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HPTLC FINGER PRINTING PROFILE OF *ELEPHANTOPUS SCABER* LINN.

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ABSTRACT

Elephantopus scaber Linn. is an important medicinal plant which is used to treat various diseases such as as melancholia, epilepsy, skin diseases, conjunctivitis, mouth ulcers, wounds, dysuria and dysentery. The whole plant is a good source of various bioactive phytochemicals which are responsible for various pharmacological activities such asanti-inflammatory, wound healing, anti-oxidant, diuretic, anti-ulcer, hepato protective, anti-viral, anti-bacterial, memory boosting , anti-anxiety etc. The High Performance Thin Layer Chromatography was carried out in chloroform, methanol and water extract of *Elephantopus scaber* Linn. HPTLC analysis was performed using Toluene: ethyl acetate: formic acid [6: 5: 1 v/v/v] as a mobile phase. HPTLC plate was scanned at 254nm and 366 nm after derivatization showing good partition and banding. Comparisons showed that maximum numbers of chemical constituents were obtained for the methanol extract followed by chloroform and water extracts. Peaks with similar Rf value obtained in various extracts are indicative of similar compounds. But the area of the peaks may differ which shows the difference in affinity of a particular chemical constituent to a solvent.

KEY WORDS: *Elephantopus scaber* Linn, HPTLC finger printing profile

INTRODUCTION

Elephantopus scaber Linn. is a rigid perennial herb found in moist, shady places as a weed. It is having pantropical distribution.[1]It is known as *Anachuvadi* in Malayalam and this name is due to the rosette shaped leaves which resembles elephant foot prints. It is used in various conditions such as melancholia, epilepsy, skin diseases, conjunctivitis, mouth ulcers, wounds, dysuria and dysentery by traditional Ayurvedic physicians of Kerala.[2]Horthus malabricus is a treatise by Van Rheede on the medicinal plants in Malabar area and their uses describes about its use in mental disorders especially depression.[1] Apart from Kerala, plant is utilized widely in various countries such as China, Pakistan, Thailand, Brazil, Malaysia, Mauritius and Srilanka.[3]It is also used as food in various countries such as India, Africa, China and Malaysia.[4] It is having various chemical constituents such as triterpenes, sequeterpene lactones, phytosterols, hydrocarbon compounds, essential oils, phenol and phenolic acids which are responsible for various pharmacological activities such as anti-inflammatory, wound healing, anti-oxidant, diuretic, anti-ulcer, hepato-protective, anti-viral, anti-bacterial, memory boosting , anti-anxiety and anti-cancer activity[5]. Validating traditional claims and bring them into frontline is the need of hour. For scientific validation of a pharmacological activity, first step to ensure the identity and quality of the plant used. HPTLC finger printing is an ideal tool for identification of raw drugs. Chemical constituents can be identified, measured and separated using this technique. Affinity of different chemical constituent towards different solvents may vary. Hence it is important to find out suitable medium for extracting maximum chemical constituents. Here present study is the comparison of HPTLC finger printing profile of chloroform, methanol and water extract of *Elephantopus scaber* Linn. at 254nm and 366nm.

EXPERIMENTAL

Materials used

Choorna of whole plant of *Elephantopus scaber Linn,* Chloroform, water, methanol, toluene, ethyl acetate, formic acid

Procedure

Test solutions were made with 128.054gram of *choorna*(powder)of whole plant of *Elephantopus scaber Linn.*in 100ml methanol and 112.9742gm in 30ml chloroform, 60.9433 in 60ml water and 5µL was applied on the stationary phase. Stationary phase was HPTLC Silica gel 60 F 254, 10 x10 cm aluminium sheet. Mobile phase was Toluene: Ethyl acetate: Formic acid (6:5:1). The plate was visualised by using CAMAG TLC scanner "Scanner-171019" under UV at 254 nm 366 nm after derivatization using anisaldehyde sulphuric acid reagent.

