AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

A COMPARATIVE ANALYSIS OF *ABHAVITHA* AND *BHAVITHA CHOORNA* OF DRIED WHOLE PLANT OF *MANDUKAPARNI - CENTELLA ASIATICA* (LINN.) URBAN. THROUGH HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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Article info

Article History:
Received: 04-03-2023
Revised: 24-03-2023
Accepted: 14-04-2023

KEYWORDS:
High Performance Thin Layer Chromato-graphy, *Choorna*, *Mandukaparni, Centella asiatica* (Linn.) Urban.

ABSTRACT

HPTLC (High Performance Thin Layer Chromatography) is a powerful analytical technique in the process of drug discovery and evaluation. Due to its dependability and simplicity, it is used as a tool for the identification, authentication, and quality control of herbal drugs. *Mandukaparni- Centella asiatica* (Linn.) Urban. is a significant and well-known drug expound discussed in the Ayurvedic classics. It is a prostrate, stoloniferous herb with enriched therapeutic potential. The herb is rich in terpinoids, flavonoids, iso-flavonoids, alkanes tannins, saponins, aminoacids and phenylpropanoids, which accounts for its multiple beneficial action in the therapeutics. It has strong anti-inflammatory and antioxidant properties. The *Abhavitha choorna* (dried powder) and *Bhavitha choorna* (powder soaked in *Mandukaparni swarasa*) of dried whole plant of *Mandukaparni- Centella asiatica* (Linn.) Urban. was subjected to High performance thin layer chromatography and comparative analysis of different peaks in both the *Choornas* were obtained. The results suggest that change in concentration of phytochemical constituents occur on *Abhavitha choorna* (powder) to *Bhavitha* (soaked in *Mandukaparni swarasa*).

INTRODUCTION

For the qualitative, quantitative, and semi-quantitative analysis of herbal drugs, one of the most dependable and highly sophisticated analytical techniques is HPTLC (High Performance Thin Layer Chromatography). Due to its consistency and dependability, it acts as the most effective quality control for herbal medicine and is therefore regarded as a tool for identification, authentication, and quality control. It functions according to the idea of absorption. To check for adulteration and guarantee drug purity, HPTLC can be used. The number of peaks indicates the presence of a particular phyto-constituents, and the peak's area indicates how much of that phytoconstituents is present in the sample.[1]

Access this article online	
Quick Response Code	
	https://doi.org/10.47070/ayushdhara.v10i2.1172
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Mandukaparni, botanically identified as *Centella asiatica* (Linn.) Urban.[2] Acharya Charaka has included the drug '*Mandukaparni'* under *Vayasthapana Mahakasaya.*[3] Phytoconstituents such as flavonoids like quercetin and rutin, flavonoids, iso flavonoids, cardiac glycosides, pterocarpans, saponins, phenols, tannins, ascorbic acid, triterpinoids and vitamins like nicotinic acid are seen in the different plant parts of the drug.[2,4,5] Previous research works suggests that the plant possess hypouricemic,[6] anti-arthritic,[7] anti-inflammatory and antioxidant activities.[8] The oral administration of the drug in the form of *Choorna* (powder) and *Swarasa* (freshly extracted juice) has been mentioned in the Ayurvedic classics.[2,9] The drug when totally dried, powdered and filtered is called *Choorna Kalpana* (powder) and is widely utilized in therapeutics. The *Choorna* (powder) is a modified form of *Kalka kalpana* (paste obtained after grinding the drug). *Swarasa* is the juice extracted from the fresh *Kalka* (paste) of the drug. The whole drug powder was processed three times under *Bhavana* process by soaking it into *Swarasa* of the same drug to potentiate

the drug in its chemical constituents and therapeutic efficacy. The HPTLC technique was employed to compare the peaks obtained for *Abhavitha choorna* (powder) and *Bhavitha choorna* (powder underwent the *Bhavana* process) of the whole plant of *Centella asiatica* (Linn.) Urban. using same solvent, mobile phase and stationary phase. The present study aims at comparing the peak obtained from HPTLC chromatogram of *Abhavitha choorna* (powder) and *Bhavitha choorna* (powder underwent the *Bhavana* process) of the whole plant of the drug *Mandukaparni -Centella asiatica* (Linn.) Urban.

