

## SIMULTANEOUS DETERMINATION OF AYURVEDIC HERBS FOR PRELIMINARY SCREENING AND QUALITY CONTROL THROUGH HPTLC

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### Abstract

**Objective:** To perform simultaneous estimation of four Ayurvedic herbs for preliminary screening and quality control through HPTLC

**Method:** Simple and precise HPTLC methods was used for simultaneous determination of four Ayurvedic herbs viz., *Foeniculum vulgare*, *Syzygium aromaticum*, *Trachyspermum ammi*, and *Cinnamomum zeylanicum*. HPTLC profiling was performed over precoated TLC plates (60 F254, 20 cm × 10 cm, 250 µm thickness, Merck, Darmstadt, Germany) via a linear ascending technique using Toluene: Ethyl Acetate (9:1 v/v) as mobile phase and derivatization is performed using anisaldehyde sulphuric acid reagent. Detection was achieved at 254 nm, 366nm and 545 nm through spectro-densitometric analysis.

**Results:** Results of the experiments conducted provided diagnostic characteristics to identify and standardize the raw herbs and Ayurvedic formulations like Mahasudarshan churna. A compact band was obtained for *Foeniculum vulgare* at Rf value of 0.42 and 0.48 at 254 nm and Rf value of 0.28 and 0.39 was obtained after derivatization with Anisaldehyde sulphuric acid reagent. Similarly results for *Syzygium aromaticum*, *Trachyspermum ammi*, and *Cinnamomum zeylanicum* are mentioned in table 1. **Conclusion:** The present investigation would serve as a document to control the quality of raw material like *Foeniculum vulgare*, *Syzygium aromaticum*, *Trachyspermum ammi*, and *Cinnamomum zeylanicum* and traditional Ayurvedic formulation like Mahasudarshan Churna in various herbal industries.

### KEYWORDS:

HPTLC, Quality Control, Raw Herbal Material, Ayurvedic Formulation.

## 1. INTRODUCTION:

For thousands of years, nature has been a treasure and a very good source of medicinal agents, a number of modern drugs have been inspired from the natural sources, many of these formulations were based on the uses of the agents used in traditional medicine [1]. Herbal medicine is defined as a branch of science in which plant-based formulations are used to alleviate diseases. Phytotherapy has been introduced as a more accurate synonym of herbal or botanical medicine. Herbal medicine was a prime healthcare system in the early twentieth century but with the advent of an allopathic system of medicine to treat the ailments, herbal medicines gradually lost its popularity among people, which is based on the fast therapeutic actions of synthetic drugs [2].

Recently there has been a shift in universal trends from synthetic to herbal medicine. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments [3]. Ayurvedic medicinal products are time-tested over centuries and together with recent extensive research on the medicinal chemistry, pharmacology and clinical aspects of many of these herbal products, their beneficial properties are continuously proving to be useful. The components used in ayurvedic medicine provide an attractive basis for the development of novel pharmaceutical products that can either act as active single-molecule medications by themselves or as components of multimodal (combination of western and Ayurvedic medicines) therapeutic regimens [4]. But the main problem associated with the herbal industries is lack of quality control parameters [5]. The quality of herbal medicine depends upon the concentration of active ingredient and its purity. There are several factors which affect the quality of herbal drugs like adulteration, misidentification, collecting season, region of cultivation, toxic metals etc. Therefore, to ensure the quality of herbal drug there is a need of standardization [6]. The present investigation would serve as a document to control the quality of raw material help in standardization of different Ayurvedic formulation like Mahanarayana oil, Maha Sudarshan churna, Maharasnadi churna in various herbal industries.

## 2. MATERIALS AND METHODS<sup>7</sup>.

### 2.1 Chemicals and Materials:

Methanol, Acetonitrile was purchased from Merck Chemicals Private Limited, Toluene, Ethylacetate, Chloroform, n-Hexane, Petroleum-ether were purchased from Rankem chemicals. Herbal raw material was taken from the store house of Minor Forest Produce Processing and Research Centre, Bhopal.

### 2.2 PREPARATION OF TEST SOLUTION FOR FOENICULUM VULGARE (SAUF).

10 grams of coarsely powdered drug was taken in 250 ml stoppered conical flask and extracted with 100 ml alcohol for 24 hours by maceration technique with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is filtered through Whatman filter paper no.1.25 ml of the extract was taken from stock solution and dried on a water bath. The dried mark was extracted with Petroleum ether 60-80°C (4x5ml).

Concentrate the pooled Petroleum ether 60-80 °C extract to 5ml.

### **2.3 PREPARATION OF TEST SOLUTION FOR SYZYGIUM AROMATICUM (LAUNG).**

10 grams of coarsely powdered drug was taken in 250 ml stoppered conical flask and extracted with 100 ml alcohol for 24 hours by maceration technique with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is filtered through Whatman filter paper no.1. 25 ml of the extract was taken from stock solution and dried on a water bath. The dried mark was extracted with Petroleum ether 60-80°C (4x5ml). The pooled Petroleum ether 60-80 °C was concentrate and extracted to 5ml.