RESULTS

Area and peaks of Chloroform extract at 254nm

Total 12 peaks were obtained for chloroform extract of powder of whole plant at 254 nm. This 12 peaks were defined with max Rf value of 0.02 with area 1292.6AU, max Rf value of 0.02 with area 6706.8 AU, max Rf value of 0.26 with area 513.3AU, max Rf value of 0.29 with area 906.7AU, max Rf value of 0.34 with area 987.5 AU, max Rf value of 0.56 with area 1289AU, max Rf value of 0.60 with area 1249.3AU, max Rf value of 0.64 with area 1853.3AU, max Rf value of 0.71 with area 977.6 AU, max Rf value of 0.78 with area 227.3 AU, max Rf value of 0.84 with area 282.9 AU, max Rf value of 0.95 with area of 7128.5 AU respectively.(Table No 1)

Table No: 1 Area and peaks of chloroform extract of powder of whole plant at 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.03	-0.02	0.00	1292.6	5.52
2	0.00	0.02	0.08	6706.8	28.62
3	0.24	0.26	0.27	513.3	2.19
4	0.28	0.29	0.32	906.7	3.87
5	0.32	0.34	0.40	987.5	4.21
6	0.52	0.56	0.58	1289.0	5.50

7	0.58	0.60	0.62	1249.3	5.33
8	0.62	0.64	0.69	1853.3	7.91
9	0.70	0.71	0.77	997.6	4.26
10	0.77	0.78	0.82	227.3	0.97
11	0.82	0.84	0.86	282.9	1.21
12	0.90	0.95	1.00	7128.5	30.42

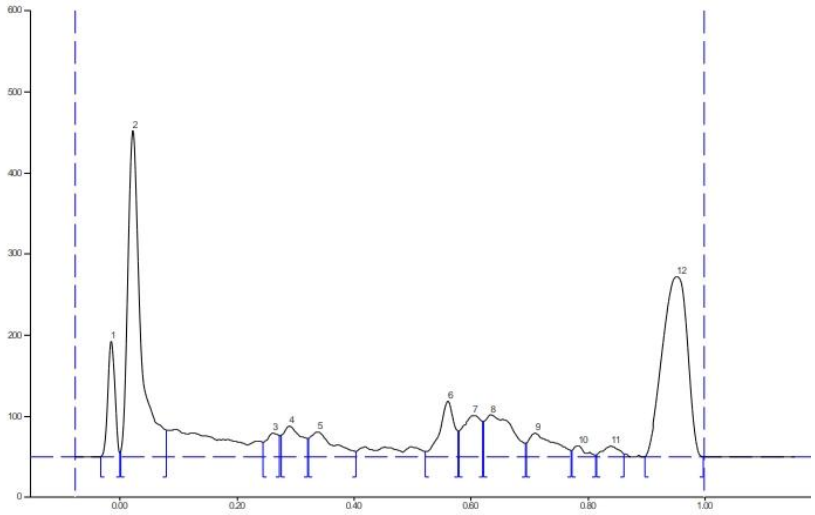


Diagram No: 1 Overview graph of chloroform extract of powder of whole plant at 254nm

Area and peaks of Methanol extract at 254 nm

Total 16 peaks were obtained for methanol extract of powder of whole plant. The peaks were obtained with max Rf value of 0 with area 1292.6, max Rf value of 0.06 with area 21404.9AU, max Rf value of 0.17 with 7549.6 AU, max Rf value of 0.25 with area 3269 AU, max Rf value of 0.36 with 8801.8 AU, max Rf value of 0.42 with area 2481AU, max Rf value 0.47 with area 1528 AU, max Rf value of 0.53 with area 4404.3AU, max Rf value 0.58 with area 4350 Au, max Rf value of 0.61 with area 4877.7, max Rf value of 0.67 with area

1709.7, max Rf value of 0.71 with area 1120.6, max Rf value 0.75 with area 1316.6, max Rf value 0.80 with area 575.2, max Rf value of 0.90 with area 6161, max Rf value of 0.93 with area 6294.4 respectively.(Table No 2)

