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A Comparative Analysis of *Choorna* and *Bhavitha Choorna* of Dried Rhizome of *Haridra* (*Curcuma Longa* Linn.) Through High Performance Thin Layer Chromatography

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ABSTRACT:

The drug *Haridra* (*Curcuma longa* Linn.) has been extensively used as medicine and strongly relates to the socio-cultural life of people. The rhizome of the plant is rich in phytoconstituents which are responsible for the pharmacological actions of the drug. HPTLC serve as a potent tool for identification, authentication and quality control of herbal medicines in order to get the genuine drug. The present study aimed to compare the *choorna* (powder) and *bhavitha choorna* (processed powder) of the methanol extract of the dried rhizome of *Haridra* (*Curcuma longa* Linn.) through high-performance thin layer chromatography. HPTLC plates Silica gel 60 F 254, 4.0 x 9.0 cm aluminum sheet was the stationary phase. Mobile phase comprising Toluene: Ethyl acetate: formic acid (6:3:0.1). The development of the plate was done by using CAMAG 20 x 20 cm automatic developing chamber. After derivatization using anisaldehyde sulphuric acid reagent, it was visualized under UV at 254 nm and 366 nm. The number of peaks in the chromatogram of *bhavitha choorna* (processed powder) was more when compared to *choorna* (powder) at 254nm indicating a greater number of phytoconstituents in *bhavitha choorna* (processed powder). The total area of *bhavitha choorna* (processed powder). is more than *choorna*(powder) of the drug at 366nm indicates that the concentration of phytoconstituents has been increased by the process of *bhavana* which substantiate that *bhavana* process can increase the potency and efficacy of the drug.
Key words: High-Performance thin layer chromatography, *Haridra*, *choorna*, *bhavitha choorna*.

INTRODUCTION

The drug *Haridra* (*Curcuma longa* Linn.) is one of the most outstanding herbs that strongly relates to the sociocultural life of people in India. *Haridra*, botanically identified as *Curcuma longa* Linn. belongs to the family

Zingiberaceae[1], which is extensively used by Ayurvedic practitioners and is well documented in the literature of Ayurveda since the Vedic period. In *Charaka Samhita*, the drug has been mentioned under *lekhaneeya* dasemani (A group of 10 drugs having scraping like action).[2] The drug can be utilized in different dosage forms according to the *yukti* (logical reasoning) of the physician. The *choorna Kalpana* (powder preparation) is the *upakalpana* (secondary preparation*)* of *kalka Kalpana* (herbal paste preparation). The term *choorna*(powder) refers to finely powdered dry drugs that are filtered through a cloth. *Samskara* can be defined as the process or tool by which drug is modified as required. *Bhavana* (processing) is one of the *samskara*, in which the *choorna* (powder) is kept submerged in liquid media like *swarasa* (juice) or it is triturated in the *dravya* for specific time. In *Bhaishajya ratnavali*, *bhavana* (processing) is that, the powdered drugs should be soaked in the liquid in the night and it should be kept in the sun in the day and this procedure has to be repeated for seven times.[3] The *choorna* (powder) after subjecting to *bhavana* (processing) can be called as *bhavitha choorna* (processed powder). In the present study, the *bhavana* process done for seven times with *swarasa*(juice) prepared from its rhizome. The *bhavana*(processing) procedure helps to enhances the therapeutic properties, increase the efficacy of the drug and thereby to decrease the dose of the drug. The rhizome of the plant is rich in phytoconstituents such as Alkaloids, flavonoids, saponin, sterols, Glycosides, phenols, volatile oils etc.[4,5] These phytoconstituents are responsible for the pharmacological actions of the plant.