### **2.4 PREPARATION OF TEST SOLUTION FOR TRACHYSPERMUM AMMI (AJWAIN):**

10 grams of coarsely powdered drug was taken in 250 ml stoppered conical flask and extracted with 100 ml alcohol for 24 hours by maceration technique with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is then filtered through Whatman filter paper no.1. 25 ml of the extract was taken from stock solution and dried on a water bath. The dried mark was extracted with Petroleum ether 60-80°C (4x5ml). The pooled Petroleum ether 60- 80 °C was concentrate and extracted to 5ml.

### **2.5 PREPARATION OF TEST SOLUTION FOR CINNAMOMUM ZEYLANICUM (DALCHINI):**

10 grams of coarsely powdered drug was taken in 250 ml stoppered conical flask and extracted with 100 ml alcohol for 24 hours by maceration technique with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is filtered through Whatman filter paper no.1. 25 ml of the extract was taken from stock solution and dried on water bath. The dried mark was extracted with chloroform (4x5ml). The pooled chloroform was concentrated and extracted to 5ml.

### **2.6 PREPARATION OF TEST SOLUTION FOR MAHA SUDARSHAN CHURNA.**

10 grams of coarsely powdered drug was taken in 250 ml stoppered conical flask and extracted with 100 ml alcohol for 24 hours by maceration technique with occasional shaking. The extract was decanted and made up to 100 ml in volumetric flask. The solution is then filtered through Whatman filter paper no.1. 25 ml of the extract was taken from stock solution and concentrate on water bath to 5ml

### **METHOD:**

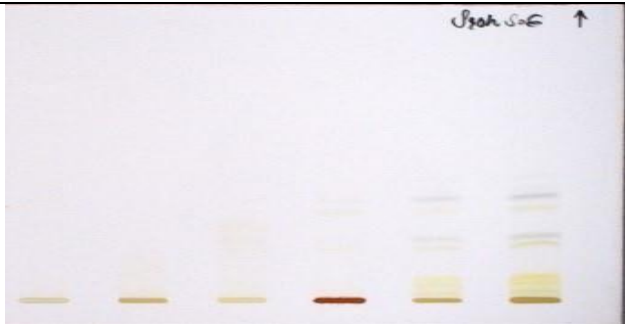
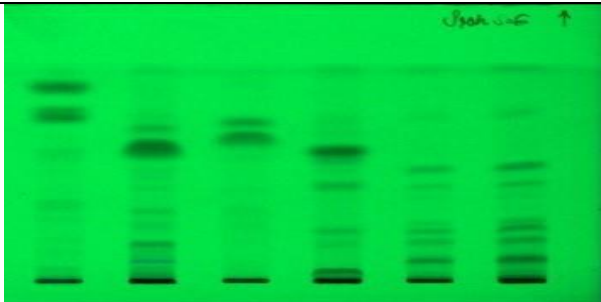
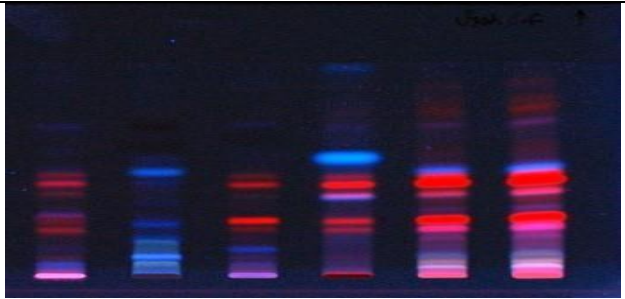
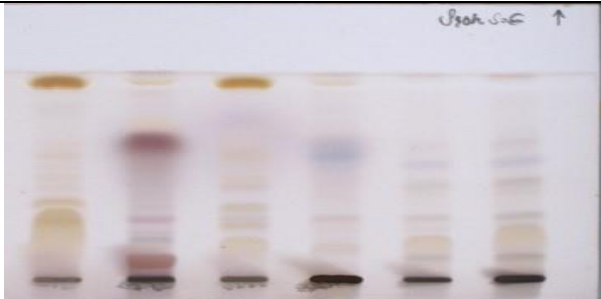
#### **Instrumentation and chromatographic conditions**

Total 5 samples having 6 bands were spotted having width of 8 mm with a Camag microliter syringe on pre-coated silica gel aluminum Plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat 5 (Switzerland). A constant application rate of 150nl/s was employed and space between two bands was 8 mm. The slit dimension was kept 6 mm × 0.30 mm micro; 100nm/s scanning speed was employed. The mobile phase consisted of Toluene: Ethyl Acetate (9:1 v/v) and derivatization are through anisaldehyde sulphuric acid reagent. Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature.

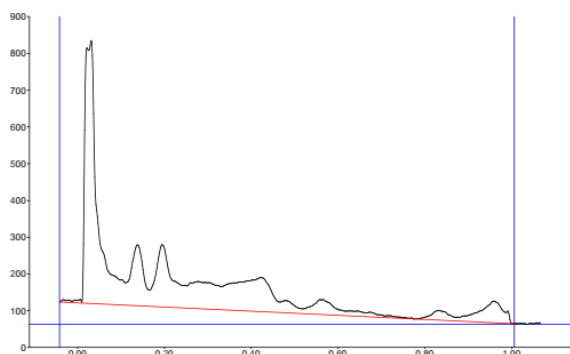
The length of the chromatogram run was approximately 70 mm. Subsequent to the development; the TLC plate was dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner 4 in the absorbance mode at 254 nm and 545 nm while at 366 nm fluorescence mode was used. The source of radiation utilized was Deuterium lamp, Tungsten lamp, and Mercury lamp respectively.