Table No: 2 Area and peaks of methanol extract of powder of whole plant at 254nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.06	0.00	0.06	27705.8	26.76
2	0.06	0.06	0.16	21404.9	20.67
3	0.16	0.17	0.24	7549.6	7.29
4	0.24	0.25	0.29	3269.0	3.16
5	0.29	0.36	0.39	8801.8	8.50
6	0.41	0.42	0.46	2481.0	2.40
7	0.46	0.47	0.49	1528.0	1.48
8	0.50	0.53	0.55	4404.3	4.25
9	0.56	0.58	0.60	4350.0	4.20
10	0.60	0.61	0.65	4877.7	4.71
11	0.66	0.67	0.69	1709.7	1.65
12	0.69	0.71	0.73	1120.6	1.08
13	0.73	0.75	0.78	1316.6	1.27
14	0.78	0.80	0.82	575.2	0.56
15	0.84	0.90	0.92	6161.0	5.95
16	0.92	0.93	1.00	6294.4	6.08

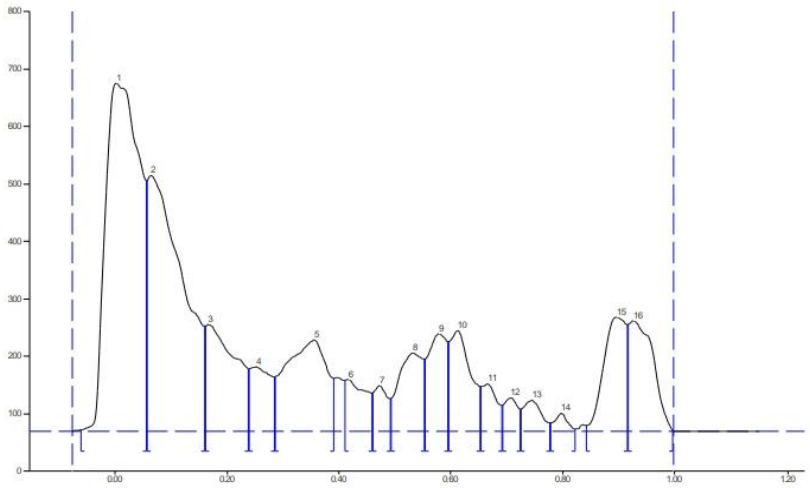


Diagram No: 2 Overview graph of methanol extract of powder of whole plant at 254nm

Area and peaks of water extract at 254nm

At 254nm, water extract of whole plant powder showed 12 peaks. The peaks were obtained with max Rf value of-0.03 with area 1182.5 AU, max Rf value of 0 with area 1999.7 AU, max Rf value 0.14 with area 311.4AU, max Rf value 0.23 with area 547.6AU, max Rf value 0.39 with area 1716 AU, max Rf value of 0.42 with area 2381.8AU, max Rf value of 0.58 with area 4595.4 AU, max Rf value of 0.62 with area 2091.1 AU, max Rf value of 0.72 with area 1371.9AU, max Rf value of 0.76 with area 721.4 AU, max Rf value of 0.83 with area 729.9AU, max Rf value of 0.94 with area 292.5 AU respectively.(Table No 3)

Table No: 3 Area and peaks of water extract of powder of whole plant at254nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.05	-0.03	-0.02	1182.5	6.59
2.	-0.01	0.00	0.04	1999.7	11.14
3.	0.14	0.14	0.19	311.4	1.74
4.	0.20	0.23	0.24	547.6	3.05

5.	0.34	0.39	0.40	1716.0	9.56
6.	0.40	0.42	0.46	2381.8	13.27
7.	0.51	0.58	0.61	4595.4	25.61
8.	0.61	0.62	0.66	2091.1	11.65
9.	0.70	0.72	0.76	1374.9	7.66
10.	0.76	0.76	0.80	721.4	4.02
11.	0.81	0.83	0.87	729.9	4.07
12.	0.93	0.94	0.98	292.5	1.63