MATERIALS AND METHODS

Preparation of *Choorna* (powder)

Mandukaparni -Centella asiatica (Linn.) Urban. was collected from Puthiyakavu village, Ernakulam district. Bulk of the fresh whole plant of the drug was visually inspected for foreign matter and sorted. Then it was washed with water thoroughly to remove physical impurities like soil, mud etc. The fresh drug was shade dried (to avoid the loss of volatile oil content). After proper drying it was chopped into small pieces. It was then made into fine powder using a pulverizer and sieved through mesh with size-120. The finely powdered drug was stored in an air tight container.

Preparation of *Bhavitha choorna* (processed powder)

Bhavitha choorna (processed powder) of whole plant of *Mandukaparni -Centella asiatica* (Linn.) Urban. was prepared according to the reference of *Bhavana vidhi* mentioned in *Bhaishajya Ratnavali.*[10] The *Choorna* (powder) of the drug was taken in a wide tray. It was spread uniformly in the tray so that it forms a thin layer. The freshly extracted *Swarasa* (juice) of the drug was then gradually poured into the fine powder so that the *Swarasa* gets absorbed into the powder. Using a sharp thin rod, it was ensured that each fine particle of the *Choorna* gets completely soaked in the *Swarasa*. Thus pouring of *Swarasa* was continued until a thin layer of *Swarasa* was seen on the surface of the drug. To ensure uniform spreading of *Bhavana dravya* in the fine particles of the powder the tray was slowly and uniformly shake on both sides to check the particles were moving uniformly on both sides and then kept it overnight. It was covered with a clean thin cloth to avoid dust or any other contamination from the external environment. On the next day morning the tray was kept under sunlight at the moderate temperature between 20 to 35°Celsius. When the top layer of the *Choorna* was completely dried, it was mixed with a thin sharp rod for uniform drying of all the areas of the fine powder and ensured that there was no contamination. The properly dried powder was then made into fine powder and sieved through the mesh size 120. Likewise the dried powder obtained after each *Bhavana* was finely powdered. Thus the entire process of *Bhavana* was repeated for three times (to restrict the symptoms like- drowsiness etc. as reported by previous studies). The powdered drug was stored in air tight containers. Figure 1 & Figure 2: *Abhavitha choorna* (Unprocessed *Choorna*), *Bhavitha choorna* (processed powder)

PROCEDURE

Test solutions were made with 2gm of *Abhavitha choorna* (powder) and 2gm of *Bhavitha choorna* (processed powder) of dried whole plant of *Mandukaparni- Centella asiatica* (Linn.) Urban. extracted in 5ml methanol and 8µL was applied on the stationary phase. Stationary phase was HPTLC Silica gel 60F 254, 5.0x10.0cm aluminium sheet. Mobile phase was Toluene: Ethyl acetate: Formic acid (6:2:1). Plates were then dried at 60°C for 5 minutes and transferred to CAMAG TLC scanner (Scanner-171019) under UV 254nm and 366nm. Post chromatographic derivatization was done using Vanillin - Sulphuric acid 100ml and the plates were dried using oven at 120°C for 20 minutes. Densitometry was done using slit dimension of 8.00×0.0.90mm, macro and the scanning speed was 20mm/seconds.

Table 1: Area and peaks of methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm wavelength

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.08	-0.07	-0.05	154.9	0.85
2	-0.05	-0.02	0.08	5746.3	31.63
3	0.18	0.28	0.29	2478.7	13.64
4	0.30	0.31	0.38	1987.9	10.94
5	0.69	0.73	0.79	1495.7	8.23
6	0.81	0.87	0.91	2950.3	16.24
7	0.91	0.94	1.05	3354.0	18.46

Table 2: Area and peaks of methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 366nm wavelength

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.05	-0.02	0.08	4467.7	8.23
2	0.13	0.16	0.19	1322.7	2.44
3	0.19	0.21	0.22	685.4	1.26
4	0.22	0.28	0.37	6149.9	11.32
5	0.37	0.40	0.43	941.9	1.73
6	0.43	0.47	0.48	992.1	1.83
7	0.49	0.54	0.59	3292.9	6.06
8	0.59	0.69	0.76	5734.4	10.56
9	0.76	0.80	0.84	2219.9	4.09
10	0.84	0.92	0.99	27361.9	50.38
11	0.96	0.99	1.05	1140.1	2.10

Table 3: Area and peaks of methanol extract of dried whole plant powder (*Bhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm wavelength

