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# High performance thin layer chromatography fingerprint profile of ingredients and formulations of Shatavaryadi churna: an ayurvedic formulation

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## Abstract

*Objective:* To develop the finger print of medicinally important ayurvedic formulation Shatavaryadi churna and its ingredients for presence of phytoconstituents in different extracts. *Methods:* Petroleum ether, chloroform, ethyl acetate and methanolic extract of all the ingredients and formulations were developed in the mobile phase n-hexane: diethyl ether (3:1), n-hexane: ethyl acetate: formic acid (6:4:0.2) n-hexane: ethyl acetate: formic acid (6:4:0.2) and toluene: ethyl acetate: formic acid (5:4:0.2) respectively using standard procedures and scanned under UV at suitable wavelength. *Results:* The HPTLC fingerprinting of all the extracts has shown several peaks with different $R_f$ values. *Conclusion:* This fingerprint profile would be helpful in the identification and authentication of the selected ingredients and the formulations.

**Keywords:**Shatavaryadi churna, High Performance Thin Layer Chromatography (HPTLC), Fingerprinting, *Asparagus racemosus,* Ayurvedic formulation.

## Introduction

Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening; plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result, people are more favorable to use natural compounds obtained from plants [1]. It has been estimated that 56% of the lead compounds for medicines in the British National Formulary are natural products [2]. Phytochemical analysis of plants which were used in folklore has yielded a number of compounds with various pharmacological activities. Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations. [3,4]

Shatavaryadi churna is a traditional Ayurvedic powdered formulation used for centuries with claimed efficacy and safety in immunomodulator, galactagogue, aphrodisiac and rejuvenator activities. The churna contains powder of tubers of *Asparagus racemosus* Willd.-1part, tubers of *Chlorophytum tuberosum* Baker.-1part, roots of *Withania somnifera* Dunal.-1part, seeds of *Mucuna pruriens* Linn.-1part, fruits of *Tribulus terrestris* Linn.-1part [5].

In the recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines [6]. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products and therefore be used for authentication and identification of herbal plant [7,8]. HPTLC is more efficient, faster method and the results are more reliable and reproducible. In combination with digital scanning profiling, HPTLC also provides accurate and precise $R_f$ values with purity of the individual spots as well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters i.e. $R_f$, height and area [9]. The main objective of this study was to evaluate and optimize the HPTLC fingerprint method in standardization of Shatavaryadi churna and its ingredients to provide beneficial information with reference to the standardization.

## Materials and Method

### Plant Materials

The plant materials of Shatavaryadi churna were collected from the different sources and authenticated by Dr. E. Roshini Nayar, Principal Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic Resources, Indian Council of Agricultural Research, Pusa Campus, New Delhi (India) i.e. Shatavari *(Asparagus racemosus* Willd.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-7/); Safed *Musli (Chlorophytum tuberosum* Baker.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-8/), Ashwagandha *(Withania somnifera* Dunal.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/2011-10/), Atmagupta *(Mucuna pruriens* (L.) DC) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.:

NHCP/NBPGHR/2011-6/) and Goksura *(Tribulus terrestris* Linn.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/2011-9/). Parts of the ingredients were crushed and powdered using grinder and passed through sieve number#85. In-house Shatavaryadi churna was prepared from these powders by mixing them in one part for each ingredient and named as IH. Marketed Shatavaryadi churna was also procured from local market and named as M.

## Sample preparation

The powders of sample were extracted with different solvents (analytical grade and were purchased from Merck Chemicals, India) ranging from non-polar to polar solvents. About 100g of the crude drug powder was subjected for soxhlet extraction with petroleum ether (40-60$^0$C) for 12 h. The extract was concentrated under reduced pressure at 40$^0$C. The dried marc were again subjected to successive extraction with different solvents i.e. chloroform, ethyl acetate and methanol. Same procedures were followed for extraction of all the samples. 10 mg of concentrated extract was dissolved in 10 ml of respective solvent in which they were extracted and all samples were prepared with same amount of extract and solvents.