High-performance thin-layer chromatography (HPTLC) is a simple, fast and precise technique for the detection of phytochemicals present in the plant. HPTLC fingerprints are useful in the authentication and identification of plant and a fingerprint of a plant extract is a chromatographic pattern of some common chemical constituents of pharmacologically active[6]. The number of peaks signifies specific phytoconstituents and the area of peak implies the quantity of constituents present in the sample.[7] HPTLC finger printing profile of rhizome of *Haridra* (*Curcuma longa* Linn.) was done previously by Kurup et al[8] and Sikha A et al[9] using ethanolic extract and Sai Prasad et al[10] using aqueous extract. The present study aims at comparing the peaks obtained by HPTLC of methanol extract of *choorna* (powder) and *bhavitha choorna* (processed powder) of dried rhizome of *Haridra (Curcuma* longa Linn.).

MATERIALS AND METHODS

Collection of plant material

The fresh rhizome of *Curcuma longa* Linn. were collected from Puranattukara village, Thrissur district, Kerala. The harvesting was done manually when the leaves turn dry and light brownish in colour. The collected drug specimen was identified by the faculty in Department of Dravyaguna vijnanam, Govt. Ayurveda College, Tripunithura, Ernakulam, Kerala

Preparation of *choorna* (Powder)

The fresh rhizomes of the drug were washed thoroughly with water to remove the physical impurities. It was boiled until froth and fumes were released with a typical turmeric aroma, and the rhizomes become soft. It was then allowed to cool gradually, cut into small pieces and shade dried. The dried rhizomes were then made into fine powder and sieved through mesh with size-120.

Preparation of *swarasa* (juice) for *Bhavana* (processing)

Swarasa (juice) of the rhizome of the drug was prepared based on the reference mentioned in *Sarangadhara samhitha*.[11] The fresh rhizome of *Haridra* (*Curcuma longa* Linn.) was collected and washed thoroughly to remove physical impurities like soil, mud etc. The rhizome was cut into small pieces, crushed and ground. The expressed juice of the drug was filtered and collected in a clean container.

Preparation of *Bhavitha choorna* (processed powder)

Bhavitha choorna (processed powder) of rhizome of *Haridra* (*Curcuma longa* Linn.) was prepared according to the reference of *bhavana vidhi* (processing method) mentioned in *Bhaishajya ratnavali.*[3] The *choorna* (powder) of the drug was taken in a clean wide-mouthed tray. It was uniformly spread so that it forms a thin layer. The *swarasa* (juice) taken from the drug *Haridra* (*Curcuma longa* Linn.) was filtered and gradually poured into the fine powder so that the *swarasa* gets absorbed into the powder. Using a sharp thin rod 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. The tray was slowly shaken on both sides to ensure uniform spreading of *bhavana dravya* in the fine particles of the powder and was kept 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 taken and then dried in shade. 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. Ensured that there was no contamination. The

properly dried powder was then made into fine powder to remove lumps and sieved through mesh size 120. Likewise, the dried powder obtained after each *bhavana* was finely powdered. The bhavana (processing) was done in the same manner for 7 times as per reference told in Bhaishajya Ratnavali.[3] The powdered drug was stored in airtight containers**.** Figure 1: *Choorna* (powder) of dried rhizome of *Haridra* (*Curcuma longa* Linn.) Figure 2: *Bhavitha Choorna* (powder) of dried rhizome of *Haridra* (*Curcuma longa* Linn.)

Procedure

Test solution were made with 1 gm each of *choorna* and *bhavitha choorna* of dried rhizome of *Haridra* (*Curcuma longa* Linn.) respectively. Both the powder of the drug was then extracted with 10 ml methanol and 3.0 µl was applied on the stationary phase. Stationary phase includes HPTLC plates Silica gel 60 F 254, 4.0 x 9.0 cm aluminium sheet. Mobile phase includes Toluene: Ethyl acetate: formic acid (6:3:0.1). The development of the plate was done by using CAMAG 20 x 20 cm automatic developing chamber. It was visualized under UV at 254 nm and 366 nm after derivatization using anisaldehyde sulphuric acid reagent.