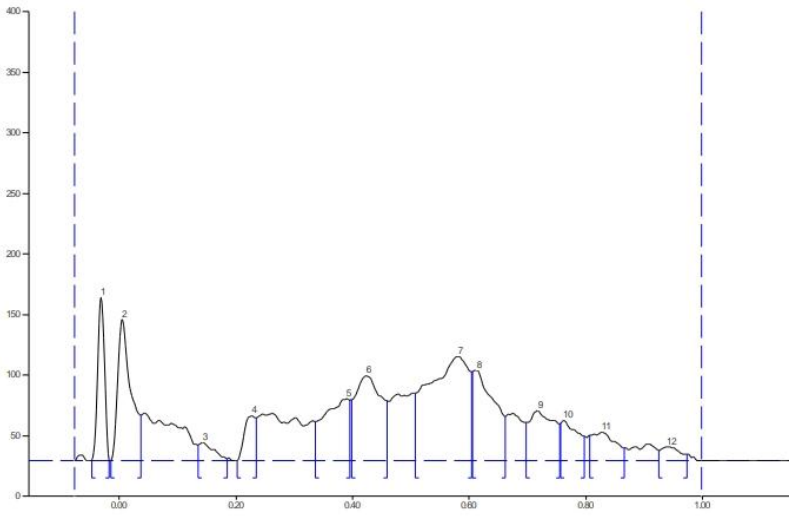


Diagram No: 3 Overview graph of water extract of powder of whole plant at 254nm

Area and peaks of chloroform extract at 366nm

Chloroform extract of whole plant showed 13 peaks at 366nm and these peaks were having max Rf value of -0.05 with area 1021.9AU, max Rf value of 0.00 with area 8540.4AU, max Rf value of 0.08 with area 1558.8AU, max Rf value of 0.24 with area 756.7AU, max Rf value of 0.32 with area 308.1AU, max Rf value of 0.46 with area 275.2AU, max Rf value of

0.52 with area 2013.2AU, max Rf value of 0.58 with area 2995.8AU, max Rf value of 0.63 with 4850.5AU, max Rf value of 0.70 with area 1679.2AU, max Rf value of 0.77 with area 398.5AU, max Rf value of 0.82 with area 697.4AU, max Rf value of 0.90 with area 9421.4AU respectively.(Table No 4)

Table No: 4 Area and peaks of chloroform extract of whole plant powder at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.05	-0.01	0.00	1021.9	2.96
2.	0.00	0.02	0.08	8540.4	24.74
3.	0.08	0.09	0.22	1558.8	4.52
4.	0.24	0.28	0.32	756.7	2.19
5.	0.32	0.34	0.37	308.1	0.89
6.	0.46	0.50	0.52	275.2	0.80
7.	0.52	0.56	0.58	2013.2	5.83
8.	0.58	0.60	0.63	2995.8	8.68
9.	0.63	0.66	0.70	4850.5	14.05
10.	0.70	0.71	0.77	1679.2	4.86
11.	0.77	0.78	0.81	398.5	1.15
12.	0.82	0.85	0.88	697.4	2.02
13.	0.90	0.95	1.00	9421.4	27.30

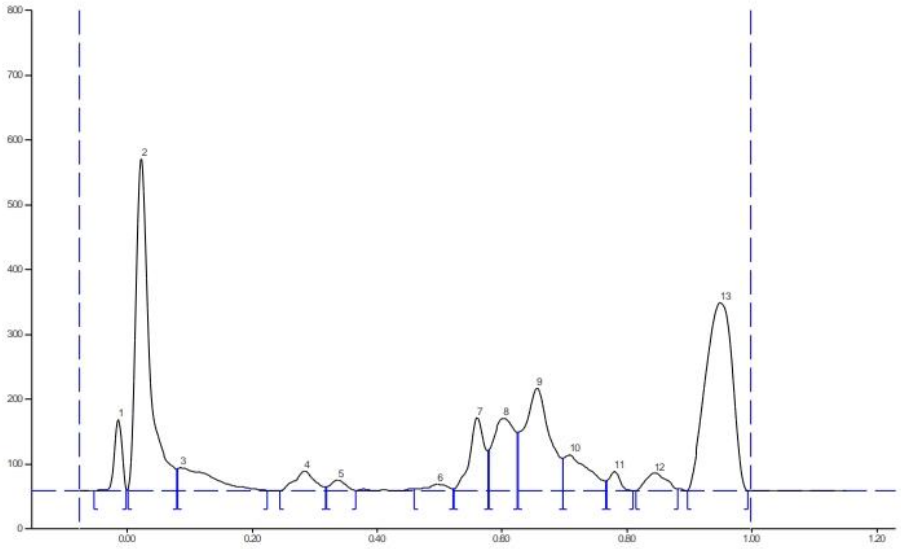


Diagram No: 4 Overview graph of chloroform extract of powder of whole plant at 366nm