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.05	-0.02	0.05	5941.7	21.16
2	0.18	0.33	0.38	6040.4	21.51
3	0.43	0.49	0.51	3308.7	11.78
4	0.52	0.53	0.57	1984.9	7.07
5	0.76	0.83	0.87	4392.9	15.65
6	0.87	0.92	0.94	2272.4	8.09
7	0.94	0.99	1.05	4134.9	14.73

Table 4: Area and peaks of methanol extract of dried whole plant powder (*Bhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 366nm wavelength

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.06	-0.02	0.08	6540.9	7.00
2	0.21	0.28	0.33	4830.7	5.17
3	0.38	0.44	0.45	2548.6	2.73
4	0.45	0.50	0.53	5769.0	6.18
5	0.53	0.62	0.68	10752.3	11.51
6	0.68	0.71	0.75	4959.0	5.31
7	0.78	0.83	0.86	14129.1	15.13
8	0.86	0.90	0.92	7046.4	7.54
9	0.92	0.98	0.97	13708.3	14.75
10	0.97	0.99	1.08	23113.6	24.75

Figure No.1: *Abhavitha choorna* **Figure No.2: *Bhavitha choorna***

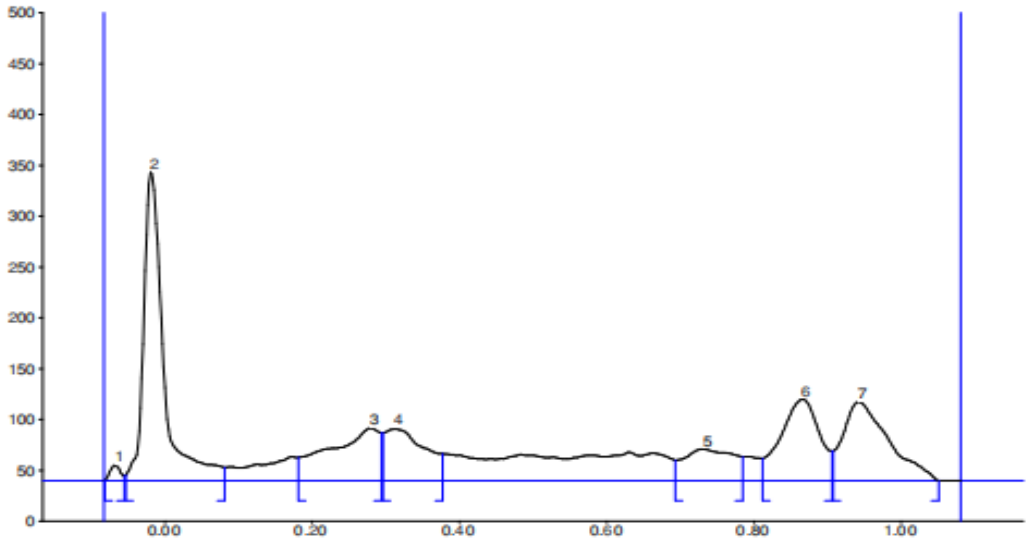

Figure 3: Overview graph of methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm

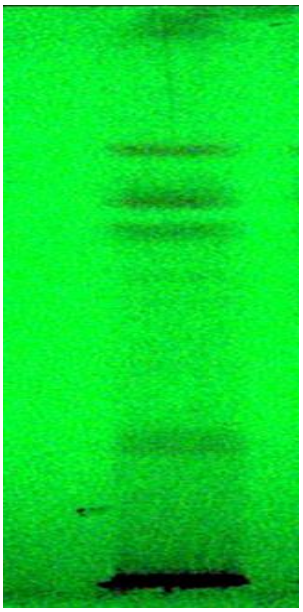

Figure 4: TLC views of methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm

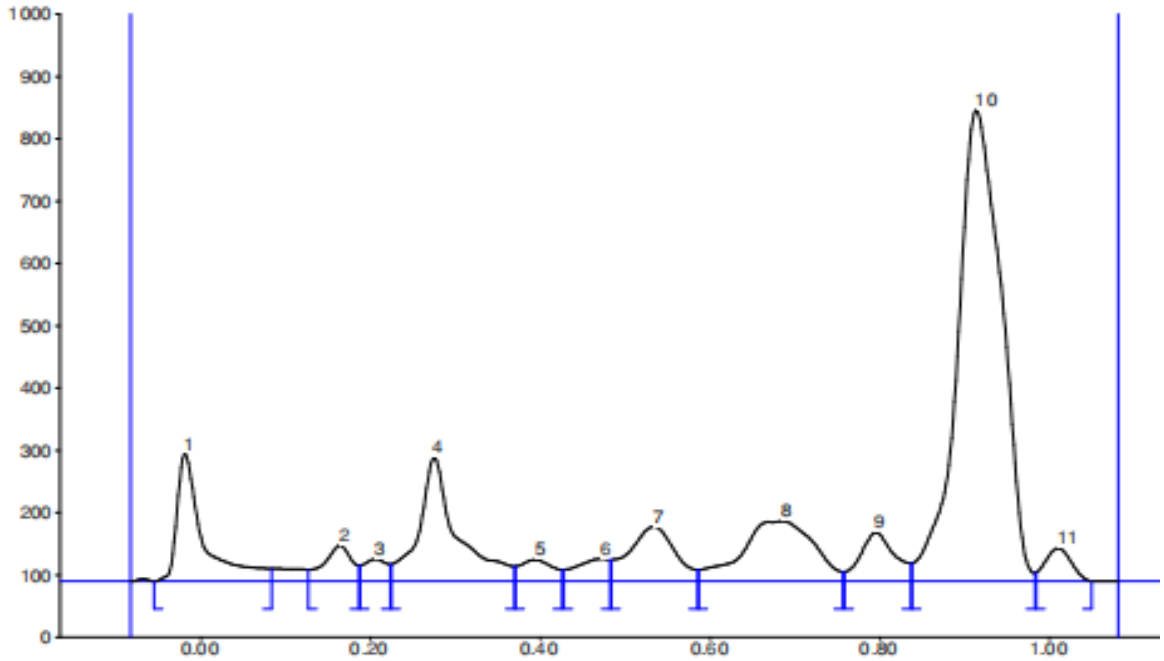

Figure 5: Overview graph of methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 366nm

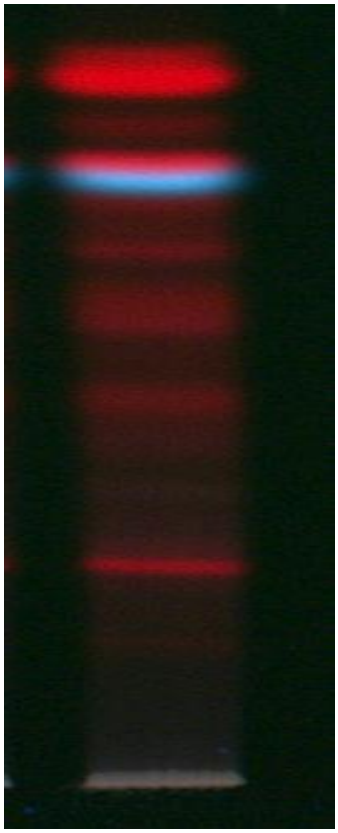

Figure 6: TLC views of methanol extract of *Abhavitha choorna* (unprocessed powder*)* of dried whole of *Centella asiatica* (Linn.) Urban. at 366nm