## Chromatography

The finger printing of different extracts of *Asparagus racemosus* Willd. (AR), tubers of *Chlorophytum tuberosum* Baker. (CT), roots of *Withania somnifera* Dunal. (WS), seeds of *Mucuna pruriens* Linn. MP), fruits of *Tribulus terrestris* Linn. (TT), Shatavaryadi churna *In-house* formulation (IH) and Marketed formulation (M) were carried out using CAMAG HPTLC system (Switzerland) with a Linomat IV auto sample applicator. The analysis was performed in air-conditioned room maintained at 22$^0$C. HPTLC was performed on precoated silica gel HPTLC aluminium plates 60 F$^{254}$ (20cm 10cm/10cm 10cm, 0.2mm thickness, 5-6µm particle size, E. Merck, Germany). 5-10µl of the sample (1µg/µl) solutions were spotted as band of 4mm or 5mm width by using auto samples fitted with a 100 µl Hamilton syringe. The plates were developed using optimized solvent system i.e. n-hexane: diethyl ether (3:1), n-hexane: ethyl acetate: formic acid (6:4:0.2) and n-hexane: ethyl acetate: formic acid (6:4:0.2) for petroleum ether (40-60$^0$C), chloroform, ethyl acetate and methanolic extract respectively in a CAMAG twin trough plate development chamber which was lined with filter paper and pre-saturated with 30ml mobile phase. The developed plates were air dried and scanned. A spectro-densitometer (Scanner 3, CAMAG) equipped with WINCATS planar chromatography manager software was used for the densitometry Measurement and data processing. Absorbance/emission was the measurement mode at a scan speed of 20 mm/sec. Spots of fraction were scanned from 200 to 800 nm so as to record their UV-VIS spectrum and to obtain wavelengths of maximum absorption. Densitogram were recorded at the wavelength of maximum absorption of the different sample spotted.

## Results

All the ingredients and formulations were subjected to HPTLC analysis by specific solvent systems for respective extracts and detected under UV at 254nm. The chromatograms shown in Figure 1-7 that all sample constituents were clearly separated.

