



ORIGINAL ARTICLE

High-Performance Thin Layer Chromatography (HPTLC) Profiling of Methanolic Extract of *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.)

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ARTICLE HISTORY

Received: 21 January 2026

Accepted: 01 September 2026

Available online

Version 1.0: 30 September 2026

Keywords

Asparagus racemosus Willd., *Shatavari*, High-performance thin layer chromatography, *Ksheerapaka*

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Nayana M, Ansary PY, Shincymol VV. High-Performance Thin Layer Chromatography (HPTLC) Profiling of Methanolic Extract of *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.). Kerala Journal of Ayurveda. 2026;5(3):01-05. <https://doi.org/10.55718/kja.573>



Abstract

High-Performance Thin Layer Chromatography (HPTLC) is a refined chromatographic technique offering improved resolution and precision for the analysis of plant-based formulations. It is extensively used for identifying and quantifying phytochemical constituents in herbal drugs, supporting their standardization and quality control. *Shatavari* (*Asparagus racemosus* Willd.), a key Ayurvedic medicinal plant, contains diverse bioactive compounds with significant therapeutic properties. *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.), a classical milk-based Ayurvedic formulation, is widely used in clinical practice; however, its analytical standardization using modern techniques remains inadequate. The present study aimed to establish an HPTLC fingerprint profile of methanolic extract of *Shatavari ksheerapaka*. Chromatographic analysis was performed on a 5 × 10 cm pre-coated silica gel 60 F254 plate, with separation achieved using a mobile phase consisting of toluene, ethyl acetate, and hexane in the ratio of 6:3:1. Following chromatographic development, the plate was analyzed at 366 nm. The methanolic extract of *Shatavari ksheerapaka* exhibited four distinct peaks, with a total area of 14838.1 AU at 366 nm, indicating the presence of distinct phytochemical constituents. This chromatographic profiling offers a comprehensive overview of the chemical composition of the formulation, contributing to its quality evaluation and offering a reliable reference for future research.

Introduction

The increasing use of herbal medicines has emphasized the need for effective standardization and quality-control approaches, particularly in Ayurveda, to ensure their authenticity and consistency. High-Performance Thin-Layer Chromatography (HPTLC) is a reliable analytical technique widely used for the standardization and quality control of herbal drugs and plant-based preparations. HPTLC enables simultaneous analysis of samples under standardized conditions and generates characteristic phytochemical fingerprints useful for authentication, quality assessment, detection of adulteration, and identification of potential marker compounds. Its rapid, simple, and cost-effective nature makes it suitable for routine evaluation of

herbal materials.¹ *Shatavari* is an important medicinal plant widely used in Ayurveda and traditionally recognized for its *rasayana* (rejuvenative), *vayasthapana* (longevity promoting), *balya* (strength-promoting) and other properties. Botanically, it is identified as *Asparagus racemosus* Willd.,² belonging to the family Asparagaceae. It is a perennial climber widely distributed in India, with its tuberous roots constituting the principal medicinal part. Its phytochemical profile includes steroidal saponins such as shatavarins, along with flavonoids, polyphenols, tannins, alkaloids, polysaccharides, and phytosterols.³ This diverse phytochemical composition may contribute to the biological activities reported for the plant.⁴ *Ksheerapaka* (milk decoction) is a classical Ayurvedic preparation in which milk serves as the processing medium for medicinal drugs. Although *Shatavari ksheerapaka* is widely used in Ayurvedic practice, standardized analytical data on the formulation remain limited, highlighting the need for systematic quality evaluation. The present investigation aimed to establish an HPTLC fingerprint profile of *Shatavari ksheerapaka* prepared from dried tuberous roots and evaluated at 366nm. The resulting chromatographic profile may serve as a reference for authentication, comparative analysis, and quality assessment of *Shatavari*-based formulations.

Materials and Methods

Plant material collection and authentication

Fresh tuberous roots of *Asparagus racemosus* Willd. were collected from its natural habitat in the month of December, when the above-ground parts had begun to turn pale yellow.⁵ The collected plant material was authenticated by the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, and further confirmed by the Department of Botany, St. Albert's College (Autonomous), Ernakulam. A voucher specimen (No. 606) was deposited for future reference. The tuberous roots were rinsed thoroughly under running water to remove adhering soil and other physical impurities, sorted, and carefully inspected for insect infestation. Any damaged or affected samples were discarded.