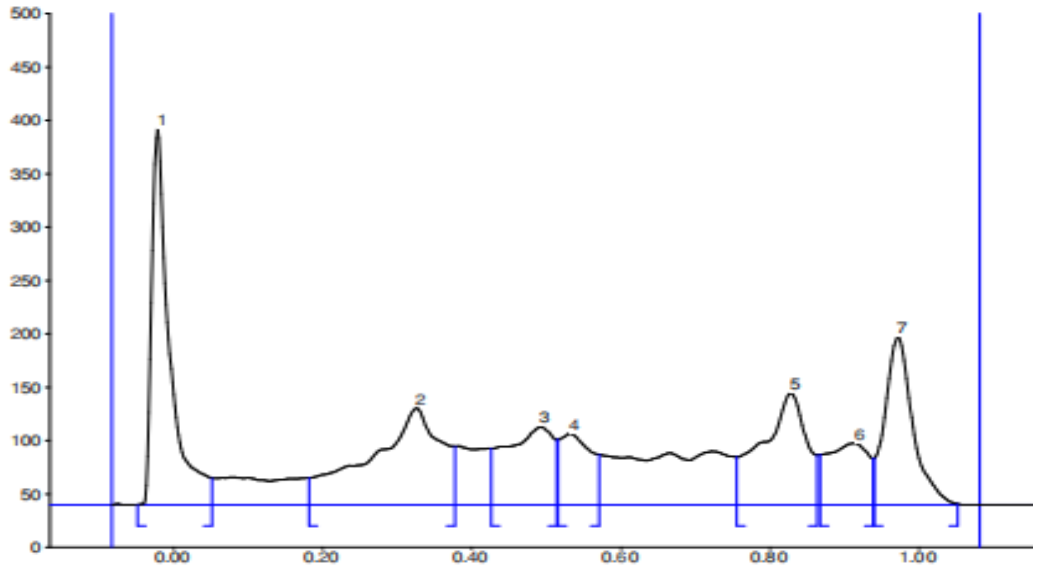

Figure 7: Overview graph of methanol extract of dried whole plant powder (*Bhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm

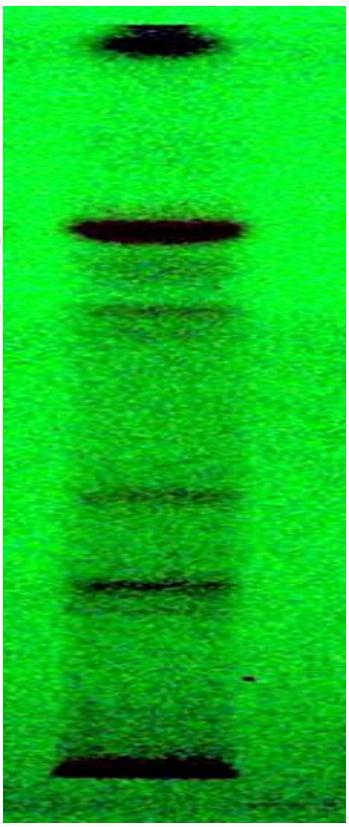

Figure 8: TLC views of methanol extract of *Bhavitha choorna* (unprocessed powder) of dried whole of *Centella asiatica* (Linn.) Urban. at 254nm

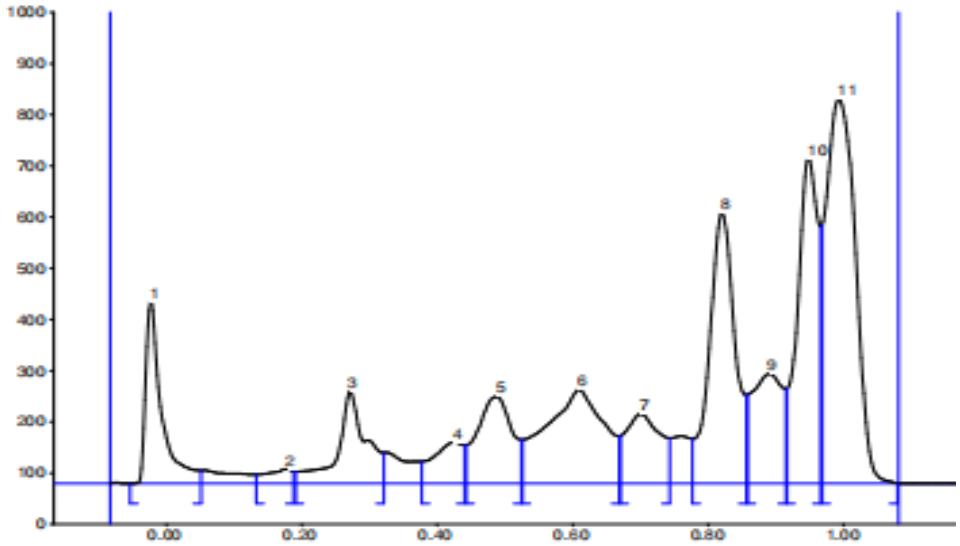

Figure 9: Overview graph of methanol extract of dried whole plant powder (*Bhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 366nm

Figure 10: TLC plate view of *Bhavitha choorna* of the whole plant of *Mandukaparni* (*Centella asiatica* Linn. Urban) at 366nm	**Figure 11: TLC plate view of *Bhavitha choorna* of the whole plant of *Mandukaparni* (*Centella asiatica* Linn. Urban) after derivatisation**

RESULT

A. HPTLC finger printing profile of whole plant powder (*Abhavitha choorna*) of *Mandukaparni-Centella asiatica* (Linn.) Urban.

