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HPTLC Fingerprint Profiling of the Tuberous Roots of Shatavari (*Asparagus racemosus* Willd.)

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ABSTRACT: The increasing global acceptance of Ayurvedic medicines necessitates reliable quality control and standardization methods to ensure their safety, efficacy, and authenticity. Due to the complex phytochemical composition of herbal formulations, systematic analytical evaluation is essential for establishing their identity and consistency. High-Performance Thin-Layer Chromatography (HPTLC) is a sensitive and reproducible analytical technique that facilitates efficient separation and visualization of bioactive constituents in plant-based preparations. *Shatavari* (*Asparagus racemosus* Willd.) is a well-recognized medicinal plant widely used in Ayurveda for its diverse therapeutic potential. The present study aims to establish an HPTLC fingerprint profile of the methanolic extract of *Shatavari choorna* (powder), prepared from the dried tuberous roots of *Asparagus racemosus* Willd. A precoated silica gel 60 F 254 plate (5 × 10 cm) served as the stationary phase, while Toluene:chloroform:methanol (6:3:1) was used as the mobile phase and visualized at 425 nm under UV light. The HPTLC analysis revealed twelve distinct peaks with a total peak area of 54757.8 AU at 425 nm, indicating the presence of multiple phytochemical constituents. The established HPTLC fingerprint profile serves as a reliable reference for the identification, quality evaluation, and standardization of *Shatavari choorna* and provides scientific support for its future research and quality control applications.

KEYWORDS: *Asparagus racemosus* Willd., HPTLC fingerprint, *Shatavari choorna,* Methanolic extract

INTRODUCTION

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced analytical technique that provides high resolution, accuracy, and reproducibility for the separation and analysis of complex plant-based formulations. It is extensively applied in pharmaceutical, clinical, forensic, biochemical, cosmetic, food, and environmental fields.[1] In the current era, the increasing need for standardized and quality-assured herbal medicines has further emphasized the importance of HPTLC. It enables rapid comparison of multiple samples, produces reliable fingerprint profiles, and supports the detection of impurities or adulteration, making it a valuable tool for quality control. HPTLC works on the principle of separating components based on their different affinities between a stationary phase coated on a plate and a mobile phase that moves by capillary

action. As the solvent travels upward, the compounds migrate at varying rates, resulting in distinct bands that can be detected and analyzed.

Shatavari, botanically identified as *Asparagus racemosus* Willd.[2] of the Asparagaceae family, is a well-recognized medicinal herb valued for its extensive therapeutic benefits and has been traditionally used in Ayurveda since ancient times. It is a perennial climbing species widely distributed across India, and its tuberous roots constitute the primary medicinally utilized part. *Shatavari* is attributed with *madhura tikta rasa* (sweet and bitter taste), *guru snigdha guna* (heavy and unctuous qualities), *madhura vipaka* (sweet post-digestive effect), and *sita virya* (cold potency), which together define its therapeutic nature. Traditionally, it is valued for its *rasayana* (rejuvenative), *vayasthapana* (longevity-promoting), *balya* (strength-enhancing), and *pushtivardhaka* (nourishing) properties, along with its beneficial effects on female reproductive health and overall well-being. The plant is rich in diverse phytoconstituents, notably steroidal saponins such as Shatavarin I–IV, along with alkaloids including asparagamine A. It also contains flavonoids like quercetin, rutin, and kaempferol, in addition to tannins, phenolic substances, sapogenins, polysaccharides, and trace levels of volatile components.[3] The combined presence of these bioactive compounds is believed to be responsible for the plant's documented antioxidant, diuretic, immunomodulatory, cytoprotective, anti-apoptotic, anti-inflammatory, analgesic, adaptogenic and other activities.[4]

A reliable chromatographic fingerprint of a genuine herbal drug is essential for confirming the presence of characteristic constituents and for safeguarding against adulteration or inferior quality materials. Owing to the complex phytochemical composition of *Shatavari choorna* (powder), prepared from the dried tuberous roots of *Asparagus racemosus* Willd., HPTLC offers an effective analytical approach for resolving its constituents. The present investigation aimed to establish a characteristic HPTLC fingerprint profile of the methanolic extract of *Shatavari choorna* with analysis carried out at 425nm, to support its authentication and quality evaluation. The developed fingerprint profile serves as a reliable reference for the identification, validation, and future analytical studies of *Shatavari choorna.*

MATERIALS AND METHODS

Collection of the Drug

Fresh tuberous roots of *Asparagus racemosus* Willd. were collected from the Mararikulam region of Alappuzha district, Kerala, in the month of December, when the aerial parts began turning pale yellow.[5] The roots were thoroughly washed, sorted, and inspected to remove damaged or infested samples. Botanical identification of the collected drug specimen was confirmed by the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura. Further plant authentication was performed at St. Albert's College (Autonomous), Ernakulam, Department of Botany, and a voucher specimen was deposited under the number 606.

Preparation of the dosage form: *Choorna* (powder)

The freshly collected tuberous roots of *Asparagus racemosus* Willd. were carefully washed with running water to remove soil, debris, and other impurities. They were then sun-dried for one to two days before being soaked in lukewarm water for an hour to soften the outer layer. Once softened, the outer skin was gently peeled off by hand, and the peeled roots were shade-dried for about two days, until completely dry, as indicated by the characteristic cracking sound. The dried roots were then cut into smaller pieces and ground into a fine powder using a pulverizer. The powdered material was passed through an 85-mesh sieve to ensure uniform particle size and finally stored in an airtight container to maintain its quality and prevent contamination. Figure 1 depicts the *Shatavari choorna* prepared from its dried tuberous roots.

