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ORIGINAL RESEARCH ARTICLE

A Comparative Analysis of *Choorna* and *Bhavitha Choorna* of Flower Buds of *Japa* (*Hibiscus rosa-sinensis* Linn.) through High-performance Thin-layer Chromatography

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ABSTRACT

Introduction: High-performance thin-layer chromatography (HPTLC) is nowadays adopted by the major pharmacopoeias of the world for analysis of herbal drugs and preparations. The current use is generally limited to the visual observation of the fingerprints for identification and detection of adulterations and falsifications. It makes sense to use HPTLC to expand chromatographic fingerprints to identify the main active ingredients in medicinal plants. Compared to thin layer chromatography, the separation and resolution are significantly superior, and the outcomes are far more consistent and repeatable.

Materials and Methods: *Japakusuma mukula* (flower buds of *Japa*) which is botanically identified as *Hibiscus rosa-sinensis* Linn. contains various phytoconstituents such as flavonoids, terpenoids, steroids, polysaccharides, alkaloids, amino acids, lipids, sesquiterpene, quinones, and naphthalene groups. HPTLC fingerprinting profile of *choorna* (powder) and *bhavitha choorna* (triturated powder) of flower buds of *Hibiscus rosa-sinensis* Linn. is demonstrated in methanolic extract in this study and bands are analyzed at 254 nm and 366 nm using CAMAG Linomat V Automatic Sample Spotter. The peaks and area are compared.

Results and Conclusion: Similarity in the peak areas even in different Max Rf values, it could be inferred that the phytoconstituent obtained in both conditions will be having similarity. Since the *bhavitha choorna* possess, the greater number of large value peak areas in both the wavelength of visualization, it is clear that *bhavitha choorna* is more potent in comparison with *choorna.*

1. INTRODUCTION

One of the most often used analytical techniques in the pharmaceutical, clinical, forensic, biochemistry, cosmetology, food and drug, and environmental sectors is high-performance thin-layer chromatography (HPTLC). It is the only chromatographic method that allows the findings to be presented as an image. Additional benefits include ease of use, affordability, sample analysis in parallel, high sample capacity, quick findings, and numerous detection potential. HPTLC, or high-performance thin-layer chromatography, is an effective method for separating phytoconstituents and aids in the verification of raw pharmaceuticals. The herbal drugs HPTLC fingerprint assists in confirming its identification and uses the adsorption concept to separate chemicals.[1] HPTLC is a useful tool for quantifying marker chemicals, monitoring purity, and detecting adulteration. This method of separation is quantitative as well as qualitative. Particular phytoconstituents are indicated by the number of peaks, and the area of a peak suggests the quantity of components in the sample.

Japakusuma mukula (flower buds of *Japa*) which is botanically identified as *Hibiscus rosa-sinensis* Linn. contains various phytoconstituents such as flavonoids including Cyanidin -3-sophoroside Cyanidin -3-sophoroside-5-glycoside, Flavylium, Quercetin -3-diglucoside, Quercetin, Quercetin 3-sophorotrioside, Cyanin chloride, Cyanidin chloride, Cyanin, Kaempferol 3-xylosylglucoside Quercetin-3,7-diglucoside,

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Rutin, Kaempferol, Myricetinanthocyanins, terpenoids, steroids, polysaccharides, alkaloids, amino acids, lipids, sesquiterpene, quinones, and naphthalene groups.[2-6] The flowers of *Japa* exhibit various pharmacological effects in its different *kalpanas* (dosage forms) such as *swarasa* (juice), *kalka* (paste), *choorna* (powder), and *kwatha* (decoction). The *Japakusuma mukula* is used in the modified dosage form as *bhavitha choorna* (triturated powder) along with *ksheera* (milk) in the case of *Asrigdhara* (abnormal uterine bleeding) as per the authentic Malayalam ayurvedic book Chikitsamanjeri.[7] This medicinal plant had immense potential in the cure of various pathological condition.

