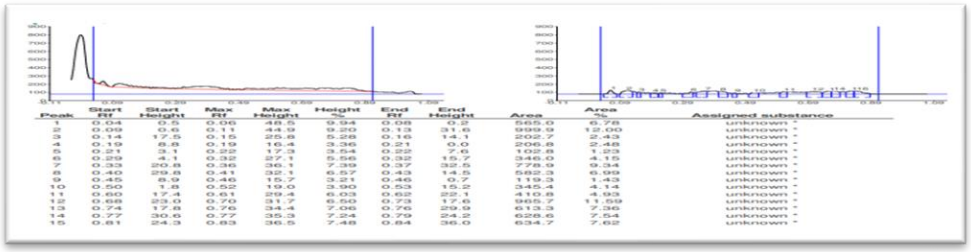


Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.1	0.08	11.5	5.67	0.08	5.4	77.9	2.14	unknown *
2	0.16	1.7	0.17	14.8	7.31	0.17	0.1	96.8	2.66	unknown *
3	0.26	6.0	0.26	16.1	7.92	0.28	7.8	166.5	4.57	unknown *
4	0.28	7.8	0.29	13.5	6.66	0.30	1.2	155.4	4.27	unknown *
5	0.35	10.2	0.35	24.1	11.86	0.36	19.2	201.7	5.54	unknown *
6	0.48	15.7	0.49	25.2	12.43	0.50	14.2	339.1	9.31	unknown *
7	0.53	17.3	0.55	29.3	14.42	0.58	17.2	806.2	22.13	unknown *
8	0.59	16.9	0.61	24.7	12.14	0.63	13.8	664.1	18.23	unknown *
9	0.65	9.4	0.68	24.5	12.04	0.69	19.3	491.5	13.49	unknown *
10	0.82	15.0	0.83	19.4	9.54	0.88	9.5	644.2	17.68	unknown *

C



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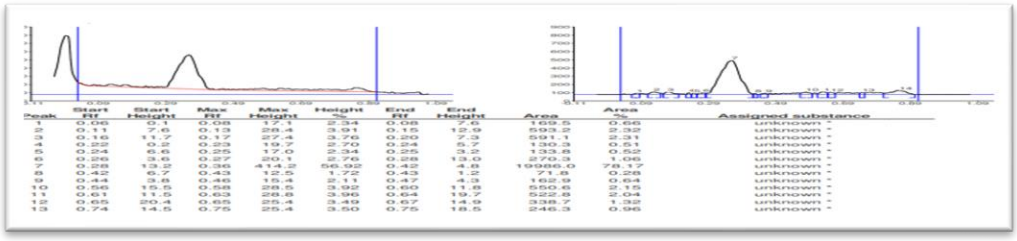


E

Figure 4: HPTLC fingerprinting of extracts at 250nm- A- extracts of *Ficus raacemosa*, B- extract of *Ficus religiosa*, C- extract of *Ficus benghalensis*, D- extract of *Ficus lacor*, E- extract of *Thespesia populnea*

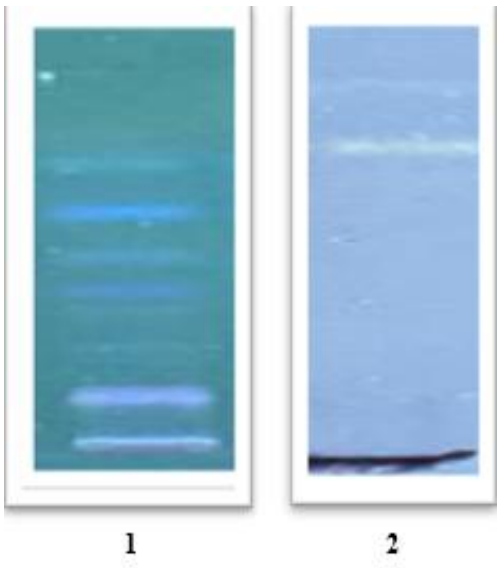
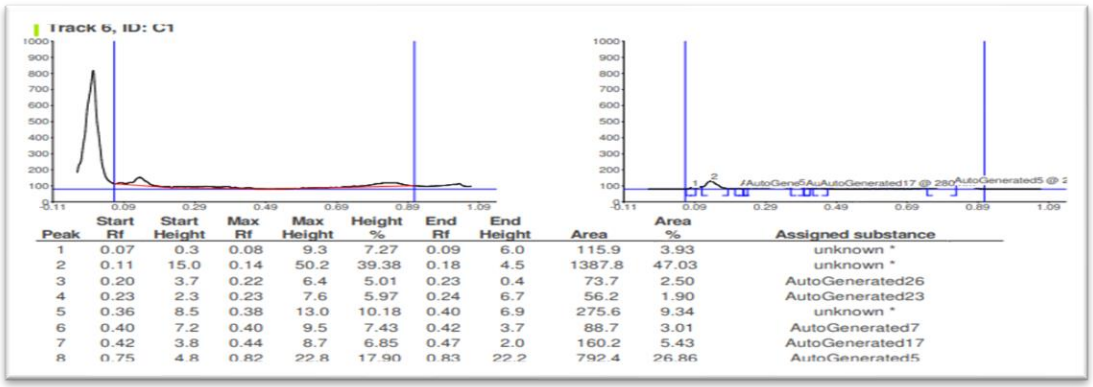
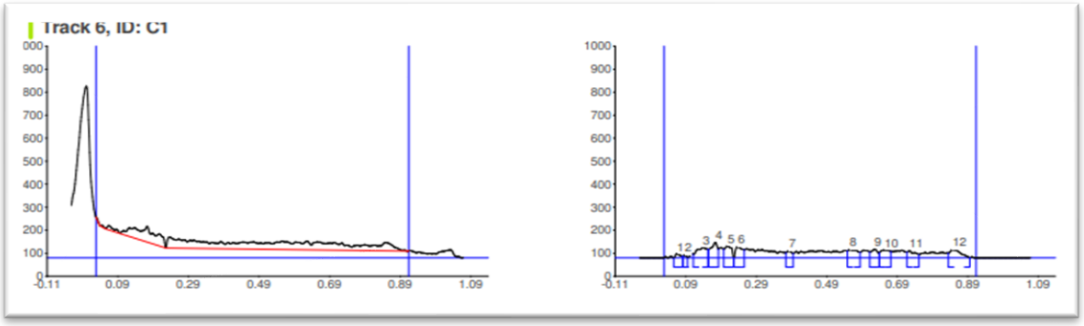