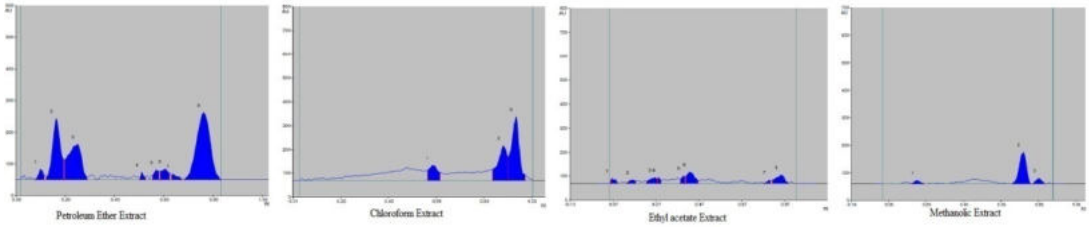


**Figure 1** HPTLC chromatogram of various extracts of *Asparagus racemosus* Willd.

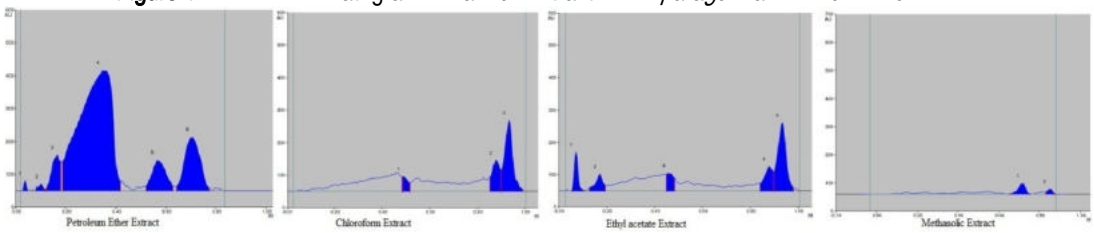


**Figure 2:** HPTLC chromatogram of various extracts of *Chlorophytum tuberosum* Baker.

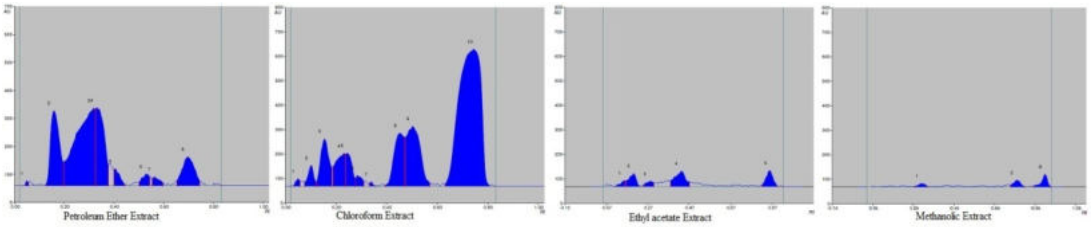


**Figure 3:** HPTLC chromatogram of various extracts of *Withania somnifera* Dunal.

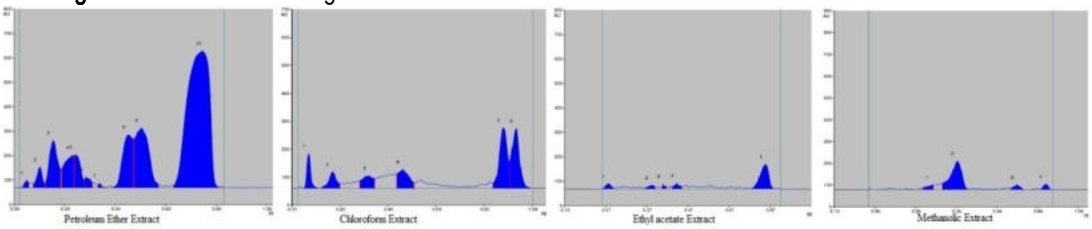


**Figure 4** : HPTLC chromatogram of various extracts of *Mucuna pruriens* Linn.

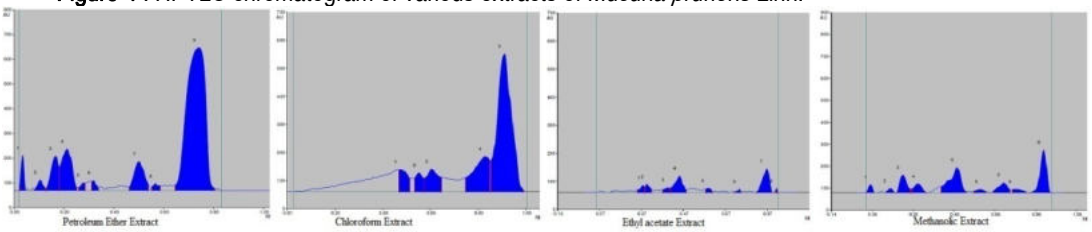


**Figure 5** : HPTLC chromatogram of various extracts of *Tribulus terrestris* Linn.

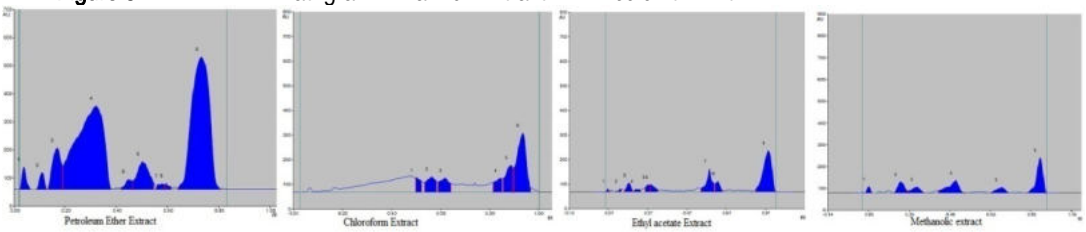


**Figure 6** :HPTLC chromatogram of various extracts of Shatavaryadi churna *In-house* formulation.

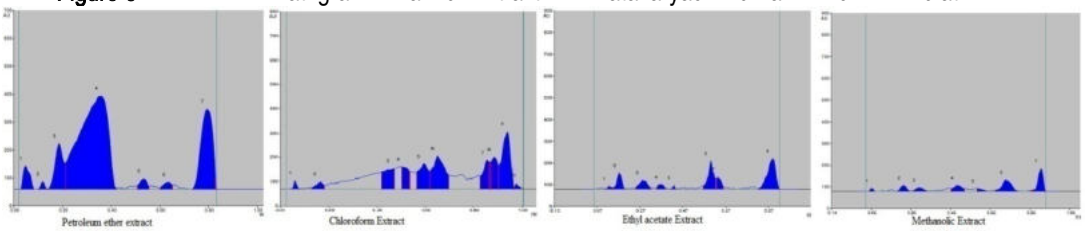


**Figure 7** : HPTLC chromatogram of various extracts of Marketed formulation

It is evident from Table 1-2 that in the petroleum extracts there were 8, 6, 8, 10, 9, 9 and 7 spots respectively for AR, CT, WS, MP, TT, IH and M (Figure 1-7). Table 1 and 2 indicates the presence of minimum number of components in petroleum ether extract of respective sample, the components with $R_f$ value 0.76, 0.35, 0.32, 0.75, 0.74, 0.73 and 0.36 respectively for AR, CT, WS, MP, TT, IH and M were found to be more predominant as the percentage area is more.''