The *choorna* (powder) is a modified dosage form of *kalka kalpana* (bolus). *Bhavana* (trituration) is a pharmaceutical process done for potentiating or purification of drugs. The process is done by soaking or triturating the powdered drugs (*choorna*) in an appropriate *drava* (liquid) for a specific period. It helps to improve the therapeutic property of the drug. Generally soaking method of *bhavana* is done for herbal drugs. Through this process the qualities of liquid media are impregnated into the *bhavitha* material (drug material that has undergone the *bhavana* process). This increases the efficacy of the drug and hence the effective dose can be reduced. *Bhavana* is done for various purposes such as purification of drugs, removing the adverse effect of a drug, enhancing the therapeutic activity of raw drugs, to reduce the excessive concentration of active principles that can be harmful, based on disease condition, remove toxic effect of the drug, decrease the *theekshnatha* (sharpness) of a drug, increase the potency of the drug, to act as binders, and as a vehicle for administration of medicine.[8]

The study drug *Japakusuma mukula* was undergone *bhavana* with its *swarasa* itself for 7 times. Here, in this study, the methodology of *bhavana* explained in *Bhaishajya Ratnavali* is executed.[9] By doing this *samskara* (procedure), the properties of drug enhanced which helped to reduce the dosage. Furthermore, the drug underwent modification both pharmacognostically and phytochemically with the process of *bhavana*. The HPTLC technique was employed to compare the peaks obtained for *choorna* (powder) and *bhavita choorna* (triturated powder) of the drug developed using same solvents, mobile phase, and stationary phase. The present study aims at comparing the peaks obtained in HPTLC chromatogram of *choorna* (powder) and *bhavita choorna* (processed powder) of flower buds of *Hibiscus rosa-sinensis* Linn. and to substantiate increased potency of *bhavita choorna* (processed powder) when comparison with *choorna* (powder).

2. MATERIALS AND METHODS

2.1. Preparation of *Choorna* (Powder)

The freshly collected flower buds of *Hibiscus rosa sinensis* Linn. were thoroughly washed to remove the physical impurities and scrutinized for any worm infestation. Then, the whole flower bud was dried in the shade until it is ready to get powdered finely. After accomplishing proper dryness (4–5 days), drug was powdered finely using mixer grinder and was sieved through mesh size-120. The powder was subjected to 7 times *bhavana* (trituration) in the *swarasa* (juice) of *Japakusumamukula* itself and is used for therapeutic administration.

2.2. Preparation of *Bhavitha Choorna* (Triturated Powder)

Bhavitha choorna (triturated powder) of *Japakusuma mukula* (flower buds of *Hibiscus rosa -sinensis* Linn.) was prepared according to the reference of *bhavana vidhi* (process of trituration) mentioned in *Bhaishajya ratnavali*.[9] The *choorna* (powder) of the drug prepared earlier was taken in a tray used in driers. Powder was spread uniformly in the tray so that it forms a thin layer. The freshly prepared *swarasa* (juice) of the drug was then gradually poured into the fine powder so that the *swarasa* gets absorbed into the powder. By means of a clean stainless-steel rod, it was mixed to confirm that each fine particle of the *choorna* (powder) gets completely soaked in the *swarasa* (juice). Thus, pouring of *swarasa* was continued until a thin layer of *swarasa* persisted on the surface of the entire powder. It was then kept in the sunlight for drying. Often, the contents of the tray were stirred using a stainless-steel rod. It was then covered with a clean thin cloth to avoid dust or any other contamination from the external environment and kept undisturbed overnight. On the next day morning the tray was taken and again poured freshly prepared *swarasa* (juice) of *Japakusumamukula* (flower buds of *Hibiscus rosa -sinensis* Linn.) into the *choorna* (powder) by mixing it with the rod and until it is completely soaked. Then the same procedure of first day *bhavana* (trituration) was repeated. 3rd–7th *bhavana* was performed continuously with sufficient quantity of *swarasa* (juice) needed each time. After 7th *bhavana* the *choorna* (powder) was kept in sunlight until it was completely dried (3 days). When the top layer of the *choorna* (powder) was completely dried, it was mixed with a rod for uniform drying of all the areas. At each stage, it was ensured that there was no contamination. During night time, it was kept undisturbed covering with a clean cloth. Then, properly dried powder after *bhavana* was made into fine powder using mixer grinder and sieved through the mesh size 120 [Figures 1 and 2].

2.3. Procedure of HPTLC

2.3.1. HPTLC conditions

The material used was HPTLC plates percolated with silica gel 60 F 254 thin-layer chromatography (TLC) plates manufactured by E. MERCK KGaA, having plate size 5.0 × 10.0 cm and 0.2 mm thickness with aluminum sheet support. The instrument used to execute the sample application was CAMAG Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland) with syringe size of 100 µL (from Hamilton). The developing chamber consists of twin trough chamber having 20 × 10 cm dimension belonging to CAMAG. The post chromatographic derivatization was done with the chromatographic sprayer with vanillin sulphuric acid reagent as the solution. The detection of the tracks was done with CAMAG TLC Scanner, linked to WINCATS software.