Figure 5: HPTLC Profile of Panchavalkala combination at A- 366nm and B- 250nm post derivatisation



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.3	0.08	9.3	7.27	0.09	6.0	115.9	3.93	unknown *
2	0.11	15.0	0.14	50.2	39.38	0.18	4.5	1387.8	47.03	unknown *
3	0.20	3.7	0.22	6.4	5.01	0.23	0.4	73.7	2.50	AutoGenerated26
4	0.23	2.3	0.23	7.6	5.97	0.24	6.7	56.2	1.90	AutoGenerated23
5	0.36	8.5	0.38	13.0	10.18	0.40	6.9	275.6	9.34	unknown *
6	0.40	7.2	0.40	9.5	7.43	0.42	3.7	88.7	3.01	AutoGenerated7
7	0.42	3.8	0.44	8.7	6.85	0.47	2.0	160.2	5.43	AutoGenerated17
8	0.75	4.8	0.82	22.8	17.90	0.83	22.2	792.4	26.86	AutoGenerated5

Figure 6: Chromatogram of Panchavalkala at 280nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.3	0.07	18.8	4.14	0.08	8.1	216.9	2.59	unknown *
2	0.08	8.6	0.09	13.0	2.86	0.09	1.7	88.9	1.06	unknown *
3	0.11	16.3	0.14	45.8	10.06	0.15	37.6	1115.7	13.32	unknown *
4	0.15	37.7	0.17	68.1	14.95	0.18	39.8	1053.1	12.58	unknown *
5	0.20	38.7	0.21	52.4	11.50	0.23	0.1	854.5	10.21	unknown *
6	0.23	0.0	0.24	48.5	10.64	0.26	35.7	824.4	9.85	unknown *
7	0.37	22.9	0.38	31.2	6.86	0.39	20.1	412.5	4.93	unknown *
8	0.55	31.1	0.56	36.6	8.04	0.58	25.3	865.5	10.34	unknown *
9	0.61	26.3	0.63	37.4	8.21	0.64	21.3	605.9	7.24	unknown *
10	0.64	22.9	0.66	35.3	7.74	0.67	28.4	762.1	9.10	unknown *
11	0.72	25.9	0.72	32.2	7.07	0.75	15.0	604.0	7.21	unknown *
12	0.84	21.0	0.85	36.1	7.93	0.90	2.3	970.0	11.58	unknown *

Figure 7: Chromatogram of Panchavalkala at 250nm

DISCUSSION

The present study was taken up to standardise a distinctive ayurvedic combination- Panchavalkala and its individual drugs. This combination is very popular in Ayurveda. Therapeutic utility of these drugs have been highlighted in classical texts of Ayurveda and all these have been said to be wound healing, styptic, best in gynaecological disorders [8]. Panchavalkala combination has been used and formulated into various formulations like- Panchavalkala choorna, Lepa, Kashaya, kwatha, ointment, gel hand wash, decoction etc. [9-13]. Hence there is a need for HPTLC Standardisation. HPTLC fingerprinting of all the extracts and Panchavalkala combination was established to identify them. The solvent system- Toluene: ethyl acetate : methanol : formic acid (4:4:1:0.1) was selected. The chromatograms were scanned at 254 and 366nm and at λ max 280nm and number of peaks with rf values were noted. The chromatograms were scanned at 250nm after derivatization with vanillin sulphuric acid. All the extracts were found to exhibit common peak with Rf value 0.82-0.86 at 254nm and 280nm. All the tracks showed a common peak with Rf value 0.64 at 366nm. The common peaks at Rf value 0.82-0.86 and 0.61-0.65 were observed in all the extracts post derivatization at 250nm. The presence of these peaks was selected as, a criteria for standardization of combinations The results of our study maybe useful to develop analytical profile for the individual drugs as well as the combination Panchavalkala.

CONCLUSION

In our present study, HPTLC fingerprint profile of individual drugs of Panchavalkala and Panchavalkala combination along with their Rf values were recorded at four different wavelengths- 254, 366, 280, post derivatisation at 250nm which can serve as a reference standard for the scientists engaged in research on the medicinal properties of Panchavalkala.

Conflict of interest

None declared.

Financial support

None declared.

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HOW TO CITE THIS ARTICLE

Khanam S, Neha KB. HPTLC Fingerprint of an ayurvedic combination-Panchavalkala. J Ayu Herb Med 2022;8(4):228-235. DOI: 10.31254/jahm.2022.8403