**Table 1:** Peak table for petroleum ether extract of ingredients of Shatavaryadi churna.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *Asparagus racemosus* | 1 | 0.10 | 537.2 | 2.23 | 0.978 | 0.975 | OK |
| | 2 | 0.16 | 5552.3 | 23.04 | 0.999 | 0.998 | OK |
| | 3 | 0.25 | 5432.9 | 22.55 | 0.995 | 0.989 | OK |
| | 4 | 0.51 | 251.3 | 1.04 | 0.992 | 0.992 | OK |
| | 5 | 0.57 | 515.8 | 2.14 | 0.991 | 0.994 | OK |
| | 6 | 0.61 | 867.5 | 3.60 | 0.992 | 0.987 | OK |
| | 7 | 0.64 | 354.0 | 1.47 | 0.994 | 0.995 | OK |
| | 8 | 0.76 | 10583.5 | 43.92 | 0.998 | 0.986 | OK |
| *Chlorophytum Tuberosum* | 1 | 0.04 | 275.7 | 0.45 | 0.996 | 0.995 | OK |
| | 2 | 0.10 | 374.1 | 0.62 | 0.959 | 0.967 | OK |
| | 3 | 0.17 | 3233.3 | 5.33 | 0.975 | 0.985 | OK |
| | 4 | 0.35 | 42998.7 | 70.86 | 0.996 | 0.998 | OK |
| | 5 | 0.57 | 4686.2 | 7.72 | 0.999 | 0.996 | OK |
| | 6 | 0.71 | 9115.1 | 15.02 | 0.998 | 0.990 | OK |
| *Withania somnifera* | 1 | 0.05 | 158.7 | 0.36 | 0.999 | 0.999 | OK |
| | 2 | 0.16 | 8883.2 | 19.91 | 0.999 | 0.974 | OK |
| | 3 | 0.32 | 19141.0 | 42.89 | 0.990 | 0.999 | OK |
| | 4 | 0.33 | 8655.0 | 19.39 | 0.986 | 0.997 | OK |
| | 5 | 0.41 | 1280.3 | 2.87 | 0.992 | 0.987 | OK |
| | 6 | 0.53 | 1106.3 | 2.48 | 0.995 | 0.971 | OK |
| | 7 | 0.56 | 783.6 | 1.76 | 0.994 | 0.989 | OK |
| | 8 | 0.70 | 4617.6 | 10.35 | 0.996 | 0.990 | OK |
| *Mucuna pruriens* | 1 | 0.05 | 419.6 | 0.52 | 0.995 | 0.978 | OK |
| | 2 | 0.10 | 1681.4 | 2.07 | 0.998 | 0.986 | OK |
| | 3 | 0.16 | 5814.6 | 7.17 | 0.994 | 0.999 | OK |
| | 4 | 0.23 | 4499.5 | 5.55 | 0.959 | 0.978 | OK |
| | 5 | 0.24 | 2866.8 | 3.54 | 0.996 | 0.987 | OK |
| | 6 | 0.29 | 906.3 | 1.12 | 0.994 | 0.973 | OK |
| | 7 | 0.34 | 183.8 | 0.23 | 0.999 | 0.999 | OK |
| | 8 | 0.46 | 7858.6 | 9.69 | 0.998 | 0.998 | OK |
| | 9 | 0.51 | 12079.0 | 14.90 | 0.998 | 0.996 | OK |
| | 10 | 0.75 | 44761.1 | 55.21 | 0.989 | 0.993 | OK |
| *Tribulus terrestris* | 1 | 0.03 | 1554.5 | 2.70 | 0.999 | 0.992 | OK |
| | 2 | 0.11 | 811.5 | 1.41 | 0.977 | 0.988 | OK |
| | 3 | 0.17 | 3624.3 | 6.29 | 0.996 | 0.997 | OK |
| | 4 | 0.21 | 6242.5 | 10.83 | 0.999 | 0.998 | OK |
| | 5 | 0.28 | 505.9 | 0.88 | 0.995 | 0.997 | OK |
| | 6 | 0.32 | 630.2 | 1.09 | 0.992 | 0.980 | OK |
| | 7 | 0.50 | 4295.8 | 7.46 | 0.997 | 0.979 | OK |
| | 8 | 0.57 | 581.2 | 1.01 | 0.998 | 0.995 | OK |
| | 9 | 0.74 | 39376.8 | 68.34 | 0.999 | 0.996 | OK |