RESULT

A. HPTLC finger printing profile of powder of dried rhizome of *Haridra* (*Curcuma longa* Linn.)

Peaks and Area at wavelength 254nm

HPTLC chromatogram of powder of dried rhizome of *Haridra* (*Curcuma longa* Linn.) were recorded. Total 9 peaks at 254 nm with total area of 157945.2 AU were obtained for the powder of the drug. These 9 peaks were defined with max Rf value of 0.03 with area 1603.1 AU, max Rf value of 0.05 with area 2291.7 AU, max Rf value of 0.09 with area 2713.0 AU, max Rf value of 0.12 with area 1015.1 AU, max Rf value of 0.17 with area 9890.9 AU, max Rf value of 0.21 with area 6474.7 AU, max Rf value of 0.29 with area 42630.9 AU, max Rf value of 0.45 with area 15636.3 AU, max Rf value of 0.65 with area 75689.5 AU respectively. (Table 1: Peak and area of powder of dried rhizome of Curcuma longa Linn. at 254nm. Figure 3: Overview graph of powder of dried rhizome of *Curcuma longa* Linn. at 254nm wavelength. Figure 5: TLC plate view of *choorna* ofdried rhizome of *Curcuma longa* Linn.at 254nm wavelength)

Peaks and Area at wavelength 366nm

Total of 14 peaks with total area of 148288.4 AU were obtained for dried powder of Curcuma longa Linn. These 14 peaks were defined with max Rf value of 0.02 with area 902.9 AU, max Rf value of 0.04 with area 547.1 AU, max Rf value of 0.09 with area 3926.9 AU, max Rf value of 0.11 with area 5045.7 AU, max Rf value of 0.16 with area 14476.7 AU and max Rf value of 0.22 with area 36014.3 AU, max Rf value of 0.33 with area 10896.5 AU, max Rf value of 0.37 with area 18627.6 AU and max Rf value of 0.42 with area 22315.0 AU, max Rf value of 0.48 with area 10471.3 AU and max Rf value of 0.52 with area 23327.7 AU, max Rf value of 0.61 with area 753.3 AU, max Rf value of 0.68 with area 319.0 AU and max Rf value of 0.72 with area 664.4 AU respectively. Table 2: Peak and area of dried powder of rhizome of Curcuma longa Linn. at 366nm. Figure 4 Overview graph of Choorna of rhizome of Curcuma longa Linn. at 366nm wavelength. Figure 6,7,8: TLC plate view of choorna of dried rhizome of Curcuma longa Linn.).

B. HPTLC finger printing profile of *bhavitha choorna* of dried rhizome of *Haridra (Curcuma longa* Linn.)

Peaks and Area at wavelength 254nm

In *bhavitha choorna* (processed powder) of the drug, 10 peaks with total area 150367.8 AU were obtained. These 10 peaks were defined with max Rf value of -0.01with area 151.3 AU, max Rf value of 0.08 with area 3472.8 AU, max Rf value of 0.12 with area 1495.8 AU, max Rf value of 0.16 with area 4900.3 AU, max Rf value of 0.21 with area 7636.0 AU, max Rf value of 0.28 with area 37255.4 AU, max Rf value of 0.45 with area 13433.6 AU, max Rf value of 0.50 with area 4862.1 AU, max Rf value of 0.59 with area 29233.4 AU, max Rf value of 0.65 with area 47927.1 AU. (Table 3: Peak and area of bhavitha choorna of rhizome of Curcuma longa Linn. at 254nm. Figure 9: Overview graph of bhavitha choorna of rhizome of Curcuma longa Linn. at 254nm. Figure 11: TLC plate view of bhavitha choorna of dried rhizome of Curcuma longa Linn. at 254nm wavelength.)

