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A COMPARATIVE ANALYSIS OF HPTLC FINGER PRINTING PROFILE OF *CHOORNA* AND *BHAVITHA CHOORNA* OF *APAMARGA* (*ACHYRANTHES ASPERA* LINN.)

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Abstract

Samskara is a pharmaceutical process which aids in the transformation of the inherent attributes of a *dravya* which provides to the addition of a new property in them. The *samskara* or the processing helps in the qualitative and quantitative alterations for the improvement, intensification, modification and lowering the unwanted effects of the substance. *Bhavana* is one mode of *samskara* in which the powdered drugs are soaked in an appropriate *drava* (liquid medium like *swarasa*, *kashaya* etc.). *Dravya* is kept completely immersed in the *drava* or it is triturated in the *drava* for specific time period. The drug *Apamarga* (*Achyranthes aspera* Linn.) is selected as the drug for choice for the purpose of *bhavana*. The present study was aimed to compare the HPTLC finger printing profile of *choorna* and *bhavitha choorna* of the drug. Test solution is made with 10.0760gm of *choorna* and 10.4896 gm *bhavitha choorna* of *Apamarga* (*Achyranthes aspera* Linn.) respectively. Both the powder of the drug was then extracted with 1 ml methanol and 10 µl of each was applied on the stationary phase Silica gel 60 F_{254}. Mobile phase selected was toluene: ethyl acetate: methanol (6:3.8:0.2). The development of the plate was done by using CAMAG 20 x 10 cm automatic developing chamber and was visualized under UV at 254 nm and 366 nm after derivatization using anisaldehydesulphuric acid. The results revealed that there obtained different peak intensities in both sample but peaks with similar Rf values in both the *choorna* was noticed which indicates the presence of same chemical constituent. Also the maximum peak area AU obtained for similar Rf values in the *bhavitha choorna* were more compared to the *choorna* indicating the amplification of phytoconstituents in the *bhavitha choorna* by the process of *bhavana*.

Key words: *Apamarga*, *samskara*, *bhavana*, *choorna*, *bhavitha choorna*, HPTLC finger printing profile

Introduction

There is nothing in the universe which cannot be used as medicine.[1]But the necessity is to employ proper *yukti* (intelligence) according to the need of the situation for the proper and effective utilization of the medicament. *Samskara* is the pharmaceutical procedure which aids in imparting new properties in the substance.[2] *Bhavana* is one mode of *samskara* in which the powdered drugs are soaked in an appropriate *drava* (liquid medium) in which the *dravya* is kept completely immersed or it is triturated in the *drava* for specific time period.[3] The process of *bhavana* fulfil different purposes in *Ayurvedic* pharmaceutical field as the procedure helps in the purification of drugs, removing the adverse effect, amplifying the therapeutic activity of drugs, removing the toxicity in the drug or to decrease the *tikshnatha* of a drug and to increase the potency of the drug.[4] As the process of *bhavana* intensifies the efficacy of a drug a small dose of that particular drug can be made to produce a very therapeutic effect. *Apamarga* is a well-known drug that has been widely used in *Ayurvedic* classics. The plant was extensively used since *Vedic* period and has been renowned for immense medicinal value such as *rakshogna, rasayana, asmarinasana, ojovardhana* etc.[5] In *Samhithas* the drug is mentioned in various single and compound preparations like gruel preparation of *apamargabija* along with *godharasa* (inguana meat) in treatment of *kshuda*.[6] Various researches on phytochemical and pharmacological studies of the drug was conducted by various scholars. Several active principles such as alkaloids, flavonoids, steroids, saponins, terpinoids etc. having potent pharmacological actions like antihyperlipidemic, antihyperglycemic and antiobesity effect has been isolated from the plant.[7,8,9] Considering the medicinal values of the drug the was selected for the process of *bhavana*. High performance thin layer chromatographic technique is used to separate, identify and quantify the active constituents in a drug. As the *bhavana* can potentiate the drug the present study aimed to compare the findings of HPTLC finger printing profile obtained for the *choorna* and *bhavithachoorna* of the drug *Apamarga* (*Achyranthesaspera* Linn.)

Experimental

Material and methods

Collection and preparation of drug

i. Collection

The mature fresh plant *Apamarga* (*Achyranthesaspera*Linn.) devoid any contamination and insect infestation was uprooted and collected as whole plant from cultivated lands of kozhinjampara Panchayat in Palakkad district.