#### RESULTS:

HPTLC was performed on aluminum-packed silica gel 60 F-254 HPTLC plates (Merck). A total of 5 samples were spotted in the form of bands of width 8 mm. The selective mobile phase was poured into the chamber and left to equilibrate for 20 min. Samples were applied to the plates as sharp bands by means of Camag Linomat 5 samples applicator using 100  $\mu$ l syringe. After drying the bands in a current of air, the plates were placed in one trough of the Camag twin trough chamber. The plate was then developed in an ascending one-dimensional model in a saturated chamber. After separation, the plates were dried and photo-documented in TLC Visualizer, and then chromatographic bands were detected through Scanner-4 before and after spraying the anisaldehydesulphuric acid reagent. The plates were observed for various bands and the R<sub>f</sub> value was recorded at 254 nm, 366 nm, and 545 nm. The experimental data obtained are mentioned in Table 1.

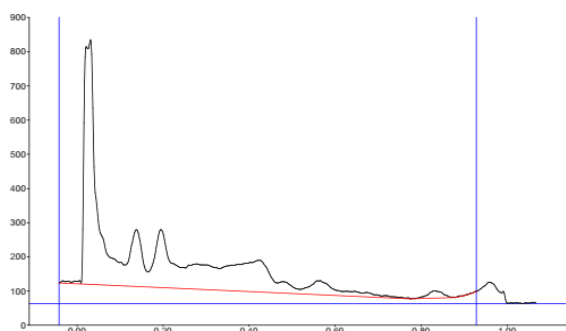
<b>Table 1</b>	
<b>TLC plate under white light</b>	<b>TLC plate under 254 nm</b>
	
<b>TLC plate under 366 nm</b>	<b>TLC plate at 545 nm after Derivatization</b>
	

## Track 1:Foeniculum vulgare, Chromatogram at 254 nm



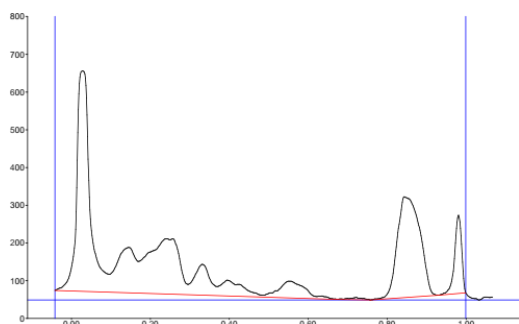
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	2.0	0.03	715.7	51.88	0.10	67.9	16995.9	42.81
2	0.11	61.2	0.14	166.3	12.05	0.17	44.2	4088.0	10.30
3	0.17	44.3	0.20	169.6	12.29	0.24	61.0	5318.5	13.40
4	0.25	60.6	0.28	73.9	5.36	0.29	71.3	1891.0	4.76
5	0.37	74.9	0.42	93.2	6.76	0.47	28.4	5450.2	13.73
6	0.47	28.5	0.48	33.4	2.42	0.52	13.2	935.2	2.36
7	0.52	13.3	0.57	41.2	2.99	0.61	14.9	1664.9	4.19
8	0.78	1.0	0.83	27.1	1.96	0.87	10.7	917.9	2.31
9	0.90	15.2	0.96	59.2	4.29	0.99	29.3	2442.6	6.15

## Track 1:Foeniculum vulgare, Chromatogram at 366 nm



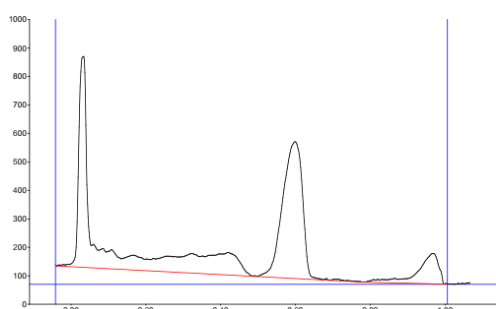
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	2.0	0.03	715.7	54.45	0.10	67.8	16994.5	46.02
2	0.11	61.2	0.14	166.2	12.65	0.17	44.1	4086.3	11.06
3	0.17	44.2	0.20	169.5	12.90	0.24	60.9	5315.3	14.39
4	0.25	60.6	0.28	73.9	5.62	0.29	71.2	1889.0	5.11
5	0.37	74.8	0.42	93.1	7.08	0.47	28.3	5442.4	14.74
6	0.47	28.4	0.48	33.3	2.53	0.52	13.0	930.5	2.52
7	0.52	13.2	0.57	41.1	3.12	0.61	14.7	1656.3	4.48
8	0.78	0.1	0.83	21.7	1.65	0.87	1.9	617.4	1.67