**Table 2** :Peak table for petroleum ether extract of Shatavaryadi churna formulations.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *In-house* formulation | 1 | 0.04 | 1235.1 | 1.75 | 0.971 | 0.961 | OK |
| | 2 | 0.11 | 929.9 | 1.32 | 0.978 | 0.980 | OK |
| | 3 | 0.17 | 4497.2 | 6.38 | 0.956 | 0.983 | OK |
| | 4 | 0.32 | 28381.7 | 40.27 | 0.999 | 0.998 | OK |
| | 5 | 0.45 | 858.8 | 1.22 | 0.973 | 0.997 | OK |
| | 6 | 0.50 | 4152.3 | 5.89 | 0.998 | 0.957 | OK |
| | 7 | 0.58 | 431.5 | 0.61 | 0.984 | 0.992 | OK |
| | 8 | 0.60 | 270.9 | 0.38 | 0.998 | 0.984 | OK |
| | 9 | 0.73 | 29728.7 | 42.18 | 0.999 | 0.993 | OK |
| Marketed formulation | 1 | 0.05 | 2038.7 | 3.60 | 0.997 | 0.975 | OK |
| | 2 | 0.12 | 361.8 | 0.64 | 0.989 | 0.975 | OK |
| | 3 | 0.19 | 4924.5 | 8.70 | 0.993 | 0.983 | OK |
| | 4 | 0.36 | 35101.2 | 62.03 | 0.993 | 0.998 | OK |
| | 5 | 0.53 | 993.7 | 1.76 | 0.979 | 0.956 | OK |
| | 6 | 0.64 | 728.9 | 1.29 | 0.997 | 0.996 | OK |
| | 7 | 0.79 | 12434.0 | 21.97 | 0.999 | 0.995 | OK |

It is revealed from Table 3-4 that in chloroform extracts there were 3, 3, 5, 6, 5, 6 and 10 spots respectively for AR, CT, WS, MP, TT, IH and M (Figure 1-7). Table 3 and 4 indicates the presence of minimum number of components in chloroform extract of respective sample, the components with $R_f$ value 0.93, 0.93, 0.93, 0.88, 0.91, 0.93 and 0.93 respectively for AR, CT, WS, MP, TT, IH and M were found to be more predominant as the percentage area is more.

**Table 3:** Peak table for chloroform extract of ingredients of Shatavaryadi churna.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *Asparagus racemosus* | 1 | 0.58 | 2461.9 | 13.70 | 0.997 | 0.993 | OK |
| | 2 | 0.88 | 6070.7 | 33.77 | 0.992 | 0.999 | OK |
| | 3 | 0.93 | 9442.6 | 52.53 | 0.984 | 0.987 | OK |
| *Chlorophytum Tuberosum* | 1 | 0.48 | 1204.6 | 9.45 | 0.963 | 0.985 | OK |
| | 2 | 0.87 | 3374.2 | 26.48 | 0.994 | 0.996 | OK |
| | 3 | 0.93 | 8164.4 | 64.07 | 0.976 | 0.984 | OK |
| *Withania somnifera* | 1 | 0.07 | 2008.2 | 12.14 | 0.999 | 0.999 | OK |
| | 2 | 0.17 | 1736.5 | 10.50 | 0.997 | 0.997 | OK |
| | 3 | 0.45 | 1766.5 | 10.68 | 0.978 | 0.966 | OK |
| | 4 | 0.87 | 2945.0 | 17.81 | 0.996 | 0.979 | OK |
| | 5 | 0.93 | 8079.6 | 48.86 | 0.973 | 0.985 | OK |
| *Mucuna pruriens* | 1 | 0.06 | 2126.9 | 8.35 | 0.999 | 0.999 | OK |
| | 2 | 0.16 | 2070.8 | 8.13 | 0.998 | 0.990 | OK |
| | 3 | 0.32 | 2247.9 | 8.83 | 0.995 | 0.978 | OK |
| | 4 | 046 | 3407.1 | 13.38 | 0.969 | 0.963 | OK |
| | 5 | 0.88 | 8003.0 | 31.44 | 0.999 | 0.999 | OK |
| | 6 | 0.93 | 7603.0 | 29.86 | 0.973 | 0.986 | OK |
| *Tribulus terrestris* | 1 | 0.47 | 3202.5 | 6.25 | 0.993 | 0.983 | OK |
| | 2 | 0.55 | 2295.2 | 4.48 | 0.989 | 0.951 | OK |
| | 3 | 0.60 | 4458.9 | 8.70 | 0.991 | 0.979 | OK |
| | 4 | 0.82 | 9000.6 | 17.56 | 0.987 | 0.999 | OK |
| | 5 | 0.91 | 32288.6 | 63.01 | 0.996 | 0.992 | OK |