Preparation of the dosage form: *Ksheerapaka* (milk decoction)

Ksheerapaka was prepared according to the method described in *Sarangadhara Samhita*,⁶ using dried tuberous roots of *Shatavari* for the present study. Although *Sarangadhara Samhita* recommends *Shatavari* should be used in fresh state, dried tuberous roots were used to facilitate preservation, storage, and consistent handling of the raw material. Comparative evaluation of fresh and dried *Asparagus racemosus* Willd. roots has demonstrated differences in their phytochemical profiles, indicating that drying may influence the chemical characteristics of the drug.⁷ Therefore, dried roots were selected to ensure uniformity and reproducibility during *ksheerapaka* preparation and subsequent HPTLC analysis.

Freshly collected tuberous roots were washed thoroughly and sun-dried for one to two days. They were then soaked in lukewarm water for an hour to soften the outer layer, which was subsequently peeled manually. The peeled roots were shade-dried for about two days until completely dry, confirmed by the characteristic cracking sound on breaking.⁵ The fully dried roots were cut into smaller pieces and coarsely crushed. For the preparation of *ksheerapaka*, the crushed drug, milk, and water were taken in the ratio 1:8:32 in an earthen pot. Specifically, 48 g of the crushed drug was mixed with 384 mL of milk and 1536 mL of water. The preparation was subjected to boiling and reduced to the original volume of the milk (384 mL), then filtered through ten layers of muslin cloth to remove sediments. Figure 1 shows the prepared *ksheerapaka* of *Asparagus racemosus* Willd.



Figure 1: *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.)

Preparation of Test Solution

20 mL of *Shatavari ksheerapaka* was concentrated under vacuum to remove the water content. The obtained residue was dissolved in 10 mL of methanol and sonicated for 10 minutes. The sample was then centrifuged, and the clear supernatant was collected and stored at -20 °C for subsequent HPTLC analysis.

HPTLC Analysis

HPTLC analysis was carried out on the methanolic extract prepared from *Shatavari ksheerapaka* at CARE KERALAM Ltd., Koratty, under standardized chromatographic conditions. The developed plate was visualized under UV light at 254 and 366 nm, followed by post-chromatographic derivatization using anisaldehyde-sulphuric acid reagent. The 366 nm profile was selected for presentation as it provided clearer visualization and better differentiation of the fluorescent bands, thereby facilitating interpretation of the chromatographic fingerprint. The chromatographic conditions employed for the analysis are detailed in Table 1.

Table 1: Chromatographic conditions for HPTLC Analysis

Parameters	Specification
Plate	Silica gel 60 F254, 5 × 10 cm, 0.2 mm (E. MERCK KGaA)
Sample/Test solution	Methanolic extract of <i>Shatavari ksheerapaka</i>
Sample volume applied	6 µL
Sample applicator	CAMAG Linomat 5 Automated Sample Spotter (CamagMuttentz, Switzerland), syringe- 100µL (Hamilton)
Mobile phase	Toluene: Ethyl acetate: Hexane (6:3:1)
Development chamber	CAMAG twin-trough glass chamber (20 × 10 cm)
Chamber saturation	30 min
Development distance	8.5 cm
Drying conditions	Oven drying at 60 °C for 5 min
Post-chromatographic derivatization	Anisaldehyde- sulphuric acid reagent
Scanner	CAMAG TLC Scanner 3(Scanner_171019)
Software	WinCATS
Detection	UV 254 nm and 366 nm
Fingerprint evaluation	366 nm

Procedure

The development chamber was pre-saturated with the mobile phase for 30 minutes before chromatographic development. The application syringe was rinsed twice with methanol prior to use. The methanolic extract was applied to the HPTLC plate, and the plate was developed in the saturated twin-trough chamber to a migration distance of 8.5 cm. Following development, the plate was dried in an oven at 60 °C for 5 minutes and then subjected to scanning. A new method file was opened, and all scanning, integration, and spectral parameters were entered. The plate was scanned in absorption-reflection mode at 254 and 366 nm using the appropriate light sources for each wavelength. Each track was scanned to obtain its corresponding UV spectrum, and chromatographic fingerprints were generated. Spectral comparison of the detected bands was performed using the spectrum display function.