## Track 1:Foeniculum vulgare, Chromatogram after Derivatization



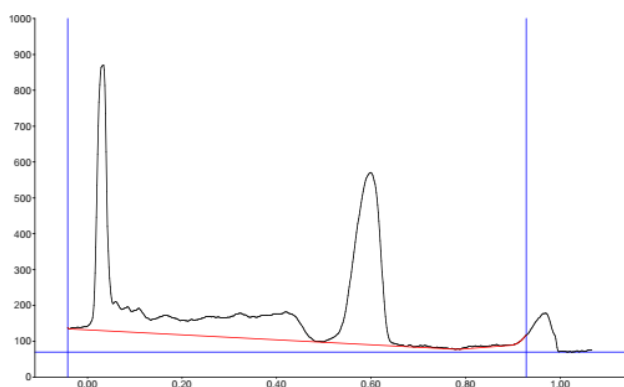
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	1.3	-0.03	13.6	1.07	0.00	0.0	218.6	0.40
2	0.00	0.4	0.03	41.9	3.30	0.05	36.8	1068.0	1.94
3	0.07	34.5	0.12	59.5	4.68	0.13	59.2	2074.2	3.76
4	0.13	58.9	0.15	63.2	4.97	0.20	33.1	2755.8	5.00
5	0.20	33.3	0.28	104.9	8.26	0.31	57.7	4847.8	8.79
6	0.31	57.7	0.33	67.8	5.33	0.35	64.7	1742.9	3.16
7	0.35	64.8	0.39	77.3	6.08	0.41	75.7	3118.7	5.65
8	0.54	75.1	0.64	101.5	7.99	0.65	100.4	7251.6	13.15
9	0.70	106.9	0.86	178.8	14.07	0.87	170.8	16387.0	29.71
10	0.87	171.8	0.90	230.9	18.17	0.94	183.2	9698.2	17.58
11	0.94	184.6	0.96	331.3	26.07	0.99	2.5	5995.9	10.87

## Track 2: Syzygium aromaticum, Chromatogram at 254 nm



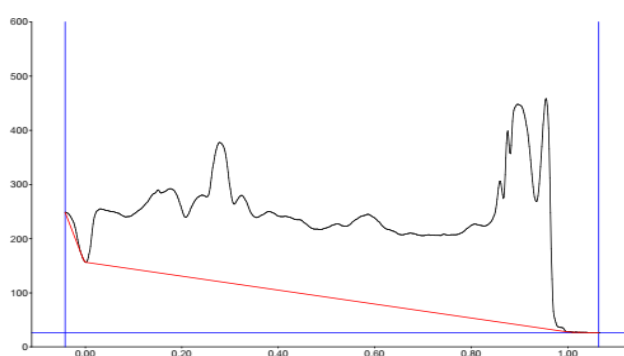
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	5.2	0.03	740.2	44.58	0.06	76.8	12662.7	25.35
2	0.09	57.8	0.11	67.5	4.06	0.13	36.6	1617.1	3.24
3	0.14	37.2	0.17	51.6	3.11	0.20	39.4	2046.6	4.10
4	0.23	43.3	0.26	55.2	3.32	0.27	53.3	1629.5	3.26
5	0.29	54.1	0.32	69.0	4.16	0.34	59.2	2403.8	4.81
6	0.38	67.0	0.42	79.3	4.78	0.49	1.4	3993.6	7.99
7	0.50	1.5	0.60	480.3	28.93	0.66	4.4	21127.8	42.29
8	0.79	0.6	0.83	11.2	0.67	0.85	10.2	347.3	0.70
9	0.90	15.4	0.97	106.0	6.38	1.00	0.2	4128.4	8.26

## Track 2: Syzygium aromaticum, Chromatogram at 366 nm



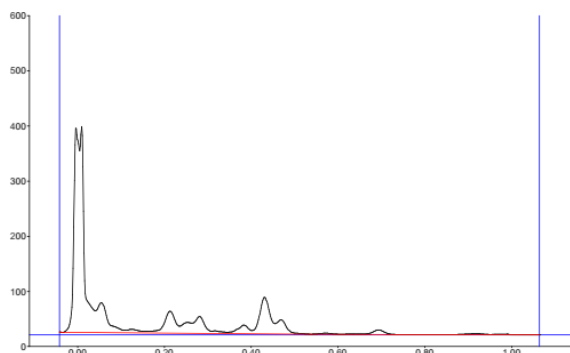
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	5.2	0.03	740.2	47.97	0.06	76.8	12662.7	27.84
2	0.09	57.8	0.11	67.5	4.37	0.13	36.6	1617.1	3.56
3	0.14	37.2	0.17	51.6	3.34	0.20	39.4	2046.6	4.50
4	0.23	43.3	0.26	55.2	3.58	0.27	53.3	1629.5	3.58
5	0.29	54.1	0.32	69.0	4.47	0.34	59.2	2403.8	5.29
6	0.38	67.0	0.42	79.3	5.14	0.49	1.4	3993.6	8.78
7	0.50	1.5	0.60	480.3	31.13	0.66	4.4	21127.8	46.45