2.3.2. Methods

The same methodology was adopted for both *choorna* and *bhavitha choorna*. Utilizing a twin trough chamber that has been previously saturated with the solvent system (toluene: ethyl acetate: acetic acid [6:3:1]) for 30 min, the plate was developed with 8 mm band length after washing the syringe twice in methanol and applying 5.0 µl of methanolic extract. Place the plate in the scanner after drying it. For absorption reflection mode, use a Deuterium, Tungsten, or Mercury lamp to scan the plate in ultraviolet (UV) at 254 nm and 366 nm, respectively. Initially, scan the UV spectrum of each scanning, then scan all the tracks. The distance between the tracks was 12.5 mm observe the fingerprint of each track. In the spectrum display, UV spectra spots can be compared. Enter all of the scanning, integration, and spectrum parameters after opening a file.

3. RESULTS

HPTLC fingerprinting profile of *choorna* (powder) and *bhavitha choorna* (triturated powder) of flower buds of *Hibiscus rosa-sinensis* Linn. were performed. The observations found were being tabulated.

3.1. HPTLC Finger Printing Profile of Methanol Extract of CHOORNA (Powder) of Flower Buds of *Hibiscus Rosa-Sinensis* Linn.

3.1.1. Area and peaks of methanol extract of choorna at 254 nm

HPTLC fingerprinting profile of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. showed 9 peaks at the wavelength of 254 nm in a total area of 31663.7 AU. These 9 peaks were defined at the maximum Rf value of −0.01 with area 16231.4 AU, maximum Rf value of 0.12 with area 3099.8 AU, maximum Rf value of 0.18 with area 753.9 AU, maximum Rf value of 0.34 with area 2837.7 AU, maximum Rf value of 0.58 with area 903.3 AU, maximum Rf value of 0.85 with area 2276.4 AU, maximum Rf value of 0.87 with area 2284.2 AU, maximum Rf value of 0.90 with area 2540.6 AU, and maximum Rf value of 1.03 with area 736.4 AU, respectively [Table 1, Chart 1 and Figure 3].

3.1.2. Area and peaks of methanol extract of choorna at 366 nm

HPTLC fingerprinting profile of *choorna* of Japakusuma mukula (powder of flower buds of *Hibiscus rosa-sinensis* Linn.) showed 12 peaks at the wavelength of 366 nm in a total area of 29211.2 AU. These 12 peaks were defined at the maximum Rf value of −0.03 with area 1953.8AU, maximum Rf value of 0.00 with area 1750.9 AU, maximum Rf value of 0.08 with area 224.3 AU, maximum Rf value of 0.13 with area 452.7 AU, maximum Rf value of 0.27 with area 111.8 AU, maximum Rf value of 0.80 with area 3528.1 AU, maximum Rf value of 0.85 with area 12230.7 AU, maximum Rf value of 1.00 with area 625.7 AU, maximum Rf value of 1.04 with area 904.5 AU, maximum Rf value of 1.08 with area 3553.9 AU, maximum Rf value of 1.13 with area 467.8 AU, and maximum Rf value of 1.20 with area 3402.0 AU, respectively [Table 2, Chart 2 and Figure 4].

3.2. HPTLC Finger Printing Profile of *Bhavitha Choorna* (Triturated Powder) of Flower Buds of *Hibiscus Rosa-sinensis* Linn

3.2.1. Area and peaks of methanol extract of bhavitha choorna at 254 nm

HPTLC fingerprinting profile of *bhavitha choorna* (triturated powder) of *Japakusuma mukula* (flower buds of *Hibiscus rosa-sinensis* Linn.) showed 12 peaks at the wavelength of 254 nm in a total area of 11574.2 AU. These 12 peaks were defined at the maximum Rf value of −0.01 with area 14464.6 AU, maximum Rf value of 0.12 with area 2887.9 AU, maximum Rf value of 0.19 with area 273.6 AU, maximum Rf value of 0.22 with area 158.4 AU, maximum Rf value of 0.34 with area 2074.1 AU, maximum Rf value of 0.54 with area 995.2 AU, maximum Rf value of 0.85 with area 3275.6 AU, maximum Rf value of 0.87 with area 2491.3AU, maximum Rf value of 0.90 with area 2527.2 AU, maximum Rf value of 0.97 with area 634.7AU, maximum Rf value of 1.02 with area 723.9 AU, and maximum Rf value of 1.07 with area 926.3 AU, respectively [Table 3, Chart 3 and Figure 5].