Peaks and Area wavelength at 366nm

A total of 11 peaks with total area of 178316.5 AU were defined. These eleven peaks were defined with max Rf value of 0.03 with area 2771.6 AU, max Rf value of 0.08 with area 5677.6 AU, max Rf value of 0.11 with area 4549.8 AU, max Rf value of 0.16 with area 28060.7 AU, max Rf value of 0.20 with area 27195.2 AU, max Rf value of 0.32 with area 13546.0 AU, max Rf value of 0.37 with area 22108.7 AU, max Rf value of 0.43 with area 26005.4

AU, max Rf value of 0.52 with area 46564.3 AU, max Rf value of 0.68 with area 1060.7 AU, max Rf value of 0.71 with area 776.5 AU respectively. (Table 4: Peak and area of bhavitha choorna of rhizome of Curcuma longa Linn. at 366nm. Figure No: 10 Overview graph of bhavitha choorna of rhizome of Curcuma longa Linn. at 366nm wavelength. Figure no. 12, 13, 14 : TLC views of bhavitha choorna of dried rhizome of curcuma longa Linn.)

DISCUSSION

Haridra has been described in Ayurvedic classics as well as traditional textbooks as alone or in combination for both internal and external applications. The drug should be genuine in order to get therapeutic effect. HPTLC, which is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. HPTLC finger printing profile of rhizome of *Haridra* (*Curcuma longa* Linn.) in ethanolic extract was documented previously by kurup et al.[8] using toluene : ethyl acetate as mobile phase showed 12 peaks under 254nm and 11 peaks under 366nm. Research work of sikha et al.[9] showed 15 peaks with different Rf in the same solvent system under 254nm. In previous work by sai prasad et al.[10] aqueous extract of the rhizome showed 2 peaks in the solvent system Toluene:Ethyl acetate:Formic acid under 500nm. In present study methanol extract of the rhizome was used for HPTLC finger printing profile at 254nm (9 peaks) and 366nm (14 peaks).

At 254nm, on comparing the present study (methanol extract) with ethanol extract done by kurup et al.[8] , 1 Peak was common (max Rf value of 0.65) with area 47.92% and 0.93% respectively. Also while comparing the present study with ethanol extract done by sikha et al.[9] showed 1 common peak (max Rf value of 0.17) with area% 6.26 and 4.40 respectively. At 366nm, on comparing the present study (methanol extract) with ethanol extract done by sikha et al.[9] showed 2 common peaks (max Rf 0.14) with area % 0.31 and 0.33 and (max Rf 0.16) with area 9.76% and 28.89% respectively.

HPTLC chromatogram of *choorna* (powder) and *bhavitha choorna* (processed powder) of dried rhizome of *curcuma longa* Linn. were carried out at wavelength 254nm and 366nm. HPTLC finger printing at 254nm revealed 9 peaks with max Rf values in the range of 0.03 to 0.65 for *choorna* (total area of 157945.2 AU) whereas *bhavitha choorna* (processed powder) showed 14 peaks with max Rf values in the range of 0.02 to 0.72 (total area 150367.8 AU). The number of peaks obtained for *bhavitha choorna* (processed powder) was more when compared to *choorna* (powder) of the drug indicating a greater number of phytoconstituents in *bhavitha choorna* (processed powder). The peak intensities were different in both samples but 4 peaks with max Rf values obtained were similar in both the *choorna* indicates the presence of same chemical constituent. 5 Individuals peaks were obtained in the *choorna* and 6 individual peaks were obtained for *bhavitha choorna* indicating the presence of different compounds in the *choorna* and *bhavitha choorna*.

At wavelength 366nm, 14 peaks were obtained in the range of max Rf 0.02 to 0.72 for *choorna* with total area of 148288.4 AU and 11 peaks with total area 178316.5AU obtained in the range of 0.03 to 0.71 for *bhavitha*. The maximum area obtained for *bhavitha choorna* (processed powder) was more when compared to *choorna* (powder) of the drug indicates more concentration of the chemical constituent in *bhavitha choorna* (processed powder) which substantiate that *bhavana* process can increase the potency and efficacy of the drug. The peak intensities were different in both samples but 5 peaks with max Rf values obtained were similar in both the *choorna* which indicates the presence of same chemical constituents.