Results

Area and peaks of methanolic extract of *Shatavari ksheerapaka* at 366nm

The HPTLC fingerprint profile of *Shatavari ksheerapaka* exhibited four peaks at 366 nm, with a total peak area of 14838.1 AU. These peaks were defined at Rf -0.02 with area 13232.0 AU, Rf 0.45 with area 1114.3AU, Rf 0.60 with area 356.7AU and Rf 0.77 with area 135.1 AU.

Figures 2 and 3 shows the HPTLC plate and the overview graph of the methanolic extract of *Shatavari ksheerapaka* at 366 nm, respectively, while Table 2 summarizes the peak positions and their corresponding areas.



Figure 2: HPTLC plate view of the methanolic extract of *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.) at 366 nm.

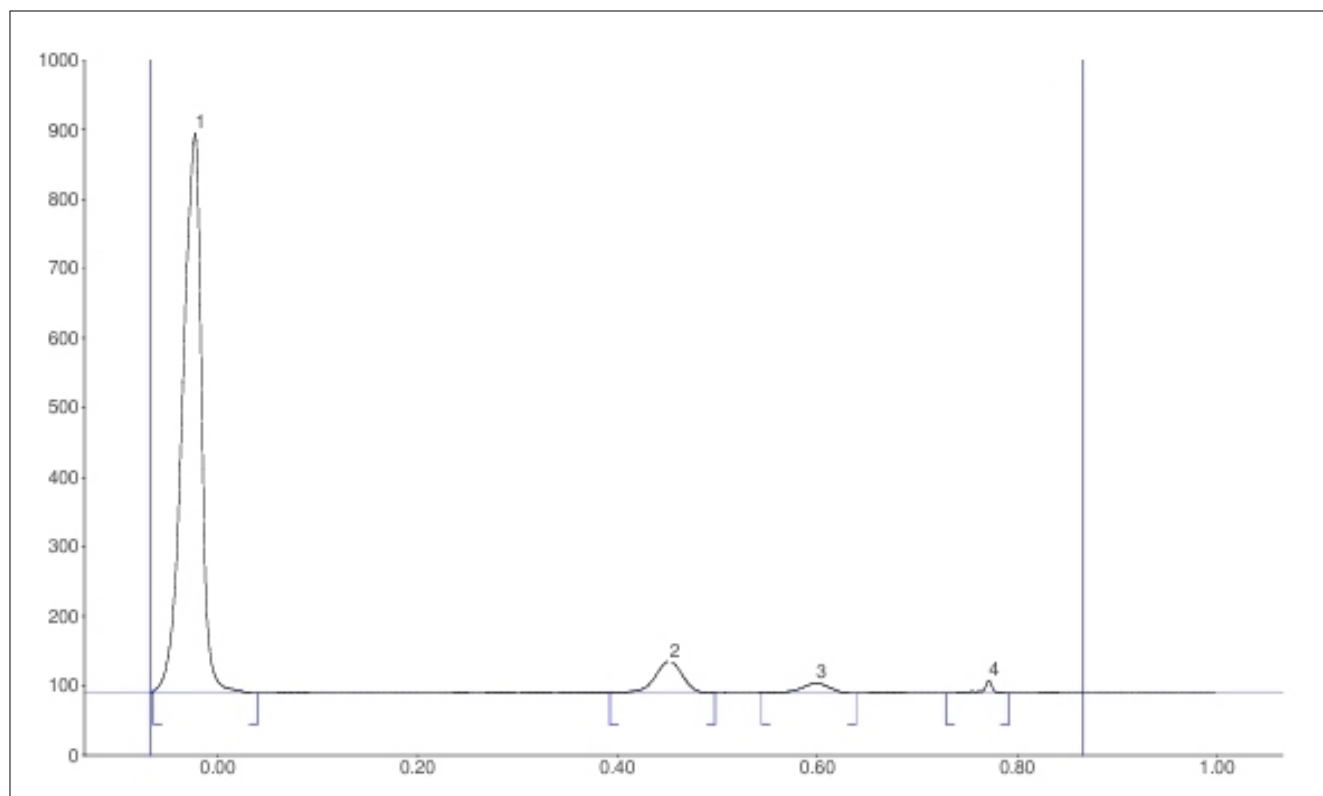


Figure 3. Overview graph of the methanolic extract of *Shatavari ksheerapaka* (milk decoction of dried tuberous roots of *Asparagus racemosus* Willd.) at 366nm