**Table 4:** Peak table for chloroform extract of Shatavaryadi churna formulations.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *In-house* formulation | 1 | 0.50 | 1295.2 | 6.21 | 0.968 | 0.975 | OK |
| | 2 | 0.56 | 2459.1 | 11.80 | 0.981 | 0.972 | OK |
| | 3 | 0.62 | 2305.9 | 11.06 | 0.982 | 0.953 | OK |
| | 4 | 0.84 | 1674.6 | 8.03 | 0.987 | 0.999 | OK |
| | 5 | 0.89 | 3296.9 | 15.82 | 0.993 | 0.999 | OK |
| | 6 | 0.93 | 9809.7 | 47.07 | 0.961 | 0.979 | OK |
| Marketed formulation | 1 | 0.06 | 421.0 | 1.17 | 0.995 | 0.988 | OK |
| | 2 | 0.16 | 858.9 | 2.39 | 0.996 | 0.991 | OK |
| | 3 | 0.46 | 3937.9 | 10.95 | 0.985 | 0.999 | OK |
| | 4 | 0.50 | 2592.6 | 7.21 | 0.962 | 0.993 | OK |
| | 5 | 0.59 | 4474.4 | 12.44 | 0.995 | 0.997 | OK |
| | 6 | 0.65 | 7120.1 | 19.79 | 0.999 | 0.979 | OK |
| | 7 | 0.85 | 3650.9 | 10.15 | 0.990 | 0.999 | OK |
| | 8 | 0.88 | 3706.2 | 10.30 | 0.999 | 0.996 | OK |
| | 9 | 0.93 | 8848.9 | 24.60 | 0.969 | 0.988 | OK |
| | 10 | 0.97 | 362.7 | 1.01 | 0.996 | 0.998 | OK |

**Table 5:** Peak table for ethyl acetate extract of ingredients of Shatavaryadi churna.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *Asparagus racemosus* | 1 | 0.06 | 322.0 | 6.77 | 0.999 | 0.996 | OK |
| | 2 | 0.16 | 331.1 | 6.96 | 0.999 | 0.999 | OK |
| | 3 | 0.26 | 683.1 | 14.35 | 0.999 | 0.999 | OK |
| | 4 | 0.28 | 233.5 | 4.91 | 0. 999 | 0.999 | OK |
| | 5 | 0.40 | 433.9 | 9.12 | 0.999 | 0.999 | OK |
| | 6 | 0.42 | 1424.3 | 29.92 | 0.999 | 0.999 | OK |
| | 7 | 0.79 | 156.7 | 3.29 | 0.999 | 0.999 | OK |
| | 8 | 0.85 | 1175.0 | 24.69 | 0.999 | 0.999 | OK |
| *Chlorophytum Tuberosum* | 1 | 0.15 | 462.0 | 6.33 | 0.999 | 0.999 | OK |
| | 2 | 0.20 | 1560.9 | 21.40 | 0.999 | 0.999 | OK |
| | 3 | 0.27 | 642.3 | 8.81 | 0.999 | 0.994 | OK |
| | 4 | 0.43 | 2756.8 | 37.80 | 0. 999 | 0.998 | OK |
| | 5 | 0.86 | 1871.6 | 25.66 | 0.999 | 0.999 | OK |
| *Withania somnifera* | 1 | 0.09 | 1712.7 | 13.28 | 0.999 | 0.999 | OK |
| | 2 | 0.15 | 934.1 | 7.24 | 0.999 | 0.999 | OK |
| | 3 | 0.19 | 287.5 | 2.23 | 0.999 | 0.999 | OK |
| | 4 | 0.23 | 341.4 | 2.65 | 0. 999 | 0.999 | OK |
| | 5 | 0.28 | 570.6 | 4.42 | 0.999 | 0.999 | OK |
| | 6 | 0.30 | 735.3 | 5.70 | 0.999 | 0.999 | OK |
| | 7 | 0.48 | 447.9 | 3.47 | 0.999 | 0.999 | OK |
| | 8 | 0.60 | 193.8 | 1.50 | 0.999 | 0.999 | OK |
| | 9 | 0.86 | 7677.1 | 59.51 | 0.998 | 0.996 | OK |
| *Mucuna pruriens* | 1 | 0.08 | 425.9 | 8.18 | 0.999 | 0.996 | OK |
| | 2 | 0.29 | 363.1 | 6.97 | 0.999 | 0.999 | OK |
| | 3 | 0.35 | 197.2 | 3.79 | 0.999 | 0.999 | OK |
| | 4 | 0.41 | 519.8 | 9,98 | 0. 999 | 0.999 | OK |
| | 5 | 0.85 | 3702.2 | 71.08 | 0.999 | 0.998 | OK |
| *Tribulus terrestris* | 1 | 0.28 | 534.7 | 8.35 | 0.998 | 0.999 | OK |
| | 2 | 0.29 | 483.2 | 7.54 | 0.999 | 0.999 | OK |
| | 3 | 0.39 | 391.8 | 6.12 | 0.999 | 0.999 | OK |
| | 4 | 0.45 | 1979.2 | 30.89 | 0. 995 | 0.999 | OK |
| | 5 | 0.58 | 314.2 | 4.90 | 0.999 | 0.999 | OK |
| | 6 | 0.73 | 152.4 | 2.38 | 0.999 | 0.999 | OK |
| | 7 | 0.86 | 2451.6 | 38.26 | 0.999 | 0.999 | OK |
| | 8 | 0.91 | 100.2 | 1.56 | 0.999 | 0.999 | OK |