3.2.2. Area and peaks of methanol extract of bhavitha choorna at 366 nm

HPTLC fingerprinting profile of *bhavitha choorna* (triturated powder) of *Japakusuma mukula* (flower buds of *Hibiscus rosa-sinensis* Linn) showed 12 peaks at the wavelength of 366 nm in a total area of 36932.2 AU. These 12 peaks were defined at the maximum Rf value of -0.01 with area 6364.1 AU, maximum Rf value of 0.09 with area 778.4 AU, maximum Rf value of 0.12 with area 644.5 AU, maximum Rf value of 0.25 with area 1276.2 AU, maximum Rf value of 0.60 with area 306.9 AU, maximum Rf value of 0.80 with area 1266.7 AU, maximum Rf value of 0.85 with area 7194.9 AU, maximum Rf value of 0.87 with area 7283.2 AU, maximum Rf value of 1.03 with area 1817.3 AU, maximum Rf value of 1.08 with area 2849.8 AU, maximum Rf value of 1.13 with area 566.5 AU, and maximum Rf value of 1.19 with area 6583.7 AU, respectively [Table 4, Chart 4 and Figure 6].

4. DISCUSSION

HPTLC fingerprinting profile of *choorna* and *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. is demonstrated in methanolic extract in this study and bands are analysed at 254 nm and 366 nm. The peaks and area are compared with the previous works in flower of *Hibiscus rosa-sinensis* Linn. It was found that nine peaks and 12 peaks are obtained, respectively, for *choorna* and *bhavitha choorna* at 254 nm, whereas both the samples showed 12 peaks at 366 nm visualization. In *choorna* at 254 nm, maximum area obtained is 16231.4 AU at Max Rf value −0.01, whereas in *bhavitha choorna* at 254 nm maximum area obtained is 14464.6 AU at Max Rf value of −0.01 itself. Hence, we can assume that the same phytoconstituent is predominant in both *choorna* and *bhavitha choorna* at the same Rf value under the same wave length. When comparing this with the previous work of Poornima et.al. in the work of "HPTLC fingerprinting profile of methanolic extract of flowers of *Hibiscus rosa-sinensis* L",, is found that at 254 nm, the maximum area was obtained as 2008.2 AU at maximum Rf value 0.04 which is almost nearer to Rf value 0.01.[10] By this comparison, it is evident that the amount of phytoconstituents in our study drug in *choorna* and *bhavitha* choorna form possess maximum amount of phytoconstituents with a large area of concentration. At 366 nm visualization, the maximum area obtained for *choorna* is 12230.7 AU at Max Rf value of 0.85 and for *bhavitha choorna* maximum area was 7283.2 AU at Max Rf value 0.87. By these evidences again, we could find that in both, the *choorna* and *bhavitha choorna* almost the same Max Rf value possesses the major phytoconstituent. As per previous study of Sumathy *et al.* 7 peaks were obtained at visualization at 360 nm.[11] In this study, at 254 nm 6 peaks with Max Rf values (0.01, 0.12, 0.34, 0.85, 0.87, and 0.90) are similar for *choorna* and *bhavitha choorna*, whereas at 366 nm 4 peaks with Max Rf values (0.80, 0.85,1.08, and 1.13) are similar in both. The area obtained in these various Max Rf values in both *choorna* and *bhavitha choorna* are almost comparable with each other which indicates the similarity in the phytoconstituents present in both these samples. In 254 nm visualization of both samples, the peak areas of Max Rf values of 1.03 and 0.58 can be compared with peak areas of Max Rf values of 1.02 and 1.07, respectively since they have almost similar values. Likewise, in 366 nm only one peak area with Max Rf of −0.00 is comparable with Max Rf value 1.03. By this similarity in the peak areas even in different Max Rf values, it could be inferred that the phytoconstituent obtained in both conditions will be having similarity. Since the *bhavitha choorna* possess the greater number of large value peak areas in both the wavelength of visualization, it is clear that *bhavitha choorna* is more potent in comparison with *choorna*.