Table 2: Peak and area of methanolic extract of *Shatavari ksheerapaka* at 366nm

Peak No	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.06	-0.02	0.04	13232.0	89.18
2	0.39	0.45	0.50	1114.3	7.51
3	0.55	0.60	0.64	356.7	2.40
4	0.73	0.77	0.79	135.1	0.91

Discussion

The present study established an HPTLC fingerprint profile of the methanolic extract of *Shatavari ksheerapaka* prepared from dried tuberous roots of *Asparagus racemosus* Willd. Four distinct peaks were observed at 366 nm, with total peak area of 14838.1 AU, providing a characteristic chromatographic profile of the formulation under the applied analytical conditions.

Previous HPTLC investigations of *Asparagus racemosus* Willd. root extracts have demonstrated diverse chromatographic profiles, depending on the extraction solvent and analytical conditions. In one such study, methanolic extract prepared from powdered *Asparagus racemosus* Willd. root showed twelve detectable chromatographic components, with the most prominent peak observed at an Rf value of 0.02.⁸ Similarly, aqueous and ethanolic root extracts have also shown distinct chromatographic profiles with multiple detectable bands, supporting the applicability of HPTLC for phytochemical fingerprinting of *Asparagus racemosus* Willd.⁹ The variation in chromatographic profiles among these studies may be attributed to differences in extraction procedures, solvent systems, sample matrices, chromatographic conditions and detection wavelengths.

In comparison with these studies, *ksheerapaka* prepared from dried tuberous roots of *Asparagus racemosus* Willd. exhibited four distinct peaks. The lower number of detectable peaks does not necessarily indicate a lower number of extractable or chromatographically detectable constituents, as the present sample represents a milk-based formulation followed by methanolic extraction rather than a direct extract of powdered roots. The formulation matrix and processing conditions may have influenced the solubility, extractability, and chromatographic behaviour of the chemical constituents present in the formulation. Thus, the observed peaks represent components resolved under the specific analytical conditions employed and provide a characteristic fingerprint of the formulation rather than a comprehensive representation of its phytochemical composition.

The highest peak area was observed near the point of application (Rf ≈ 0.00), with an area of 13232.0 AU. Additional peaks were detected at Rf values of 0.45, 0.60, and 0.77, with peak areas of 1114.3, 356.7, and 135.1 AU, respectively. These peaks represent chromatographically detectable components of the methanolic extract; however, their individual chemical identities cannot be established from the HPTLC fingerprint alone. To the best of our knowledge, published HPTLC fingerprint data specifically for *Shatavari ksheerapaka*

prepared from dried tuberous roots of *Asparagus racemosus* Willd. are limited. The present findings therefore provide preliminary chromatographic reference data for *Shatavari ksheerapaka*.

Conclusions

The HPTLC fingerprint profile of the methanolic extract of *Shatavari ksheerapaka* demonstrated four distinct chromatographic peaks at 366 nm, representing the characteristic chromatographic pattern of the formulation. The established profile provides useful baseline analytical data for *Shatavari ksheerapaka* and may serve as a reference for future quality-control and standardization studies. Further investigations using authenticated reference standards and complementary analytical techniques are warranted for the characterization and identification of individual phytoconstituents.

Acknowledgements

The authors sincerely acknowledge Dr. Madhusudhanan K., Professor and Head, Department of Botany, St. Albert's College (Autonomous), Ernakulam, for authentication of the plant material, and the staff of CARE KERALAM Ltd., Koratty, for their valuable assistance and cooperation during the study.

Funding

This study was funded by the Central Council for Research in Ayurvedic Sciences (CCRAS) under the PG-STAR Scheme Session 2.

Conflicts of interest

The authors declare no conflict of interest.

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