

## STANDARDIZATION OF *SHATAVARI* (*Asparagus racemosus*) W.S.R TO HPTLC PROFILE

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### ABSTRACT

*Shatavari* is a herbal drug used from ancient time in different compound formulations as well as single for the treatment of various diseases. In the present study the used parts of *Shatavari* tubers are assessed for its HPTLC (High performance thin layer chromatography) findings. In this study, the sample medicine is evaluated for chemical constituents present in *Shatavari* (*Asparagus racemosus* Linn) tubers. In the modern era advancement of science chromatographic and spectral fingerprints plays an important role in the quality control of herbal medicines. High Performance Thin Layer Chromatography has become a routine analytical technique due to its advantages of reliability in

quantification of analytics at micro and even in nanogram levels and cost effectiveness. It has been proved as a very useful technique because of its low operating cost, high sample throughput and need for minimum sample clean-up. The major advantage of HPTLC is in reducing analysis time and cost per analysis. *Shatavari* is rich source of Shatavarin IV present in tuber which is responsible for its pharmacological action. The data obtained is discussed critically to lay out the possible way of raw drug identification for herbal material. Hope this scientific critique will be a step ahead for drug identification of *Shatavari* in Ayurvedic system of treatment.

**KEYNOTE:** *Shatavari* Shatavarin, HPTLC, alcoholic extract.

### INTRODUCTION

*Shatavari* is a sacnadt, much branched spinous undershrub with tuberous, short tuber stock bearing numerous fusiform tuberous tubers, 30- 100 cm long and 1-2 cm thick. Leaves reduced to minute chaffy scales and spines. Cladodes acicular, 2-6 nate, falcate, finely acuminate.<sup>[1]</sup> It grows upward direction and having thorns on its stalk. Branches are

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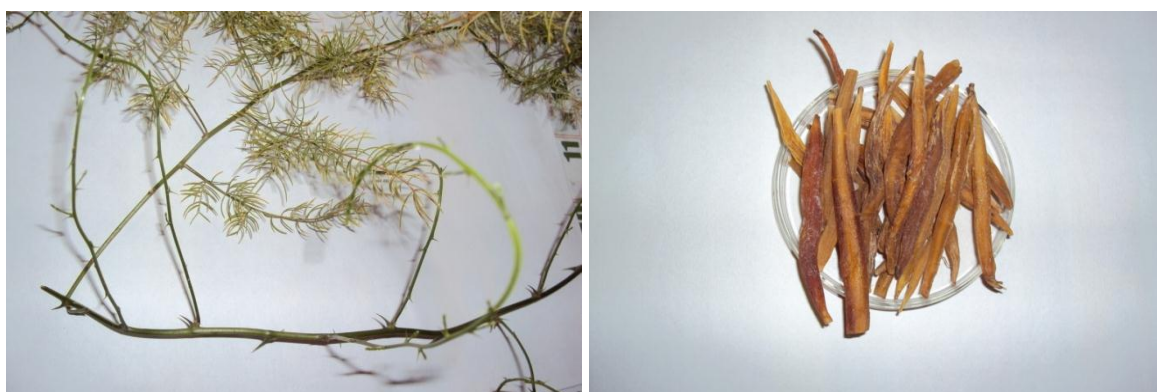
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triangular, smooth with straight line on it. Thorns are curved and 0.60 to 1.25 cm in length. Flowers are small, white and fragrant, appear in bunches. Fruits are small round shape, 1-2 seeds, which become red when ripened. There are many thick oblong tuberlets near the main tuber.<sup>[2]</sup> As per Ayurvedic classical text its pharmacodynamics properties comprises of *madhura* and *tikta-rasa*, *guru* and *snigdha guna*, *sheeta veerya* & *madhura vipak*.<sup>[3-5]</sup>

*Shatavari* is used as a galactagogue and for disorders of female genitourinary tract; as a styptic and ulcer-healing agent; as an intestinal disinfectant and astringent in diarrhoea; as a nervine tonic, and in sexual debility for spermatogenesis. Along with other therapeutic applications, The Ayurvedic Pharmacopoeia of India indicates the use of the tuberous root in gout, puerperal diseases, lactic disorders, haematuria, bleeding disorders and also recommends it for hyperacidity.<sup>[6]</sup>

For the authentication of the raw material, now a days different physico-chemical parameters are used for its quality assessment. To establish the fingerprint of a particular herbal material, its phyto-chemical findings of HPTLC is the basic tool. This tool is also facilitating the raw drug (herbal) standardization a step ahead. Though the identifying the study material i.e. *Shatavari* has been defined in the ancient texts but for facilitating the cross disciplinary debate and for global acceptance, honest efforts have been made to assess it on the above said parameters and for establishing the data obtained.

Due to its applicability in many Ayurvedic formulations, adulterations of this highly potent material become very usual. This unlawful commercialization, in turn causes decreasing of the quality of the medicine. It is essential to standardize the raw material for preparation of authentic medicines.<sup>[7]</sup> Hence the current study was undertaken to standardize the *Shatavari* sample available in market.