5. CONCLUSION

HPTLC fingerprinting profile of *choorna* and *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. is demonstrated in methanolic extract in this study and bands are analysed at 254 nm and 366 nm. Since the *bhavitha choorna* possess the greater number of large value peak areas in both the wavelength of visualization, it is clear that *bhavitha choorna* is more potent in comparison with *choorna.*

6. ACKNOWLEDGMENT

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7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

As this analytical technique was done as part of clinical study ethical clearance and CTRI registration was done.

Ethical clearance number – 05/DG/IEC/2021.

CTRI Reg no: CTRI/2022/08/044981

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAIBALITY

This is an original manuscript and all data are available for only review purposes from principal investigators.

12. PUBLISHERS NOTE

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Figure 1*: Japakusuma mukula choorna*

Figure 2: *Bhavitha Japakusuma*

Figure 3: High performance thin layer chromatography plate view of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

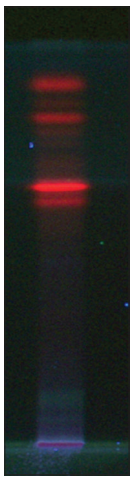

Figure 4: High-performance thin-layer chromatography plate view of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

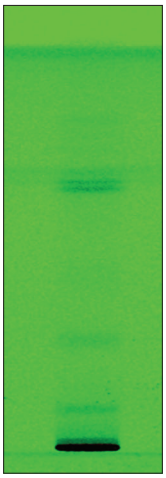

Figure 5: High-performance thin-layer chromatography plate view of methanol extract of *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

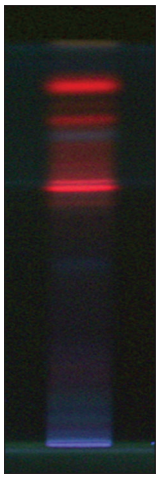

Figure 6: High-performance thin-layer chromatography plate view of methanol extract of *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

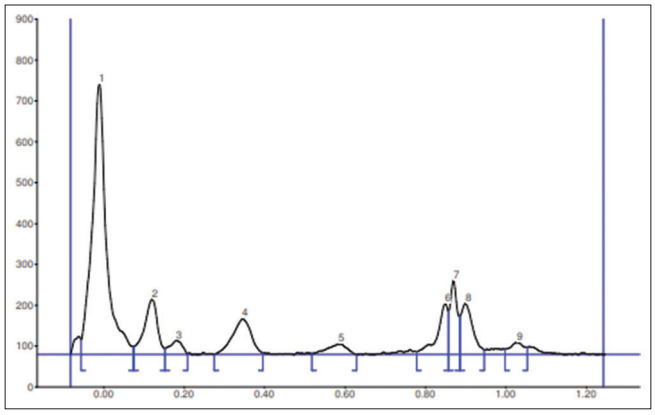

Chart 1: Overview graph of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

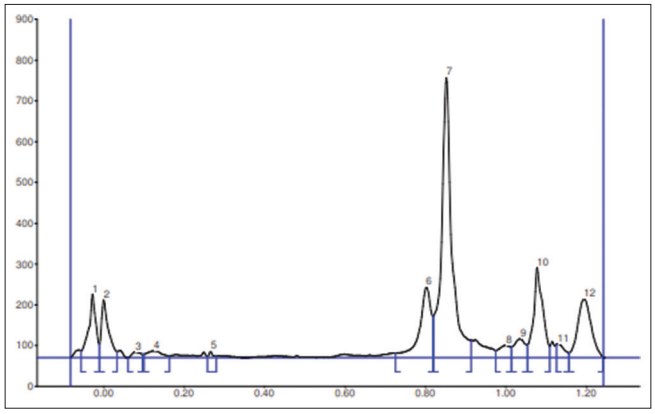

Chart 2: Overview graph of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

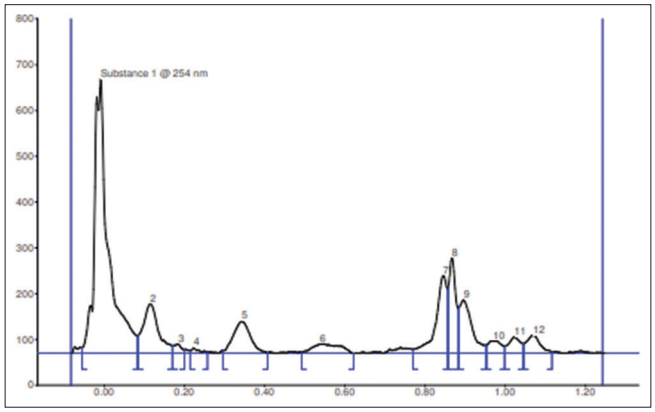

Chart 3: Overview graph of methanol extract of *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