Area and peaks of methanol extract at 366nm

Total 13 peaks were obtained in the methanol extract of powder of whole plant at 366nm. These 13 peaks were having max Rf value of -0.06 with area 70490.9 AU, max Rf value of 0.23 with area 6189.2 AU, max Rf value of 0.29 with area 11495.2AU, max Rf value of 0.39 with area 4871AU, max Rf value of 0.45 with area 2856.2AU, max Rf value of 0.49 with area 14216.9AU, max Rf value of 0.60 with area 7581.1AU, max Rf value of 0.66 with area 2431.4AU, max Rf value of 0.69 with area 1639.2AU, max Rf value of 0.73 with area 1284.6AU, max Rf value of 0.78 with area 510.1AU, max Rf value of 0.82 with area 320.5 AU, max Rf value of 0.85 with area 15908.9AU respectively.(Table No 5)

Table No: 5 Area and peaks of methanol extract of whole plant powder at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.06	0.01	0.23	70490.9	50.42
2.	0.23	0.25	0.29	6189.2	4.43

3.	0.29	0.36	0.39	11495.2	8.22
4.	0.39	0.40	0.45	4871.5	3.48
5.	0.45	0.47	0.49	2856.2	2.04
6.	0.49	0.57	0.60	14216.9	10.17
7.	0.60	0.62	0.66	7581.1	5.42
8.	0.66	0.67	0.69	2431.4	1.74
9.	0.69	0.71	0.73	1639.2	1.17
10.	0.73	0.75	0.78	1284.6	0.92
11.	0.78	0.80	0.82	510.1	0.36
12.	0.82	0.84	0.85	320.5	0.23
13.	0.85	0.93	1.00	15908.9	11.38

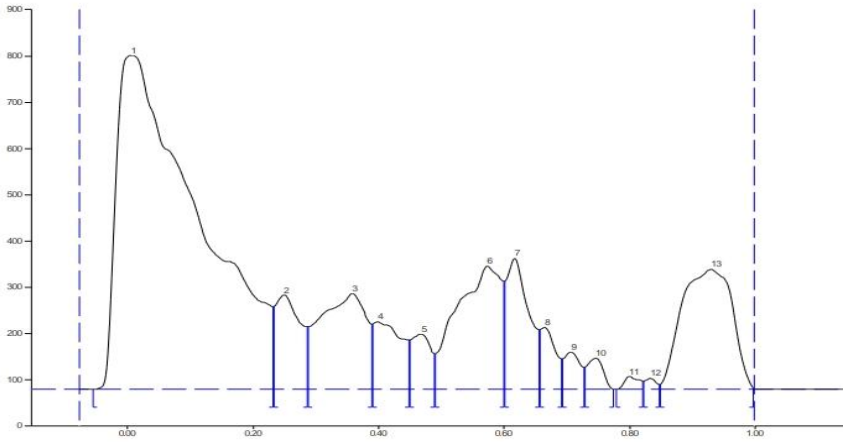


Diagram No: 5 Overview graph of methanol extract of powder of whole plant at 366nm

Area and peaks of water extract at 366nm

At 366nm, water extract showed 12 peaks with max Rf value of -0.03 with area 902.8AU, max Rf value of 0.01 with area 3645AU, max Rf value 0.07 with area 598.1 AU, max Rf value 0.12 with area 994.1Au, max Rf value of 0.22 with area 563.8AU, max Rf value of 0.30 with area 942.9AU, max Rf value of 0.42 with area 5985.7 AU, max Rf value of 0.47 with area 3424.8AU, max Rf value of 0.58 with area 13849.5AU, max Rf value of 0.72 with area 1953.5AU, max Rf value of 0.83 with area 502.3AU, max Rf value of 0.91 with area 343.2AU respectively.(Table No 6)