**Shatavari Plant Shatavari Tuber.**

## MATERIALS AND METHOD

The sample material i.e. *Shatavar* was assessed for its phyto-chemical values specially HPTLC to establish the possible fingerprints for its authentication.

### Materials

Following materials are required for HPTLC analysis.

### Drug

The tuber powder of *Shatavari* was used for HPTLC. Alcoholic extract of *Shatavari* is used for this procedure. The phytochemical analysis of the drug *Shatavari* was carried out from the “Institute of pharmaceutical science” Jalandhar. Sample material i.e. *Shatavari* was collected from authenticated shop in Delhi market. Alcoholic extract of *Shatavari* tuber powder was prepared in the laboratory.

### Chemicals and Reagents

- n-Butanol
- Glacial acetic acid
- Ethylene acetate
- Chloroform
- Methanol

### Apparatus used

- ✓ HPTLC Machine
- ✓ Handmade and cellulose plates
- ✓ Binding agent (starch).
- ✓ Cellulose ( microcrystalline)
- ✓ Cellulose (microcrystalline) with florescent indicator.
- ✓ Acetylated cellulose + CaSO<sub>4</sub>. ½ H<sub>2</sub>O
- ✓ Silica Gel.
- ✓ Glass support.
- ✓ Polyester (Plastic) sheets. (0.2 mm thick).
- ✓ Aluminium sheet (0.1 mm thick)
- ✓ Pre-coated of HPTLC Al sheets Silica gel 60 F254, Camag Cat No. 034.5554

**Method:**<sup>[3]</sup>

For HPTLC study of *Shatavari* following having specific significance were adopted

- Selection of HPTLC plates and solvents
- Sample preparation including any clean up
- Derivatisation
- Application of sample
- Development of chromatographic layers
- Detection including post chromatographic derivatisation
- Analysis and documentation of findings

A standard HPTLC machine having required features was selected for the study. After this, for preparing study sample 03gm. of *Shatavari* churna was dissolved in 20 ml of methanol. It was stirred intermittently for 6 hours. The solution thus prepared was kept for 18 hours in standstill. Then it was filtered and filtered extract (filtrate) was used as original sample for HPTLC analysis.

**Stationary phase**

TLC Al sheets Silica gel 60 F254 pre-coated Camag Cat No. 034.5554, cut to 10cm x 10cm

**Sample application – CAMAG Linomat 5**

**Instrument** - CAMAG Linomat 5 “Linomat 5\_080222” S/N 080222 (1.00.12) Executed by CT Institute of Pharmaceutical Science, Jalandhar.

**Linomat 5 application parameters**

Spray gas:	Inert gas
Sample solvent type:	Methanol
Dosage speed:	150nl/s
Predosage volume:	0.2 ul

**Sequence**

Syringe size:	100 µl
Number of tracks:	12
Application position:	8.0 mm
Band length:	8.0 mm

**Mobile phase**

n-Butanol: Water : Glacial acetic acid - 7 : 2 : 1.

**1. Development chamber**

Camag Twin Trough chamber of 10 x 10 cm with 3.5 s.s lid.

**2. Chamber Saturation**

20 minutes with paper.

**3. Plate Equilibrium**

None.

**4. Sample/ Standard application**

Apply with the help of Camag ATS-4 of sample solution on pre-coated layer 10mm from the bottom edge.

- Band length 8mm.

**5. Development distance**

80mm.

**6. Visualization**

Observe under UV cabinet at 254 nm.

**7. Photo documentation**

At 254 nm for Shatavari Visible.

**8. Measurement Mode**

UV absorbance / reflectance.

**9. Scanning****a) For Qualification**

Using Camag Scanner 3 with Win CATS software, Slit-micro, 6x.30mm, scan at 270nm.

**b) For Identification**

Record spectra between 190 to 400 nm

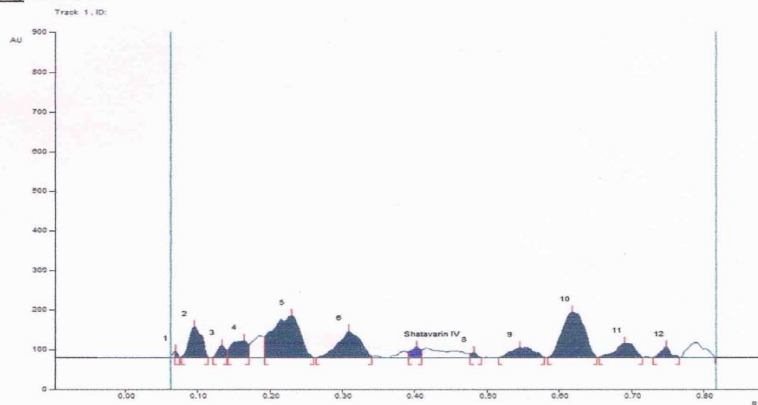
## RESULT OF SCAN HPTLC

❖ % Area of chemical constituents in alcoholic extract of *Shatavari* (scanned at 560nm).