i. Area and peaks of Methanol extract at 254nm (Table-1, Figure 3 & 4)

Total 7 peaks were obtained for methanol extract of dried whole plant powder (*Abhavitha choorna*) of *Centella asiatica* (Linn.) Urban. at 254nm wavelength with the total area of 18167.8 AU. These 7 peaks were defined with max. Rf value of -0.07 with area 154.9 AU, max. Rf value of -0.02 with area 5746.3 AU, max. Rf value of 0.28 with area 2478.7 AU, max. Rf value of 0.31 with area 1987.9 AU, max. Rf value of 0.73 with area 1495.7 AU, max. Rf value of 0.89 with area 2950.3 AU and the last max. Rf value of 0.94 with area 3354.0 AU respectively, tabulated in table no.1.

ii. Area and peaks of Methanol extract at 366 nm (Table-2, Figure -5 & 6)

At 366nm wavelength *Abhavitha choorna* of the drug showed 11 peaks with total area of 54308.9 AU. These 11 peaks were defined with max. Rf value of -0.03 with area 4467.7.0 AU, max. Rf value of 0.17 with area 1322.7 AU, max. Rf value of 0.21 with area 685.4 AU, max. Rf value of 0.28 with area 6149.9 AU, max Rf. value of 0.40 with area 941.9 AU and max Rf. value of 0.47 with area 992.1 AU, max. Rf value of 0.54 with area 3292.9 AU, max. Rf value of 0.69 with area 5734.4 AU and max. Rf value of 0.80 with area 2219.9 AU, max. Rf value of 0.92 with area 27361.9 AU and max. Rf value of 1.01 with area 1140.1 AU respectively, tabulated in table no.2.

B. HPTLC finger printing profile of *Bhavitha choorna* (processed powder) of whole plant powder of *Mandukaparni - Centella asiatica* (Linn.) Urban

i. Area and peaks of Methanol extract at 254nm (Table-3, Figure 7 & 8)

At 254nm wavelength 7 peaks with total area 28075.9 AU were obtained for *Bhavitha choorna*. These 7 peaks were defined with max. Rf value of -0.02 with area 5941.7 AU, maxi. Rf value of 0.33 with area 6040.4AU, max. Rf value of 0.49 with area 3308.7 AU, max. Rf value of 0.53 with area 1984.9 AU, max. Rf value of 0.83 with area 4392.9 AU, max. Rf value of 0.92 with area 2272.4 AU and max. Rf value of 0.99 with area 4134.9 AU respectively, which are tabulated in table no.3.

ii. Area and peaks of Methanol extract at 366 nm (Table-4, Figure 9, 10 & 11)

At 366nm wavelength 11 peaks with total area 93397.9 AU were obtained for *Bhavitha choorna* of the drug. These 7 peaks were defined with max. Rf value of -0.02 with area 6540.9AU, max. Rf value of 0.28 with area 4830.7AU, max. Rf value of 0.44 with area 2548.6 AU, max. Rf value of 0.50 with area 5769.0 AU, max. Rf value of 0.62 with area 10752.3 AU, max. Rf value of 0.71 with area 4959.0 AU and max. Rf value of 0.83 with area 14129.1AU, max. Rf value of 0.90 with area 7046.4AU and max. Rf value of 0.98 with area 13708.3 AU and max. Rf value of 0.99 with area 23113.6 AU respectively, tabulated in table no.4.

DISCUSSION

HPTLC finger printing profile for *Abhavitha* and *Bhavitha choorna* of whole plant of *Mandukaparni - Centella asiatica* (Linn.) Urban. was performed at 254nm and 366nm wavelengths. Peaks of different compounds were obtained at different max. Rf values. At 254nm wavelength 7 individual peaks were obtained for both the *Choorna* with total 18167.8 AU and 28075.9AU area respectively. Peak intensities were different but a common peak at same max. Rf value -0.02 was obtained in both the *choornas* with 5746AU and 5980.3AU area respectively. Among 7 peaks, 2 peaks found comparable between both the *Choornas,* one peak at max. Rf value 0.31 in *Abhavitha choorna* was comparable with peak at Rf value 0.32 of *Bhavitha choorna* with area of 1987.9AU and 5245.1AU respectively and second peak at max. Rf value 0.94 in *Abhavitha choorna* was comparable with peak at Rf value 0.96 of *Bhavitha choorna* with area of 3354.0AU and 4173.1AU respectively. Area increased in *Bhavitha choorna* revealed increased compound concentration after *Bhavana* process done in it.