CONCLUSION

HPTLC profiling of *choorna* (powder) and *bhavita choorna* (processed powder) of *Haridra* (Curcuma longa Linn.) was done and comparison of their peaks were discussed . The number of peaks obtained for *Bhavitha choorna* (processed powder) was more when compared to *choorna (*powder) of the drug at 254nm, indicates more number of phytoconstituents in *bhavitha choorna* (processed powder). The total area of *bhavitha choorna* is more than *choorna* of the drug at 366nm indicates that the concentration of phytoconstituents has been increased by the process of *bhavana* which substantiate that *bhavana* process can increase the potency and efficacy of the drug.

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Figure 1: *Choorna* (powder) of dried rhizome of *Haridra* (*Curcuma longa* Linn.)

Figure 2: *Bhavitha Choorna* (powder) of dried rhizome of *Haridra* (*Curcuma longa* Linn.)

Table 1: Peak and area of powder of dried rhizome of *Curcuma longa* Linn. at 254nm

Peak	Start Rf	Max Rf	End Rf	Area(AU)	Area %(AU)
1	0.00	0.03	0.04	1603.1	1.01
2	0.04	0.05	0.07	2291.7	1.45
3	0.07	0.09	0.11	2713.0	1.72
4	0.11	0.12	0.13	1015.1	0.64
5	0.13	0.17	0.20	9890.9	6.26
6	0.20	0.21	0.23	6474.7	4.10
7	0.23	0.29	0.39	42630.9	26.99
8	0.39	0.45	0.52	15636.3	9.90
9	0.52	0.65	0.73	75689.5	47.92

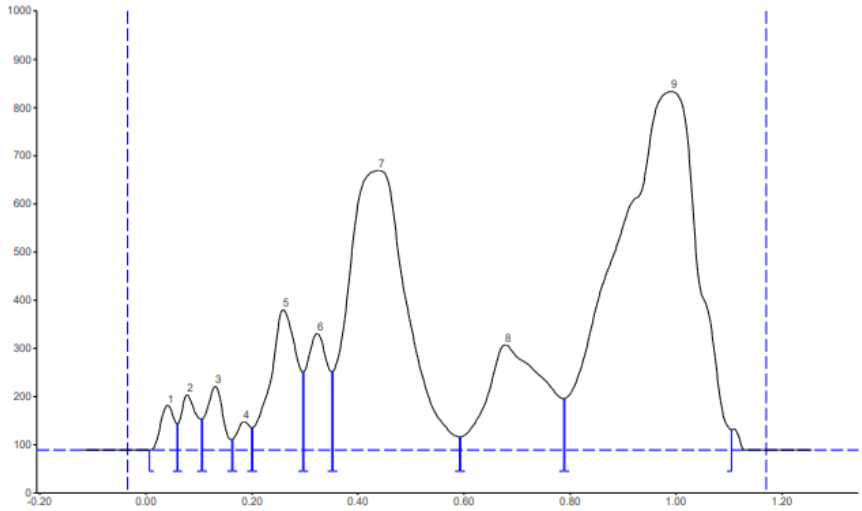
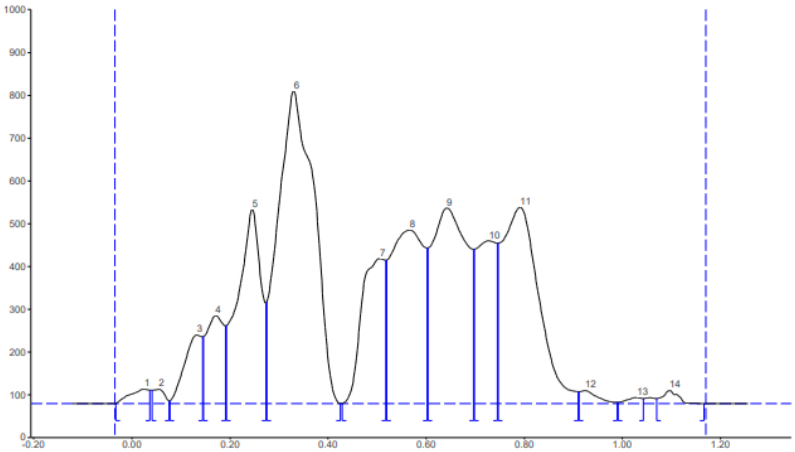