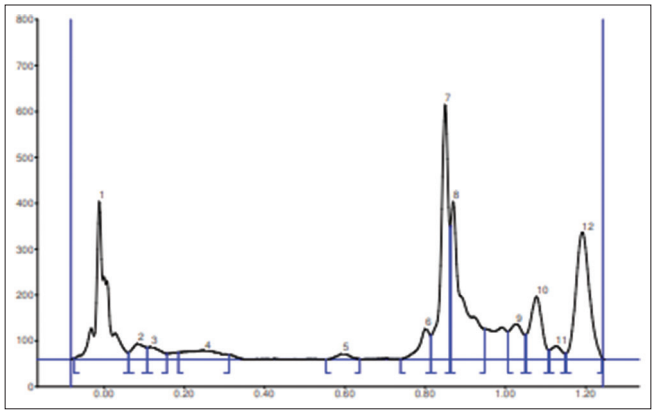

Chart 4: Overview graph of methanol extract of *bhavitha choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

Table 1: Peak and area of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

Peak No.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	−0.06	−0.01	0.07	16231.4	51.26
2.	0.08	0.12	0.15	3099.8	9.79
3.	0.15	0.18	0.21	753.9	2.38
4.	0.28	0.34	0.40	2837.7	8.96
5.	0.52	0.58	0.63	903.3	2.85
6.	0.78	0.85	0.86	2276.4	7.19
7.	0.86	0.87	0.89	2284.2	7.21
8.	0.89	0.90	0.95	2540.6	8.02
9.	1.00	1.03	1.05	736.4	2.33

Table 2: Peak and area of methanol extract of *choorna* of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

Peak No.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	−0.06	−0.03	−0.01	1953.8	6.69
2.	−0.01	0.00	0.03	1750.9	6.00
3.	0.06	0.08	0.10	224.3	0.77
4.	0.10	0.13	0.16	452.7	1.55
5.	0.26	0.27	0.28	111.8	0.38
6.	0.73	0.80	0.82	3528.1	12.08
7.	0.82	0.85	0.92	12230.7	41.88
8.	0.98	1.00	1.01	625.7	2.14
9.	1.02	1.04	1.05	904.5	3.10
10.	1.06	1.08	1.11	3553.9	12.17
11.	1.13	1.13	1.16	467.8	1.60
12.	1.16	1.20	1.24	3402.0	11.65

Table 3: Peak and area of methanol extract of *bhavitha choorna* (triturated powder) of flower buds of *Hibiscus rosa-sinensis* Linn. at 254 nm

Peak No.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	−0.05	−0.01	0.08	14464.6	46.02
2.	0.09	0.12	0.17	2887.9	9.19
3.	0.17	0.19	0.20	273.6	0.87
4.	0.22	0.22	0.26	158.4	0.50
5.	0.30	0.34	0.41	2074.1	6.60
6.	0.49	0.54	0.62	995.2	3.17
7.	0.77	0.85	0.86	3275.6	10.42
8.	0.86	0.87	0.88	2491.3	7.93
9.	0.89	0.90	0.95	2527.2	8.04
10.	0.95	0.97	1.00	634.7	2.02
11.	1.00	1.02	1.05	723.9	2.30
12.	1.05	1.07	1.12	926.3	2.95

Table 4: Peak and area of methanol extract of *bhavitha choorna* (triturated powder) of flower buds of *Hibiscus rosa-sinensis* Linn. at 366 nm

Peak No.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	−0.08	−0.01	0.06	6364.1	17.23
2.	0.06	0.09	0.11	778.4	2.11
3.	0.11	0.12	0.16	644.5	1.75
4.	0.19	0.25	0.31	1276.2	3.46
5.	0.55	0.60	0.64	306.9	0.83
6.	0.74	0.80	0.81	1266.7	3.43
7.	0.82	0.85	0.86	7194.9	19.48
8.	0.87	0.87	0.95	7283.2	19.72
9.	1.01	1.03	1.05	1817.3	4.92
10.	1.05	1.08	1.11	2849.8	7.72
11.	1.11	1.13	1.15	566.5	1.53
12.	1.15	1.19	1.24	6583.7	17.83