## Track 2: Syzygium aromaticum, Chromatogram after Derivatization



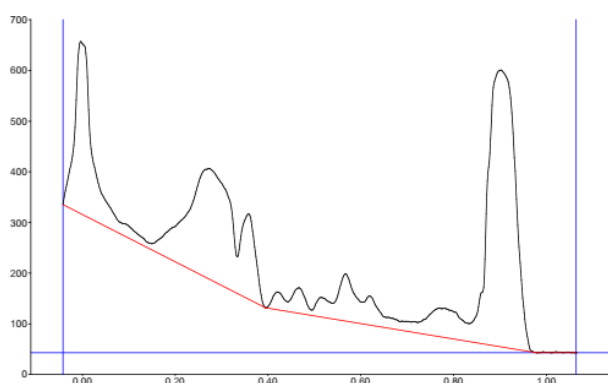
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	13.4	-0.03	22.2	0.77	-0.01	0.3	290.9	0.29
2	0.00	0.2	0.03	102.5	3.58	0.08	94.2	5041.3	5.01
3	0.08	94.4	0.15	152.8	5.35	0.16	148.0	6570.0	6.52
4	0.16	148.5	0.18	158.3	5.54	0.21	109.6	5184.2	5.15
5	0.21	109.7	0.24	154.9	5.42	0.25	153.1	4335.9	4.31
6	0.25	153.1	0.28	256.8	8.98	0.31	146.9	8744.3	8.68
7	0.31	147.6	0.32	164.9	5.77	0.35	128.0	4770.6	4.74
8	0.36	128.1	0.38	142.6	4.99	0.40	136.3	4934.0	4.90
9	0.44	134.8	0.45	135.4	4.74	0.48	122.1	3763.0	3.74
10	0.48	122.9	0.53	138.0	4.83	0.54	135.6	5125.8	5.09
11	0.54	135.6	0.59	163.8	5.73	0.65	134.9	12476.9	12.39
12	0.75	146.8	0.81	174.3	6.10	0.82	173.2	8523.1	8.46
13	0.83	173.2	0.86	260.5	9.11	0.87	229.3	6326.0	6.28
14	0.87	231.7	0.90	407.0	14.24	0.94	232.0	16881.7	16.76
15	0.94	232.3	0.96	425.0	14.86	0.99	6.0	7732.3	7.68

## Track 3: Trachyspermum ammi, Chromatogram at 366 nm



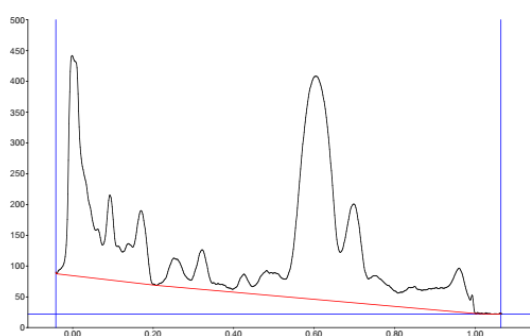
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	0.1	0.01	373.6	61.77	0.04	39.5	7069.0	59.49
2	0.04	39.9	0.06	54.0	8.93	0.11	4.5	1166.3	9.81
3	0.16	2.6	0.21	39.7	6.57	0.24	12.8	877.4	7.38
4	0.26	18.5	0.28	30.5	5.04	0.31	3.8	618.7	5.21
5	0.35	2.3	0.38	15.4	2.55	0.40	5.7	341.1	2.87
6	0.40	5.8	0.43	66.2	10.94	0.46	18.7	1312.3	11.04
7	0.46	18.8	0.47	25.4	4.20	0.54	0.0	497.9	4.19

## Track 3: Trachyspermum ammi, Chromatogram after Derivatization

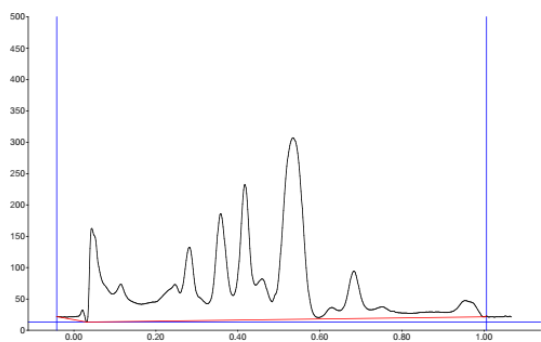


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	36.8	-0.00	339.5	20.84	0.08	22.3	10837.1	15.34
2	0.09	22.4	0.10	25.0	1.54	0.14	10.5	756.6	1.07
3	0.14	10.5	0.28	218.5	13.41	0.33	71.5	16993.3	24.06
4	0.34	74.0	0.36	168.6	10.35	0.39	0.1	4336.3	6.14
5	0.40	0.2	0.42	35.1	2.15	0.44	19.0	748.8	1.06
6	0.44	19.0	0.47	50.5	3.10	0.50	10.4	1220.1	1.73
7	0.50	10.9	0.52	38.6	2.37	0.54	30.5	990.3	1.40
8	0.54	30.8	0.57	93.4	5.73	0.60	42.4	2775.6	3.93
9	0.60	42.5	0.62	57.1	3.50	0.66	20.4	1554.0	2.20
10	0.72	20.6	0.78	57.2	3.51	0.83	35.3	3539.3	5.01
11	0.83	35.4	0.90	545.7	33.49	0.97	1.2	26880.1	38.06

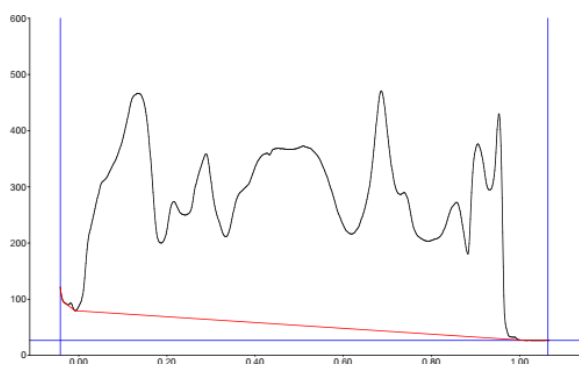
## Track 4: Cinnamomum zeylanicum, Chromatogram at 254 nm