Table No: 6 Area and peaks of water extracts of whole plant at 366nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.05	-0.03	-0.02	902.8	2.68
2.	-0.01	0.01	0.06	3645.0	10.81
3.	0.06	0.07	0.08	598.1	1.77
4.	0.10	0.12	0.19	994.1	2.95
5.	0.20	0.22	0.24	563.8	1.67
6.	0.28	0.30	0.32	942.9	2.80
7.	0.32	0.42	0.45	5985.7	17.76
8.	0.45	0.47	0.50	3424.8	10.16
9.	0.51	0.58	0.70	13849.5	41.09
10.	0.70	0.72	0.80	1953.5	5.80
11.	0.80	0.83	0.87	502.3	1.49
12.	0.87	0.91	0.93	343.2	1.02

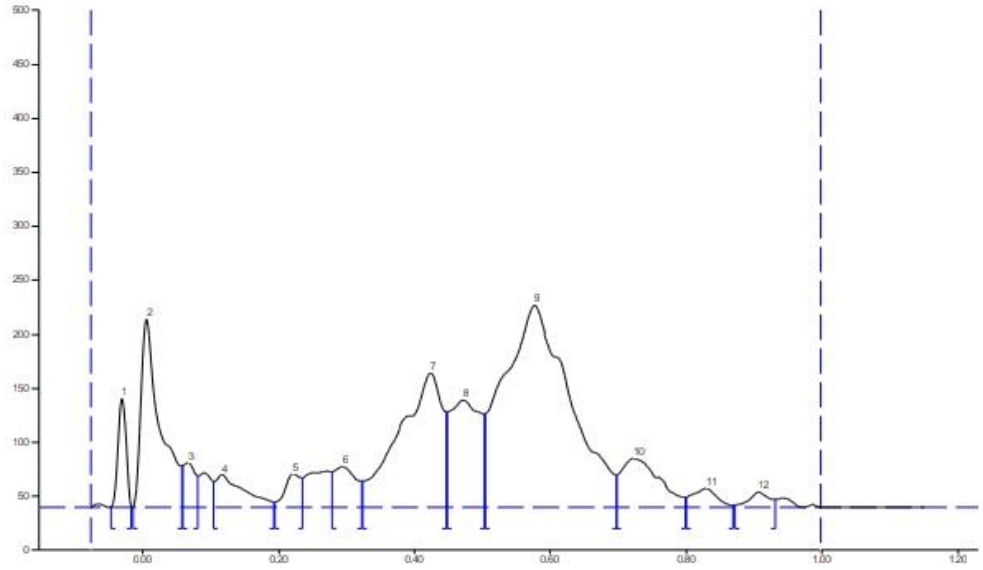
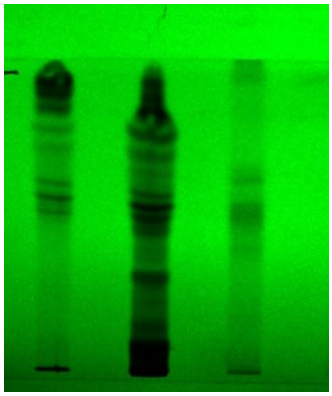
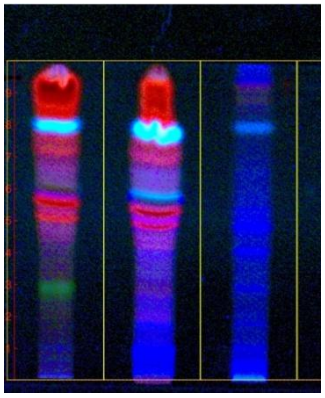


Diagram No: 6 Overview graph of water extract of powder of whole plant at 366nm



Picture No: 1 TLC views of chloroform, methanol, and water extract of whole plant at 254nm



Picture No: 2 TLC views of chloroform, methanol, and water extract of whole plant at 366 nm

DISCUSSION

HPTLC chromatogram of the chloroform, water and methanol extracts of *Elephantopus scaber* Linn at 254nm and 366nm were recorded. The Rf values of the separated compounds of each extracts were noted at 254nm, and 366nm.Each peak indicate presence of a specific chemical constituent.