Figure no. 1 Plant of *Apamarga* (*Achyranthes aspera* Linn.)

ii. Identification

Collected fresh whole plants of *Apamarga* (*Achyranthes aspera* Linn.) were identified from the department of Dravyagunavijnanam, Government Ayurveda College, Tripunithura.

iii. Preparation

The fresh whole plant was washed thoroughly with water to remove physical impurities. A sufficient quantity of the drug was used for the preparation of *swarasa* (juice) for *bhavana* and *choorna* (powder).

Preparation of *choorna* (Powder)

A sufficient quantity of the drug was properly cleaned and dried under sunlight. It was then made into fine powder and sieved through mesh with size-120.

Preparation of *Swarasa* (juice) for *bhavana*

Swarasa from the whole plant was prepared based on its method of preparation mentioned in *Sarangadhara samhitha*.[10] Fresh whole plant of the drug was washed thoroughly to remove physical impurities like soil, mud etc. and the excess water was strained out using a strainer. The whole plant was cut into small pieces, crushed and pounded. It was then placed in a cotton cloth, pressed and squeezed out through the cloth and the expressed juice of the drug was collected in a clean container.

Preparation of *bhavitha choorna* (processed powder)

Bhavitha churna (processed powder) of whole plant of *Apamarga* (*Achyranthesaspera* Linn.) was prepared according to the reference of *bhavanavidhi* mentioned in *Bhaishajya ratnavali*.[11] The *choorna* of the drug was taken in a wide mouthed plastic tray. It was spread uniformly in the tray so that it forms a thin layer of thickness 1 cm. The *swarasa* of the drug was then gradually poured into the fine powder such that the *swarasa* get drained into the powder. Thus pouring of *swarasa* was continued until a thin layer of *swarasa* was seen present on the surface of the drug. Using a clean sharp thin rod ensured the complete soaking of each and every fine particles of the drug. To ensure uniform spreading of *bhavana dravya* in the fine particles of the powder the tray was slowly and uniformly shaken on both sides. Then the tray was then left overnight. On the next day morning the tray was taken and covered with a clean thin cloth to prevent the occurrence of any contamination from the external environment. It is then dried under sunlight. 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 fungal 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 7 times.

Choorna and *bhavithachoorna* (processed powder) of the whole plant were used the assessment of HPTLC finger printing profile

Figure no. 2 *Choorna* of the whole plant of *Apamarga* (*Achyranthes aspera* Linn.)

Figure no. 3 *Bhavitha choorna* of the whole plant of *Apamarga* (*Achyranthes aspera* Linn.)

HPTLC fingerprinting profile

Procedure

Preparation of sample

For maceration 10.0760 gm of the *choorna* and 10.4896 processed powder was kept in 50 ml methanol. Then it was filtered and 0.514 gm residue of *choorna* and 0.2121gm residue of *bhavitha choorna* was collected from it and was made up to 1ml methanol. 10µl of the prepared solution was applied on stationary phase for HPTLC fingerprinting.

Method

Stationary phase used was silica gel $60F_{254}$ (E MERCK K Ga A) of 6.0 ×10.0 cm without prewashing. The sample was spotted on the stationary phase using CAMAG Linomat 5 sampler at 8.0 mm from the edge of the plate. Application rate was 150 nl/s per seconds. The mobile phase used was toluene: ethyl acetate: methanol (6:3.8:0.2). TLC plate was prepared on saturated twin through glass chamber of 20×10 cm up to a distance of 80 mm. Plates were then dried at 60^0C for 5 minutes and transferred to CAMAG visualizer under UV 254nm and 366 nm. Post chromatographic derivatization was done using anisaldehydesulphuric acid 100ml and the plates were dried using oven at 120^0C for 10 minutes. Densitometry was done using slit dimension of 8.00 ×0.0.90 mm, Macro and the scanning speed was 20mm per seconds.

Results

i. HPTLC of *choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.)