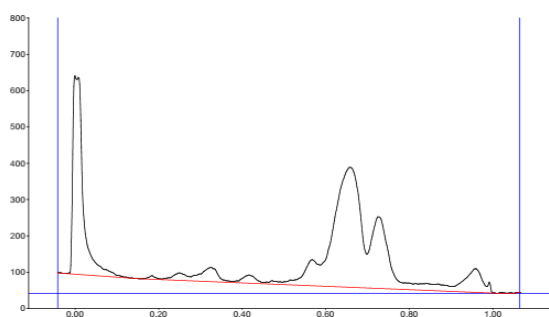
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	6.6	-0.00	357.5	23.32	0.06	77.9	11036.8	20.39
2	0.08	55.2	0.09	137.8	8.99	0.11	55.8	2451.7	4.53
3	0.13	47.9	0.14	62.3	4.06	0.15	57.6	1082.3	2.00
4	0.15	58.3	0.17	118.2	7.71	0.20	0.8	2561.2	4.73
5	0.21	0.1	0.25	46.5	3.04	0.29	14.0	1449.1	2.68
6	0.29	13.3	0.32	64.3	4.19	0.35	11.6	1471.0	2.72
7	0.40	4.5	0.43	30.6	2.00	0.45	11.7	651.9	1.20
8	0.45	12.0	0.48	39.6	2.58	0.49	35.9	836.0	1.54
9	0.53	28.3	0.61	362.4	23.84	0.67	79.9	21844.2	40.36
10	0.67	80.5	0.70	160.3	10.46	0.74	41.0	5477.0	10.12
11	0.74	41.0	0.75	47.0	3.06	0.78	33.0	1461.3	2.70
12	0.82	23.9	0.85	35.3	2.30	0.87	29.7	1105.4	2.04
13	0.91	35.1	0.96	71.1	4.64	0.99	20.9	2689.4	4.97

**Track 4: Cinnamomum zeylanicum, Chromatogram at 366 nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	0.7	0.02	18.4	1.43	0.03	0.3	257.3	0.69
2	0.03	1.1	0.04	149.5	11.64	0.09	44.6	3608.6	9.67
3	0.10	44.6	0.12	59.9	4.66	0.17	27.6	2098.5	5.62
4	0.17	27.7	0.25	58.3	4.54	0.26	44.7	2676.5	7.17
5	0.26	45.0	0.28	117.2	9.13	0.33	26.9	2982.9	7.99
6	0.33	27.0	0.36	170.4	13.27	0.39	39.4	4011.5	10.74
7	0.39	39.7	0.42	216.7	16.87	0.44	55.8	4597.2	12.31
8	0.45	56.0	0.46	65.3	5.08	0.48	28.8	1506.6	4.04
9	0.49	29.5	0.54	289.7	22.55	0.60	2.5	11203.1	30.01
10	0.60	2.6	0.63	18.2	1.42	0.65	13.2	432.6	1.16
11	0.65	13.2	0.68	76.0	5.92	0.73	13.9	2067.5	5.54
12	0.73	14.0	0.75	18.2	1.42	0.82	7.4	853.7	2.29
13	0.91	8.0	0.96	26.5	2.07	1.00	0.0	1038.2	2.78

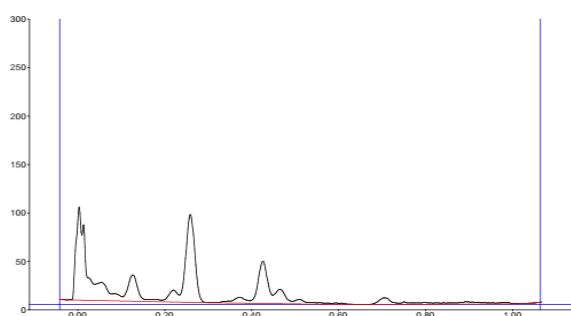
**Track 4: Cinnamomum zeylanicum, Chromatogram after Derivatization**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	0.4	0.13	394.2	11.30	0.19	130.6	33608.9	19.92
2	0.19	130.8	0.22	205.6	5.89	0.24	183.3	6940.4	4.11
3	0.24	183.4	0.29	294.8	8.45	0.33	149.7	14768.9	8.75
4	0.34	150.1	0.43	302.9	8.68	0.43	302.4	16801.1	9.96
5	0.43	300.5	0.45	313.1	8.97	0.47	312.7	7760.0	4.60
6	0.48	312.8	0.51	320.1	9.17	0.62	169.0	27082.2	16.05
7	0.62	169.0	0.69	427.4	12.25	0.73	245.2	22516.2	13.35
8	0.73	245.3	0.74	249.1	7.14	0.79	165.3	9179.0	5.44
9	0.80	165.5	0.86	237.8	6.81	0.88	147.5	12590.0	7.46
10	0.89	152.6	0.91	344.4	9.87	0.93	264.1	10448.4	6.19
11	0.94	264.5	0.96	400.5	11.48	0.98	4.4	7007.6	4.15