Figure 3: Overview graph of powder of dried rhizome of *Curcuma longa* Linn. at 254nm wavelength

Table 2: Peak and area of dried powder of rhizome of *Curcuma longa* Linn. at 366nm

Peak	Start Rf	Max Rf	End Rf	Area(AU)	Area %(AU)
1	-0.02	0.02	0.02	902.9	0.61
2	0.03	0.04	0.05	547.1	0.37
3	0.05	0.09	0.10	3926.9	2.65
4	0.10	0.11	0.13	5045.7	3.40
5	0.13	0.16	0.18	14476.7	9.76
6	0.18	0.22	0.28	36014.3	24.29
7	0.28	0.33	0.34	10896.5	7.35
8	0.34	0.37	0.40	18627.6	12.56
9	0.40	0.42	0.46	22315.0	15.05
10	0.46	0.48	0.49	10471.3	7.06
11	0.49	0.52	0.60	23327.7	15.73
12	0.60	0.61	0.65	753.3	0.51
13	0.65	0.68	0.69	319.0	0.22
14	0.71	0.72	0.77	664.4	0.45

Figure 4: Overview graph of *Choorna* of rhizome of *Curcuma longa* Linn. at 366nm wavelength



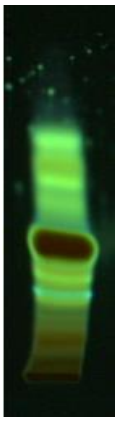


Figure 5: TLC plate view of *choorna* of dried rhizome of *Curcuma longa* Linn. at 254nm wavelength

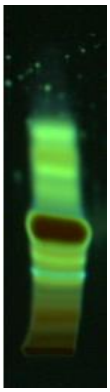


Figure 6: TLC plate view of *choorna* of dried rhizome of *Curcuma longa* Linn. at 366nm wavelength

Figure 7: TLC views of methanol extract of dried rhizome of *Curcuma longa* Linn. after derivatization at 366nm

Figure 8: TLC views of methanol extract of dried root powder of rhizome of *Curcuma longa* Linn. after derivatization in white light

Table 3: Peak and area of *bhavitha choorna* of rhizome of *Curcuma longa* Linn. at 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.02	-0.01	0.00	151.3	0.10
2	0.02	0.08	0.10	3472.8	2.31
3	0.10	0.12	0.13	1495.8	0.99
4	0.13	0.16	0.18	4900.3	3.26
5	0.18	0.21	0.23	7636.0	5.08
6	0.23	0.28	0.39	37255.4	24.78
7	0.39	0.45	0.48	13433.6	8.93
8	0.48	0.50	0.52	4862.1	3.23
9	0.52	0.59	0.61	29233.4	19.44
10	0.61	0.65	0.74	47927.1	31.87