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area%	Assigned Substance
1	0.07Rf	9.9AU	0.10Rf	66.4AU	14.09%	0.11Rf	2.3AU	1110.4AU	10.49%	Unknown
2	0.11Rf	1.9AU	0.12Rf	13.1AU	2.78%	0.13Rf	0.1AU	95.8AU	0.91%	Unknown
3	0.13Rf	1.4AU	0.14Rf	24.3AU	5.15%	0.15Rf	3.8AU	246.4AU	2.33%	Unknown
4	0.16Rf	1.9AU	0.17Rf	16.6AU	3.52%	0.19Rf	0.4AU	249.1AU	2.35%	Unknown
5	0.20Rf	0.1AU	0.23Rf	87.8AU	18.61%	0.26Rf	0.5AU	2374.4AU	22.42%	Unknown
6	0.27Rf	0.1AU	0.31Rf	50.1AU	10.62%	0.35Rf	1.3AU	1465.4AU	13.84%	Unknown
<b>7m</b>	0.38Rf	5.7AU	0.39Rf	13.2AU	2.79%	0.40Rf	11.5AU	247.3AU	2.34%	<b>Shatavarin IV</b>
8	0.53Rf	0.3AU	0.55Rf	14.0AU	2.98%	0.58Rf	0.3AU	302.0AU	2.85%	Unknown
9	0.59Rf	0.4AU	0.62Rf	111.3AU	23.59%	0.65Rf	0.1AU	3036.5AU	28.68%	Unknown
10	0.67Rf	0.3AU	0.70Rf	32.1AU	6.80%	0.72Rf	0.6AU	641.7AU	6.06%	Unknown
11	0.73Rf	0.2AU	0.75Rf	17.8AU	3.78%	0.77Rf	3.1AU	297.6AU	2.81%	Unknown
12	0.77Rf	3.9AU	0.80Rf	24.9AU	5.28%	0.81Rf	1.5AU	521.4AU	4.92%	Unknown

❖ The area of percentage shows presence of chemical constituents in the drug at that Rf values.

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SHAHPUR CAMPUS, VILLAGE: UDOPUR, PRATAPURA ROAD, JALANDHAR (Pb) 144020  
Contact: 09914504420, 0181-5055127**Solvent system:** Chloroform : Methanol (7 : 3).**Scanning:** 560 nm.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	9.9	0.10	66.4	14.09	0.11	2.3	1110.4	10.49	unknown *
2	0.11	1.9	0.12	13.1	2.78	0.13	0.1	95.8	0.91	unknown *
3	0.13	1.4	0.14	24.3	5.15	0.15	3.8	246.4	2.33	unknown *
4	0.16	1.9	0.17	16.6	3.52	0.19	0.4	249.1	2.35	unknown *
5	0.20	0.1	0.23	87.8	18.61	0.26	0.5	2374.4	22.42	unknown *
6	0.27	0.1	0.31	50.1	10.62	0.35	1.3	1465.4	13.84	unknown *
7m	0.38	5.7	0.39	13.2	2.79	0.40	11.5	247.3	2.34	Shatavarin IV
8	0.53	0.3	0.55	14.0	2.98	0.58	0.3	302.0	2.85	unknown *
9	0.59	0.4	0.62	111.3	23.59	0.65	0.1	3036.5	28.68	unknown *
10	0.67	0.3	0.70	32.1	6.80	0.72	0.6	641.7	6.06	unknown *
11	0.73	0.2	0.75	17.8	3.78	0.77	3.1	297.6	2.81	unknown *
12	0.77	3.9	0.80	24.9	5.28	0.81	1.5	521.4	4.92	unknown *

*Sub*  
24/11/14  
Dr. A K Sharma  
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## DISCUSSION

Plants have been used for medicinal purposes across history and now a days there is unprecedented interest towards use of plant based medicine. For this purpose proper identification and scientific knowledge of bioactive phyto constituent is necessary. Phytochemical screening<sup>[8]</sup> is necessary in order to establish the identity, purity, safety and quality of Ayurvedic crude drugs for which HPTLC is a standard tool Shatavari is a potent drug used as nervine tonic, galactogogue, ophthalmic, anodyne, aphrodisiac, rejuvenating, carminative, appetiser, stomachic, antispasmodic, tonic and having broad spectrum application in therapeutics. In this analytical study it is tried to establish the HPTLC findings of Shatavari to identify the raw sample for the preparation of different genuine Ayurvedic medicines and develop the fingerprint for the crude Shatavari. The sample drug contains Shatavarin IV which is presents in Shatavari tuber. In the drug sample 2.79% Shatavarin IV is present at 254 nm band, Rf 0.39 and percentage area 2.34%. This phytochemical is responsible for drug action.

## CONCLUSION

In current era, it has become challenging to find out the quality and standard raw material for manufacturing Ayurvedic medicines. As *Shatavari* tuber is used in many formulations and also used as a single drug for treating different ailments, this scientific study attempted to establish the crude Shatavari tuber interms of its HPTLC findings.

It is concluded that the sample drug contains Shatavarin IV which is presents in *Shatavari* tuber. In the drug sample 2.79% Shatavarin IV is present at 254 nm band, Rf 0.39 and percentage area 2.34%. Shatavarin IV is the chief testing constituent of *Asparagus racemosus* tuber.

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