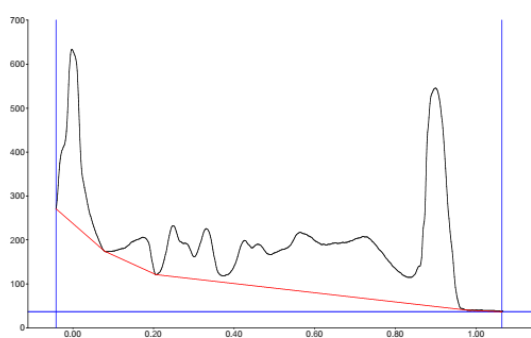
**Track 5, Mahasudarshan Churna, Chromatogram at 254 nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	1.9	0.00	546.6	40.93	0.12	2.2	11287.2	27.03
2	0.16	0.6	0.18	10.3	0.77	0.21	1.9	154.9	0.37
3	0.21	1.9	0.25	20.7	1.55	0.27	12.0	538.7	1.29
4	0.28	11.2	0.33	39.5	2.96	0.36	4.0	1378.2	3.30
5	0.39	5.3	0.42	22.9	1.72	0.45	3.9	594.5	1.42
6	0.52	13.4	0.57	71.8	5.38	0.59	58.0	2086.0	5.00
7	0.59	58.0	0.66	330.6	24.76	0.70	93.6	16643.3	39.85
8	0.70	94.6	0.73	197.4	14.78	0.79	17.8	6647.0	15.92
9	0.90	13.1	0.96	65.3	4.89	0.99	17.2	2257.4	5.41
10	0.99	18.0	0.99	30.4	2.28	1.01	0.1	174.1	0.42

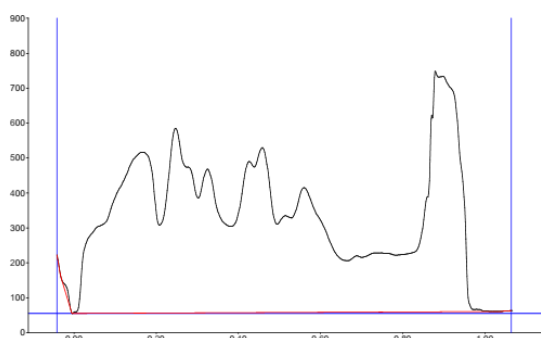


**Track 5, Mahasudarshan Churna, Chromatogram at 366 nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	0.4	0.00	95.9	31.63	0.04	16.6	1686.7	30.82
2	0.04	16.6	0.05	18.8	6.20	0.08	7.0	418.0	7.64
3	0.10	4.3	0.13	26.9	8.89	0.16	1.9	502.7	9.19
4	0.19	1.6	0.22	12.3	4.05	0.23	7.2	219.1	4.00
5	0.24	7.6	0.26	90.6	29.89	0.30	0.0	1557.1	28.45
6	0.40	2.5	0.43	43.6	14.38	0.45	8.6	796.1	14.55
7	0.45	8.8	0.47	15.0	4.95	0.49	2.6	293.0	5.35

**Track 5, Mahasudarshan Churna, Chromatogram after Derivatization at 545 nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	8.9	-0.00	394.4	23.66	0.08	0.1	12738.3	16.75
2	0.08	0.1	0.18	72.1	4.32	0.21	0.3	3225.9	4.24
3	0.21	0.2	0.25	115.7	6.94	0.30	50.6	4536.1	5.97
4	0.30	50.8	0.33	117.6	7.05	0.37	14.7	3571.0	4.70
5	0.38	14.7	0.43	100.7	6.04	0.45	85.9	3126.3	4.11
6	0.45	86.5	0.46	95.6	5.73	0.49	75.4	2864.1	3.77
7	0.49	75.7	0.57	133.8	8.03	0.62	111.6	10069.6	13.24
8	0.66	118.5	0.73	140.6	8.43	0.83	59.3	13993.3	18.40
9	0.84	59.7	0.90	497.0	29.80	0.97	2.6	21911.5	28.82

**Track 5, Mahasudarshan Churna, Chromatogram after Derivatization at 366 nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	0.9	-0.02	26.9	0.58	-0.01	2.8	236.3	0.11
2	0.01	4.5	0.17	460.9	9.89	0.21	252.1	46250.5	21.34
3	0.21	253.0	0.25	528.7	11.35	0.30	328.9	27145.0	12.53
4	0.30	329.3	0.33	411.3	8.83	0.38	247.1	18435.8	8.51
5	0.38	247.1	0.43	432.7	9.29	0.44	416.9	14161.1	6.53
6	0.44	417.0	0.46	472.2	10.13	0.50	252.9	15924.1	7.35
7	0.50	253.4	0.52	277.1	5.95	0.53	270.6	6745.1	3.11
8	0.53	270.9	0.56	357.0	7.66	0.67	147.2	24700.2	11.40
9	0.67	147.3	0.69	161.2	3.46	0.70	156.7	3889.1	1.79
10	0.70	156.8	0.75	169.3	3.63	0.78	162.0	9925.5	4.58
11	0.79	162.3	0.88	687.9	14.77	0.89	671.6	22395.1	10.33
12	0.89	671.9	0.90	673.5	14.46	0.98	5.9	26889.0	12.41

The RF value obtained through the HPTLC analysis is subsequently compared with the established standard value documented in the "Thin Layer Chromatographic Atlas of Ayurvedic Pharmacopoeia Drugs." This comparative assessment ensures the accuracy and consistency of the obtained RF value, validating the identity of the constituent compound in the Ayurvedic herb. The results of this comparison are organized and presented in Table 2,

providing a clear reference for the alignment between experimental findings and the recognized standards in the field of Ayurvedic pharmacopoeia.