Table 5-6 indicates that in ethyl acetate extracts there were 7, 5, 9, 5, 7, 9 and 8 spots respectively for AR, CT, WS, MP, TT, IH and M (Figure 1-7). Table 5 and 6 indicates the presence of minimum number of components in ethyl acetate extract of respective sample, the components with $R_f$ value 0.93, 0.93, 0.93, 0.88, 0.91, 0.93 and 0.93 respectively for AR, CT, WS, MP, TT, IH and M were found to be more predominant as the percentage area is more.

Table 7-8 indicates that in methanolic extracts there were 3, 2, 3, 4, 9, 6 and 7 spots respectively for AR, CT, WS, MP, TT, IH and M (Figure 1-7). Table 7 and 8 indicates the presence of minimum number of components in methanolic extract of respective sample, the components with $R_f$ value 0.72, 0.78, 0.91, 0.47, 0.48, 0.91 and 0.92 respectively for AR, CT, WS, MP, TT, IH and M were found to be more predominant as the percentage area is more.

**Table 6:** Peak table for ethyl acetate extract of Shatavaryadi churna formulations.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *In-house* formulation | 1 | 0.07 | 81.0 | 0.72 | 0.996 | 0.998 | OK |
| | 2 | 0.13 | 114.1 | 1.01 | 0.999 | 0.999 | OK |
| | 3 | 0.17 | 656.4 | 5.82 | 0.999 | 0.999 | OK |
| | 4 | 0.21 | 188.7 | 1.67 | 0. 999 | 0.999 | OK |
| | 5 | 0.27 | 484.9 | 4.30 | 0.997 | 0.999 | OK |
| | 6 | 0.29 | 550.5 | 4.88 | 0.997 | 0.999 | OK |
| | 7 | 0.58 | 2092.8 | 18.57 | 0.994 | 0.998 | OK |
| | 8 | 0.62 | 827.6 | 7.34 | 0.997 | 0.999 | OK |
| | 9 | 0.88 | 6275.3 | 55.67 | 0.997 | 0.995 | OK |
| Marketed formulation | 1 | 0.12 | 195.4 | 1.60 | 0.999 | 0.999 | OK |
| | 2 | 0.17 | 1426.2 | 11.70 | 0.999 | 0.997 | OK |
| | 3 | 0.28 | 1311.2 | 10.76 | 0.996 | 0.995 | OK |
| | 4 | 0.37 | 508.7 | 4.17 | 0. 999 | 0.999 | OK |
| | 5 | 0.43 | 122.4 | 1.00 | 0.999 | 0.999 | OK |
| | 6 | 0.60 | 2923.0 | 23.98 | 0.999 | 0.999 | OK |
| | 7 | 0.63 | 1023.1 | 8.39 | 0.971 | 0.992 | OK |
| | 8 | 0.89 | 4680.8 | 38.40 | 0.996 | 0.996 | OK |