Area and peaks obtained atwavelength254nm

12 peaks with total area of 99.99AU were obtained for *choorna* of the drug. These twelve peaks were defined with maximum Rf value of 0.01 with area 35434.5AU, maximum Rf value of 0.12 with area 7184.3 AU, maximum Rf value of 0.16 with area 3146.7AU, maximum Rf value of 0.24 with area 1912.0 AU, maximum Rf value of 0.32 with area 1312.0AU, maximum Rf value of 0.43 with area 4242.0AU, maximum Rf value of 0.43 with area 4242.0AU, maximum Rf value of 0.49 with area 763.3AU, maximum Rf value of 0.54 with area 1069.3AU, maximum Rf value of 0.59 with area 1069.3AU, maximum Rf value of 0.70 with area 1128.9AU, maximum Rf value of 0.79 with area 686.0AU and maximum Rf value of 0.83 with area 2051.4AU.

Table no. 1 Area and peaks of *choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.)

Peak no.	Start Rf	Max Rf	End Rf	Area(AU)	% Area(AU)
1	-0.03	0.01	0.09	35434.5	59.05
2	0.11	0.12	0.15	7184.3	11.97
3	0.15	0.16	0.21	3146.7	5.24
4	0.21	0.24	0.27	1912.0	3.19
5	0.28	0.32	0.33	1312.0	2.19
6	0.38	0.43	0.46	4242.0	7.07
7	0.48	0.49	0.51	763.3	1.27
8	0.52	0.54	0.57	1069.3	1.78
9	0.57	0.59	0.64	1072.8	1.79
10	0.64	0.70	0.73	1128.9	1.88
11	0.76	0.79	0.81	686.0	1.14
12	0.81	0.83	0.90	2051.4	3.42

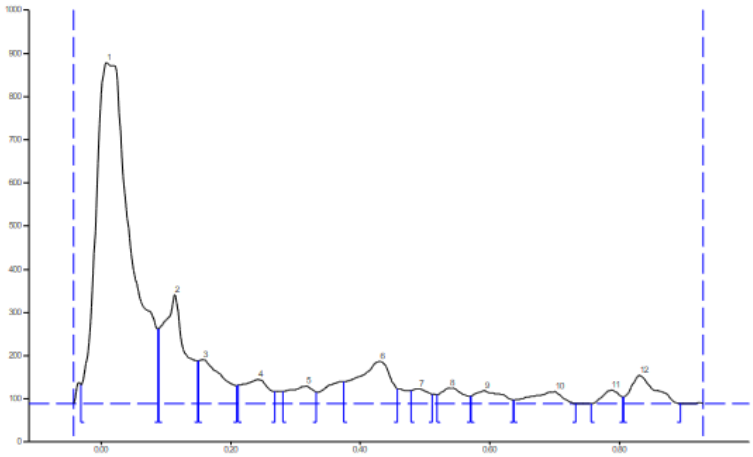

Diagram No: 1 Overview graph of *choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.) at 254nm

Figure no. 4 TLC plate view of *choorna* of *Apamarga* (*Achyranthes aspera* Linn.) at 254nm wavelength (*Achyranthesaspera* Linn.)

1Derivatization at different wavelengths of *choorna* of *Alabu*[*Lagenariasiceraria*(Mol.) Standely] 254nm

ii. HPTLC of *bhavitha choorna* of whole plant of *Apamarga* (*Achyranthesaspera* Linn.)

Area and peaks obtained at wavelength254nm

13 peaks with total area 100.01AU were obtained for *bhavitha choorna* of the drug. These thirteen peaks were defined with maximum Rf value of 0.01 with area 37427.5AU, maximum Rf value of 0.12 with area 6893.8AU, maximum Rf value of 0.17 with area 6536.3AU, maximum Rf value of 0.23 with area 3614.4AU, maximum Rf value of 0.30 with

area 5013.1AU, maximum Rf value of 0.35 with area 3279.4AU, maximum Rf value of 0.44 with area 9661.9AU, maximum Rf value of 0.49 with area 3078.3AU, maximum Rf value of 0.55 with area 3031.3AU, maximum Rf value of 0.60 with area 3031.3AU, maximum Rf value of 0.68 with area 4025.0AU, maximum Rf value of 0.72 with area 5759.0AU, maximum Rf value of 0.78 with area 7633.8AU

Table no. 2 Area and peaks of *bhavitha choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.)