At 366nm wavelength, as an average of 3 TLC tracks 11 individual peaks were obtained in both *Abhavitha* and *Bhavitha choorna* indicating that no any new chemical compound found in the *Bhavitha choorna* extra than *Abhavitha choorna.* Among 11 peaks, 3 peaks at -0.02, 0.28 and 0.99 max. Rf were noted common with area of 4467.7AU and 6540.9AU, 6149.9AU in *Abhavitha choorna* and 4830.7AU, 1140.1AU and 23113.6AU in *Bhavitha choorna* respectively. More area in case of *Bhavitha choorna* suggests increased concentration of compounds identified at max. Rf value -0.02 and 1.0 where as less area showed decreased concentration of the compound identified at max. Rf value 0.28. Furthermore, 3 comparable peaks were also noted among both the *Choornas.* One peak at max. Rf value 0.80 in *Abhavitha choorna* was comparable to the peak at max. Rf value 0.83 in *Bhavitha choorna* with an area of 2219.9AU and 14129.1AU respectively showing increased concentration of particular compound in *Bhavitha choorna*. Also peaks obtained at max. Rf values 0.69 and 0.92 for *Abhavitha choorna* was comparable with corresponding peak at max. Rf values 0.71 and 0.95 for *Bhavitha choorna* with an area of 5734.4AU, 4959AU (*Abhavitha*) and 27361.9AU, 13708.3AU (*Bhavitha*) respectively. Here drop in peak areas showed impaired concentration of respective compound in *Bhavitha choorna.* Total area of peaks was also observed more in case of *Bhavitha choorna* of the drug revealing that the concentration of phytoconstituents has been increased by the *Bhavana* process which uphold that potency and efficacy of the drug can be increased by the *Bhavana* process.

A previous research work on quantitative estimation of asiatic acid, asiaticoside & madecassoside in two accessions of *Centella asiatica*[11] by Gupta Abhishek et.al. 2014 has reported identification of asiatic acid, asiaticoside & made cassoside at maximum refrective index 0.98, 0.99 and 0.99 subsequently through their HPTLC study. In present study, increased

concentration of chemical constituents of respective same maximum refractive index (0.98, 0.99 and 0.99) in *Bhavitha choorna* supported the enhancement in pharmacological potential of the drug after *Bhavana process* by increasing chemical concentration of its main active phytoconstituents.

CONCLUSION

HPTLC profiling of *Abhavitha choorna* (plain powder) and *Bhavitha choorna* (processed powder) of *Mandukaparni- Centella asiatica* (Linn.) Urban. was done and comparison of their peaks were discussed. The number of peaks obtained for *Bhavitha choorna* (processed powder) was equal when compared to *Abhavitha choorna* (powder) of the drug at 254nm (7 peaks) and also in case of and 366nm wavelength (11 peaks), indicates same number of phytoconstituents in both the *Choorna*. Respective area and total area in case of *Bhavitha choorna* found more than *Abhavitha choorna* of the drug at 254nm and 366nm wavelength indicates that the concentration of phytoconstituents has been increased by the process of *Bhavana* which substantiate that *Bhavana* process can increase the pharmacological potency and therapeutic efficacy of the drug.

Acknowledgement

I express my sincere gratitude to Dr.T.D Sreekumar, Principal, Government Ayurveda College Tripunithura. I would also like to thank my teachers, Dr.Jilu Joy (Assistant Professor) and Dr.Honey Thomas (Assistant Professor), Department of Dravyaguna Vijnana, Government Ayurveda College Tripunithura, for their constant encouragement and help with the assignment. I also want to thank the entire staff at CARe KERALAM Ltd. in Thrissur for their assistance in getting this study done.

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Cite this article as:
Priyanka Narang, P Y Ansary, Sara Monsy Oommen, Shincymol V V, Honey Thomas. A Comparative Analysis of Abhavitha and Bhavitha Choorna of Dried Whole Plant of Mandukaparni - Centella Asiatica (Linn.) Urban. Through High Performance Thin Layer Chromatography. AYUSHDHARA, 2023;10(2):68-76.
https://doi.org/10.47070/ayushdhara.v10i2.1172
Source of support: Nil, Conflict of interest: None Declared

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