**Table 7** :Peak table for methanolic extract of ingredients of Shatavaryadi churna.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *Asparagus racemosus* | 1 | 0.21 | 305.1 | 6.85 | 0.982 | 0.989 | OK |
| | 2 | 0.72 | 3624.4 | 81.41 | 0.991 | 0.996 | OK |
| | 3 | 0.83 | 522.5 | 11.74 | 0.999 | 0.999 | OK |
| *Chlorophytum Tuberosum* | 1 | 0.78 | 1200.8 | 75.79 | 0.999 | 0.999 | OK |
| | 2 | 0.91 | 383.6 | 24.21 | 0.998 | 0.969 | OK |
| *Withania somnifera* | 1 | 0.30 | 363.9 | 15.42 | 0.990 | 0.971 | OK |
| | 2 | 0.77 | 773.8 | 32.79 | 0.984 | 0.997 | OK |
| | 3 | 0.91 | 1222.3 | 51.79 | 0.998 | 0.989 | OK |
| *Mucuna pruriens* | 1 | 0.34 | 596.9 | 7.94 | 0.999 | 0.999 | OK |
| | 2 | 0.47 | 5869.9 | 78.10 | 0.985 | 0.974 | OK |
| | 3 | 0.76 | 568.2 | 7.56 | 0.999 | 0.999 | OK |
| | 4 | 0.90 | 481.2 | 6.40 | 0. 993 | 0.996 | OK |
| *Tribulus terrestris* | 1 | 0.05 | 554.0 | 3.20 | 0.999 | 0.999 | OK |
| | 2 | 0.15 | 370.7 | 2.14 | 0.999 | 0.999 | OK |
| | 3 | 0.21 | 2242.5 | 12.95 | 0.999 | 0.999 | OK |
| | 4 | 0.29 | 1287.4 | 7.44 | 0.999 | 0.999 | OK |
| | 5 | 0.48 | 5141.5 | 29.70 | 0.999 | 0.998 | OK |
| | 6 | 0.60 | 451.7 | 2.61 | 0.996 | 0.999 | OK |
| | 7 | 0.71 | 1621.2 | 9.36 | 0.999 | 0.999 | OK |
| | 8 | 0.76 | 592.1 | 3.42 | 0.999 | 0.996 | OK |
| | 9 | 0.90 | 5051.4 | 29.18 | 0.998 | 0.996 | OK |

**Table 8** :Peak table for methanolic extract of Shatavaryadi churna formulations.

| Sample | Peak | $R_f$ value | Area | Area % | r(s,m) | r(m,e) | Purity |
|---|---|---|---|---|---|---|---|
| *In-house* formulation | 1 | 0.06 | 349.6 | 3.52 | 0.999 | 0.999 | OK |
| | 2 | 0.22 | 1369.0 | 13.68 | 0.999 | 0.999 | OK |
| | 3 | 0.30 | 891.3 | 8.97 | 0.999 | 0.998 | OK |
| | 4 | 0.49 | 2399.6 | 24.16 | 0.995 | 0.997 | OK |
| | 5 | 0.72 | 855.5 | 8.61 | 0.997 | 0.999 | OK |
| | 6 | 0.91 | 4076.7 | 41.05 | 0.996 | 0.998 | OK |
| Marketed formulation | 1 | 0.06 | 145.1 | 2.19 | 0.999 | 0.999 | OK |
| | 2 | 0.22 | 622.2 | 9.38 | 0.999 | 0.999 | OK |
| | 3 | 0.30 | 478.5 | 7.22 | 0.999 | 0.995 | OK |
| | 4 | 0.49 | 929.8 | 14.02 | 0.995 | 0.996 | OK |
| | 5 | 0.60 | 271.4 | 4.09 | 0.982 | 0.993 | OK |
| | 6 | 0.74 | 2011.9 | 30.33 | 0.984 | 0.960 | OK |
| | 7 | 0.92 | 2173.4 | 32.77 | 0.997 | 0.999 | OK |

## Conclusion

Present study proves that the HPTLC technique is feasible for development of chromatographic fingerprints to determine major active constituents of medicinal plants. The separation and resolution are much better, and the results are much more reliable and reproducible than TLC. The results obtained from qualitative evaluation of HPTLC fingerprint will be helpful in the identification and quality control of the drug. HPTLC analysis of all the ingredients and formulations of Shatavaryadi churna can provide standard fingerprints and can be used as a reference for the identification and quality control of the drug. The present study may be useful for the isolation, purification, characterization and identification of marker chemical compounds of the respective samples; the identified marker can be used for routine standardization of Shatavaryadi churna formulations.

## Author's contributions

Puspendra Kumar carried out the extraction of the samples; solvent system development and HPTLC fingerprint analyss. Dr. Shivesh Jha carried out the participated in the design of the study, interpretation of the data and drafting of manuscript. Dr. Tanveer Naved conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript

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