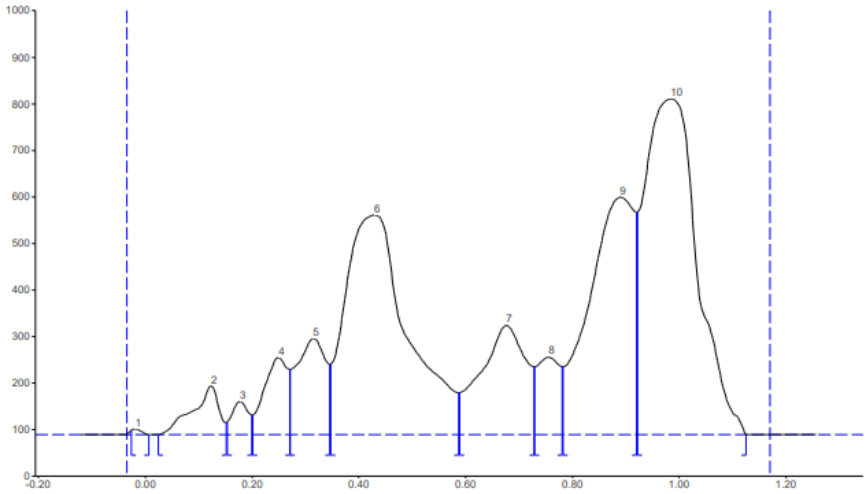


Figure 9: Overview graph of *bhavitha choorna* of rhizome of *Curcuma longa* Linn. at 254nm

Table 4: Peak and area of *bhavitha choorna* of rhizome of *Curcuma longa* Linn. at 366nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.01	0.03	0.04	2771.6	1.55
2	0.04	0.08	0.09	5677.6	3.18
3	0.09	0.11	0.12	4549.8	2.55
4	0.12	0.16	0.18	28060.7	15.74
5	0.19	0.20	0.28	27195.2	15.25
6	0.28	0.32	0.34	13546.0	7.60
7	0.34	0.37	0.40	22108.7	12.40
8	0.40	0.43	0.46	26005.4	14.58
9	0.46	0.52	0.65	46564.3	26.11
10	0.65	0.68	0.70	1060.7	0.59
11	0.70	0.71	0.76	776.5	0.44

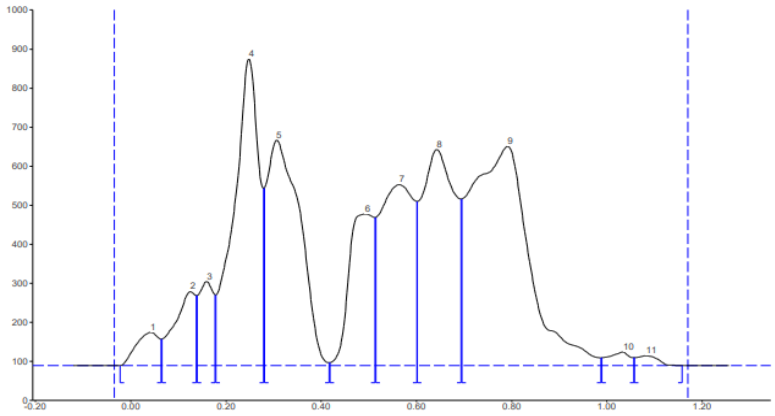


Figure No: 10 Overview graph of *bhavitha choorna* of rhizome of *Curcuma longa* Linn. at 366nm wavelength

Figure No 11: TLC plate view of *bhavitha choorna* of dried rhizome of *Curcuma longa* Linn. at 254nm wavelength

Figure No 12 : TLC plate view of *bhavitha choorna* of dried rhizome of *Curcuma longa* Linn. at 366nm wavelength

Figure 13: TLC views of methanol extract of *bhavitha choorna* of dried rhizome of Curcuma longa Linn. after derivatization at 366nm.

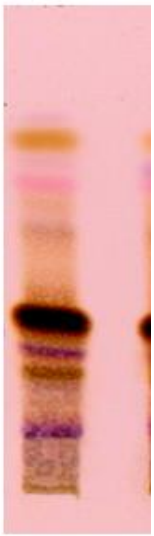


Figure 14: TLC views of methanol extract of *bhavitha choorna* of dried rhizome of curcuma longa Linn. after derivatization in white light.