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 racemosusLinn) 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 sacnadnt, 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.11 It grows upward direction and having thorns on its stalk. Branches are
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.\textsuperscript{[2]} As per Ayurvedic classical text its pharmacodynamics properties comprises of \textit{madhura} and \textit{tikta- rasa}, \textit{guru} and \textit{snigdha guna}, \textit{sheeta veerya} & \textit{madhura vipak}.\textsuperscript{[3-5]}

\textit{Shatavari} is used as a galactagogue and for disorders of female genitourinary tract; as a styptic anducer-healing agent; as an intestinal disinfectant and astringent in diarrhoea; as a nerve 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.\textsuperscript{[6]}

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. \textit{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.\textsuperscript{[7]} Hence the current study was undertaken to standardize the \textit{Shatavari} sample available in market.

\textbf{Shatavari Plant Shatavari Tuber.}
MATERIALS AND METHOD
The sample material i.e. Shatavar iwas 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 + CaSO4. ½ H2O
- 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:[3]
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

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

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Start Height</th>
<th>Max Rf</th>
<th>Max Height</th>
<th>Max%</th>
<th>End Rf</th>
<th>End Height</th>
<th>Area</th>
<th>Area%</th>
<th>Assigned Substance</th>
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<tbody>
<tr>
<td>1</td>
<td>0.07Rf</td>
<td>9.9AU</td>
<td>0.10Rf</td>
<td>66.4AU</td>
<td>14.09%</td>
<td>0.11Rf</td>
<td>2.3AU</td>
<td>1110.4AU</td>
<td>10.49%</td>
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<td>2</td>
<td>0.11Rf</td>
<td>1.9AU</td>
<td>0.12Rf</td>
<td>13.1AU</td>
<td>2.78%</td>
<td>0.13Rf</td>
<td>0.1AU</td>
<td>95.8AU</td>
<td>0.91%</td>
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<tr>
<td>3</td>
<td>0.13Rf</td>
<td>1.4AU</td>
<td>0.14Rf</td>
<td>24.3AU</td>
<td>5.15%</td>
<td>0.15Rf</td>
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<tr>
<td>4</td>
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<td>3.52%</td>
<td>0.19Rf</td>
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<tr>
<td>5</td>
<td>0.20Rf</td>
<td>0.1AU</td>
<td>0.23Rf</td>
<td>87.8AU</td>
<td>18.61%</td>
<td>0.26Rf</td>
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</tr>
<tr>
<td>6</td>
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<td>0.31Rf</td>
<td>50.1AU</td>
<td>10.62%</td>
<td>0.35Rf</td>
<td>1.3AU</td>
<td>1465.4AU</td>
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</tr>
<tr>
<td>7m</td>
<td>0.38Rf</td>
<td>5.7AU</td>
<td>0.39Rf</td>
<td>13.2AU</td>
<td>2.79%</td>
<td>0.40Rf</td>
<td>11.5AU</td>
<td>247.3AU</td>
<td>2.34%</td>
<td>Shatavarin IV</td>
</tr>
<tr>
<td>8</td>
<td>0.53Rf</td>
<td>0.3AU</td>
<td>0.55Rf</td>
<td>14.0AU</td>
<td>2.98%</td>
<td>0.58Rf</td>
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<td>0.65Rf</td>
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<tr>
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<td>0.70Rf</td>
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<td>0.72Rf</td>
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<td>11</td>
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<tr>
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<td>0.80Rf</td>
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<td>5.28%</td>
<td>0.81Rf</td>
<td>1.5AU</td>
<td>521.4AU</td>
<td>4.92%</td>
<td>Unknown</td>
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</tbody>
</table>

Solvent system: Chloroform : Methanol (7 : 3).

Scanning: 560 nm.
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\cite{8} 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, galactogouge, 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 present 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 present 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.

REFERENCES
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