Peak no.	Start Rf	Max Rf	End Rf	Area(AU)	Area % (AU)
1	-0.03	0.01	0.10	37427.5	35.90
2	0.11	0.12	0.15	6893.8	6.61
3	0.15	0.17	0.21	6536.3	6.27
4	0.21	0.23	0.25	3614.4	3.47
5	0.25	0.30	0.31	5013.1	4.81
6	0.34	0.35	0.38	3279.4	3.15
7	0.38	0.44	0.48	9661.9	9.27
8	0.48	0.49	0.52	3078.3	2.96
9	0.52	0.55	0.57	3031.3	2.91
10	0.57	0.60	0.66	8298.1	7.96
11	0.66	0.68	0.69	4025.0	3.86
12	0.70	0.72	0.75	5759.0	5.52
13	0.75	0.78	0.87	7633.8	7.32

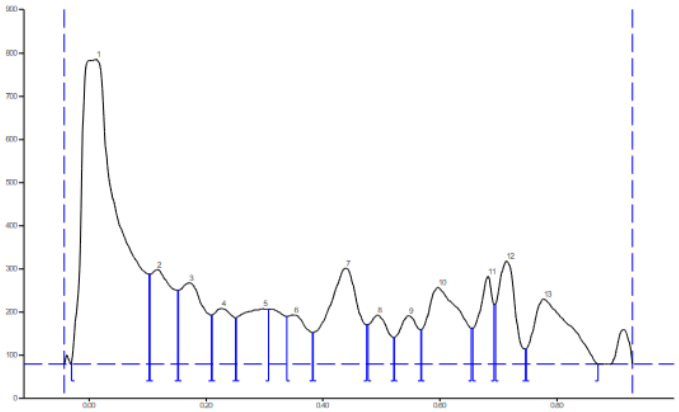

Diagram No: 2 Overview graph of *bhavitha choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.) at 254nm

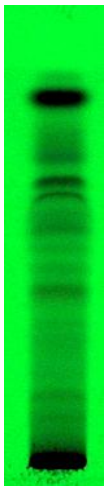

Figure no.5 TLC plate view of *bhavitha choorna* of *Apamarga* (*Achyranthes aspera* Linn.) at 254nm wavelength

Discussion

HPTLC finger printing profile of *choorna* and *bhavitha choorna* of whole plant of *Apamarga* (*Achyranthes aspera* Linn.) was carried out at wavelengths 254 nm. 12 peaks were obtained for *choorna* and 13 peaks for *bhavitha choorna.* Peak intensities were different in both sample but the presence of peaks with similar Rf values in both the *choorna* indicated the presence of same chemical constituent. 5 peaks were found common in both the drug samples. Peak with Rf value of 0.01 in *choorna* with area 35434.5AU and that in *bhavitha choorna* with area 37427.5AU were identified. Peak with Rf value 0.12 in *choorna* with area 7184.3 AU and that in *bhavitha choorna* with area 6893.8AU was also found. For *choorna* area obtained for maximum Rf 0.16 and Rf 0.17 were 3146.7AU and 6536.3AU respectively. Area obtained for maximum Rf value of 0.24 in *choorna* and maximum Rf value of 0.23 in *bhavitha choorna* were 1912.0 AU and 3614.4AU. Comparable maximum Rf value of 0.32 in *choorna* with area 1312.0AU and maximum Rf value of 0.30 with area 5013.1AU in *bhavitha choorna* were found. Thus prominent increase in the area of peak number 1, 3, 4 and 5 in *bhavitha choorna* compared to *choorna* indicates the increase in the concentration of the chemical constituent obtained at that particular peak as a result of *bhavana* process. Area obtained in maximum Rf value of 0.12 is 7184.3 AU and 6893.8AU for *choorna* and *bhavitha choorna* respectively. Here the peak area is reduced in *bhavitha choorna* indicating that the

concentration has been reduced in it. 7 Individuals peaks were obtained in the *choorna* and 8 individual peaks were obtained for *bhavitha choorna* indicating the presence of new compounds in the *choorna* and *bhavitha choorna*.

Conclusion

The process of *bhavana* increases the potency in the drug. It adds on to its therapeutic efficacy. From the HPTLC finger printing profile obtained for the *choorna* and *bhavitha choorna* more number of peaks were found in the *bhavitha choorna*. The process of *bhavana* intensified the phtyoconstuitents in the *bhavitha choorna* compared to *choorna* which is evident from the amplification of peaks areas obtained for the *bhavitha choorna*.

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Conflicts of Interest

There is no conflict of interest associated with this publication

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