At 254nm, methanol extract of the drug showed maximum number of peaks (16 peaks), followed by chloroform (12 peaks) and water extracts (12 peaks). Peak intensities were different in different extracts. Peak number 6 was common in methanol extract and water extract with max Rf value of 0.42 with area 2481AU and 2381.8 AU respectively. Chloroform and water extract obtained 8th peak as common with max Rf value of 0.62. But the area of peak was reduced in chloroform extract (area -1853.3AU) while comparing with water extract (area- 2091.1AU). Similar Rf values of different extracts indicate the presence of same chemical constituents. Chloroform, methanol and water extracts were having peaks with max Rf value 0.24, 0.25, 0.23 respectively and methanol extract was having maximum area 3269AU followed by water extract with area 547.6 AU and chloroform extract with area 513.3AU. Peak with max Rf value 0.52 was found in the chloroform extract with area 1289AU whereas peak with max Rf value of 0.53 was present in the methanol extract with area 4404.3AU. Peak with max Rf value 0.58 was present in both chloroform and methanol extract and peak obtained in the methanol extract was having more area (area- 4350AU) while comparing with that of chloroform extract (area-1249.3AU). Peaks with similar max Rf values such as 0.70, 0.71 and 0.72 were obtained in chloroform, methanol and water extract with area 997.6AU, 1120.6AU and 1374.9AU respectively. Chloroform and water extracts were having 10th peak with similar max Rf such as 0.77 and 0.76 with area 227.3AU and 721.4AU respectively. Chloroform and water extracts obtained 11th peak with max Rf values 0.82 and 0.83 respectively and water extract was having more peak area (area-729.9AU) while comparing with that of chloroform extract(area- 282.9AU). Both chloroform and methanol extract obtained peak with max Rf value of 0.90 and chloroform extract was having more peak area (7128.5AU) than methanol extract (6161AU). Peaks with similar max Rf values such as 0.93 and 0.94 were present in the methanol and water

extract and area was less in water extract (292.5AU) when compared with methanol extract (6294.4AU).

At 366 nm, methanol and chloroform extract was having maximum number of peaks (13 peaks) followed by aqueous extract (12 peaks). 12th peak present in the chloroform and methanol extract were common with max Rf value of 0.82 and peak area of chloroform extract (697.4AU) was higher than that of methanol extract (320.5AU). Chloroform extract was having more area (1558.8AU) of 3rd peak with max Rf 0.08 while comparing with 3rd peak of water extract (598.1AU) with max Rf value 0.07. Water and chloroform extracts obtained peaks with similar max Rf values such as 0.22 with area 563.8AU and 0.24 with area 756.7AU respectively. Peaks with max Rf value 0.29 with area 11495.2 AU was obtained in methanol extract whereas peaks with max Rf value 0.30 with area 942.9 AU obtained for water extract. Methanol extract was having peak with max Rf 0.45 with area 2856.2 AU whereas chloroform extract was having peak with max Rf 0.46 with area 275.2 AU. Chloroform extract and water extract were having peaks with max Rf 0.58 with area 2995.8 AU and 13849.5 AU respectively whereas methanol extract was having peak with max Rf 0.60 with area 7581.1 AU. All the three extracts were having 10th peak with similar max Rf values such as 0.70, 0.73, 0.72 for chloroform, methanol and water extracts respectively and water extract was having more peak area (1953.5AU) followed by chloroform extract(1679.2AU) and methanol extract(1284.6AU). Chloroform and methanol extracts obtained their 11th peaks with similar max Rf values such as 0.77 with area 398.5AU and 0.78 with area 510.1AU respectively. Chloroform and water extracts were having peaks with similar max Rf values such as 0.90 with area 9421.4 AU and 0.91 with area 343.2 AU respectively.

CONCLUSION

Maximum numbers of chemical constituents were found in methanol extract followed by chloroform and water extract at 254nm and 336nm.. Peaks with similar Rf value obtained in various extracts are indicative of similar compounds. But the area of the peaks may differ which shows the difference in affinity of a particular chemical constituent to a solvent. HPTLC finger printing serves a tool to identify raw drugs and prevent

adulteration. By comparing HPTLC chromatogram of various extracts, the most suitable solvent can be identified.

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CONFLICTS OF INTEREST

There are no conflicts of interest associated with this publication.

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