<b>Table 2</b>					
<b>1. <i>Foeniculum vulgare</i> (Sauf)</b>					
<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>
<b>254 nm: major spot at Rf:</b>	<b>254 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>After derivatization</b>	<b>After derivatization</b>
0.52	-	<b>0.40</b>	0.42	<b>0.27</b>	0.26
		<b>0.46</b>	0.48	<b>0.40</b>	0.40
		0.60		0.60	
				0.78	
<b>2. <i>Syzygium aromaticum</i> (Laung)</b>					
<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>
<b>254 nm: major spot at Rf:</b>	<b>254 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>After derivatization</b>	<b>After derivatization</b>
<b>0.18</b>	0.20	<b>0.18</b>	0.17	0.12	0.18
0.60		<b>0.60</b>	0.60	<b>0.18</b>	0.53
		0.79		<b>0.52</b>	0.81
				<b>0.82</b>	0.86
				<b>0.86</b>	
<b>3. <i>Trachyspermum ammi</i> (Ajwain)</b>					
<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental Value</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental Value</b>		
<b>366nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>Major spot after Derivatization</b>	<b>Major spot after Derivatization</b>		
0.16	0.38	0.32	0.62		
<b>0.39</b>		<b>0.62</b>	0.90		

0.56		0.91			
0.74					
<b>4. <i>Cinnamomum zeylanicum</i> (Dalchini)</b>					
<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>	<b>Ayurvedic Pharmacopoeia of India</b>	<b>Experimental data</b>
<b>254 nm: major spot at Rf:</b>	<b>254 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>366 nm: major spot at Rf:</b>	<b>After derivatization</b>	<b>After derivatization</b>
<b>0.09</b>	0.09	<b>0.41</b>	0.42	<b>0.22</b>	0.22
<b>0.46</b>	0.48	0.51	0.63	<b>0.29</b>	0.29
0.58	0.96	<b>0.61</b>	0.75	0.58	0.91
<b>0.95</b>		<b>0.75</b>		<b>0.89</b>	

**Discussion:**

The congruence between experimental Rf values and pharmacopoeial references carries noteworthy implications. Firstly, it reaffirms the validity of using Rf values as a reliable tool for compound identification. The fact that Ayurvedic herbs from diverse sources produce matching Rf values supports the reliability of Rf values as indicative markers of specific compounds. This mutual alignment bolsters confidence in utilizing Rf values for rapid identification and quality assessment, particularly in the herbal industry.

Furthermore, the consistent Rf values bridge the traditional and modern realms of herbal medicine. Ayurvedic texts have provided knowledge about herbs for generations, and the matching Rf values provide a tangible link between traditional wisdom and contemporary analytical methods. This synchronization fosters a deeper understanding of Ayurveda, contributing to its recognition as a valuable system of holistic healing.

**Conclusion:**

In the current study four herbal drugs were simultaneously analyzed using HPTLC. The plate development parameter used is in reference with the Thin Layer Chromatographic Atlas of Ayurvedic Pharmacopoeial Drugs and thus the experimental Rf value is compared to the reference value mentioned in Pharmacopoeia. The result corresponds to a characteristic match with the values mentioned in Ayurvedic Pharmacopoeia, which indicate the qualitative analysis of individual herbal drug and polyherbal formulation. The present investigation has been an attempt to document the qualitative HPTLC profiling of raw herbal material that can serve as an important diagnostic tool to evaluate the quality of herbal medicine thereby

saving time and cost of the manufacturer.

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**DECLARATION OF COMPETING INTEREST:**

The authors declare that there are no conflicts of interest

**REFERENCES:**

1. Cragg G M, Newman D J. Natural Product Drug Discovery in the Next Millennium. *Pharmaceutical Biology*. 2001;39:8-17.
2. Singh A. Herbal Medicine Dream Unresolved. *Ethnobotanical Leaflets*. 2007;11:195-98.
3. Kumar A, Yadav S K, Singh S, Pandeya S N. Analgesic activity of ethanolic extract of roots of *Prosopis cineraria*. *Druce. Journal of Applied Pharmaceutical Science*. 2011;01(08): 158-60.
4. Balachandran P, Govindarajan R. Ayurvedic drug discovery. *Expert Opinion. Drug Discovery*. 2007; 2(12):1631-52.
5. Anupam K Sachan, Garima Vishnoi, Roopak Kumar. Need of standardization of herbal medicine in modern era. *International Journal of Phyto medicine*. 2016:300-307.
6. Kunle, Oluyemisi Folashade, Egharevba, Henry Omoregie, Ahmadu, Peter Ochogu. Standardization of herbal medicines- A review. *International Journal of Biodiversity and Conservation*. 2012;4:101-112.
7. Thin Layer Chromatographic Atlas of Ayurvedic Pharmacopoeial Drugs, Part 1, Volume1, First Edition, New Delhi, Ministry of Health and Family Welfare, Department of Ayush, Government of India;2009.