Preparation of Sample Solutions and Chromatogram Visualization

The test solution was prepared by extracting 0.5 g of *Shatavari choorna* obtained from its dried tuberous roots with 10 mL of methanol, and filtered to obtain a clear extract. Chromatographic separation was performed using a mobile phase composed of toluene, chloroform, and methanol in the ratio of 6:3:1. A volume of 7.0 µL of the extract was applied to the HPTLC plate, which was developed to a solvent front distance of 9.5 cm. After development, the plate was dried under ambient conditions and examined under ultraviolet light at 425 nm. Rf values and color of the resolved bands were recorded.

HPTLC Conditions

HPTLC analysis was carried out on silica gel 60 F254–coated aluminum plates (5.0 × 10.0 cm). Sample application was performed using a CAMAG Linomat 5 applicator equipped with a 100 µL Hamilton syringe. Chromatographic development was conducted in a CAMAG glass twin-trough chamber (20 × 10 cm). After development, plates were derivatized with anisaldehyde–sulphuric acid using a chromatographic sprayer. Detection and documentation were accomplished using a CAMAG TLC Scanner coupled with WINCATS software.

Procedure

Chromatographic development was performed in a twin-trough chamber (20 × 10 cm) pre-saturated for 30 minutes with the mobile phase consisting of toluene, chloroform, and methanol (6: 3: 1). After rinsing the syringe twice with methanol, 7.0 µL of the methanolic extract was applied as 8 mm bands. The developed plates were dried at 60 °C for 5 min and scanned in absorption–reflection mode at 254 nm and 366 nm. Subsequently, the plates were derivatized with anisaldehyde–sulphuric acid reagent, and rescanned at 425 nm. UV spectra were recorded for individual spots followed by scanning of all tracks. A track spacing of 12.5 mm was maintained, and chromatographic fingerprints were generated after entering the required scanning and integration parameters.

RESULTS

Area and peaks of methanolic extract of *Shatavari choorna* (powder of dried tuberous roots of *Asparagus racemosus* Willd.) at 425 nm

The HPTLC fingerprint profile of the *choorna* (powder) prepared from dried tuberous roots of *Asparagus racemosus* Willd. exhibited twelve distinct peaks when scanned at 425 nm, with a total area of 54757.8 AU. These twelve peaks were defined at the maximum Rf value of -0.01 with area 28026.4 AU, maximum Rf value of 0.05 with area 4811.7AU, maximum Rf value of 0.11 with area 8036.6 AU, maximum Rf value of 0.15 with area 1046.3AU, maximum Rf value of 0.18 with area 1274.6AU, maximum Rf value of 0.21 with area 1158.5AU, maximum Rf value of 0.26 with area 913.2AU, maximum Rf value of 0.37 with area 5730.4AU, maximum Rf value of 0.52 with area 2260.8AU, maximum Rf value of 0.60 with area 866.9AU, maximum Rf value of 0.72 with area 300.9AU, maximum Rf value of 0.73 with area 331.5AU respectively.

HPTLC plate view and the overview graph of the methanolic extract of *Shatavari choorna* at 425nm are illustrated in Figures 2 and 3, respectively. The associated peak positions and area values obtained at this wavelength are summarized in Table 1.

DISCUSSION

The present investigation established an HPTLC fingerprint profile of *Shatavari choorna*, prepared from the dried tuberous roots of *Asparagus racemosus* Willd., for quality control and standardization. The methanolic extract of the formulation was analyzed at 425 nm and revealed twelve distinct and well-resolved peaks, indicating the presence of multiple phytoconstituents and reflecting the chemical diversity of the formulation.

The cumulative peak area of 54757.8 AU represents the overall phytochemical richness of the *Shatavari choorna*. Each resolved peak corresponds to a distinct phytochemical component, highlighting the suitability of HPTLC as a robust tool for the quality control and standardization of herbal medicines.

Among the resolved components, a prominent peak observed at or near the point of application (Rf ≈ 0.00), with a peak area of 28026.4 AU, indicates the presence of a major constituent in relatively high concentration. The minor negative Rf value obtained is attributed to densitometric baseline alignment and software rounding during HPTLC scanning rather than true negative migration. In *choorna* formulations, which are rich in finely powdered plant materials containing polar phytoconstituents, such retention at the origin is commonly encountered due to strong interactions with the silica gel stationary phase. Thus, the peak observed near the origin may be attributed to the chromatographic behaviour of highly polar constituents present in the formulation under the applied experimental conditions.

The use of methanol as an extraction solvent facilitated the efficient extraction of a wide range of polar and moderately non-polar bioactive constituents from *Shatavari choorna*. Furthermore, anisaldehyde–sulphuric acid derivatization enhanced the visualization and sensitivity of phytochemical detection, thereby strengthening the reliability of the developed HPTLC fingerprint profile.

The established HPTLC fingerprint can serve as a reliable analytical reference for the standardization and quality assessment of *Shatavari choorna*, aiding in the authentication of the formulation and the detection of potential adulteration or substitution, and thereby contributing to its consistent quality and rational utilization.

CONCLUSION

HPTLC analysis of *Shatavari choorna* was performed to assess its quality, purity, and consistency, as the chemical composition of powdered herbal formulations can vary due to differences in raw materials, processing, and storage. The study established a reproducible HPTLC fingerprint at 425nm, revealing twelve distinct peaks, reflecting the chemical diversity and phytochemical richness of the formulation. This method effectively resolved multiple bioactive compounds and can serve as a robust approach for detecting adulteration, ensuring standardization, and supporting the quality control of the formulation.

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Figure 1: Powder of dried tuberous roots of *Asparagus racemosus* Willd.

Figure 2: HPTLC plate view of the methanolic extract of *Shatavari choorna* (powder of dried tuberous roots of *Asparagus racemosus* Willd.) at 425 nm.

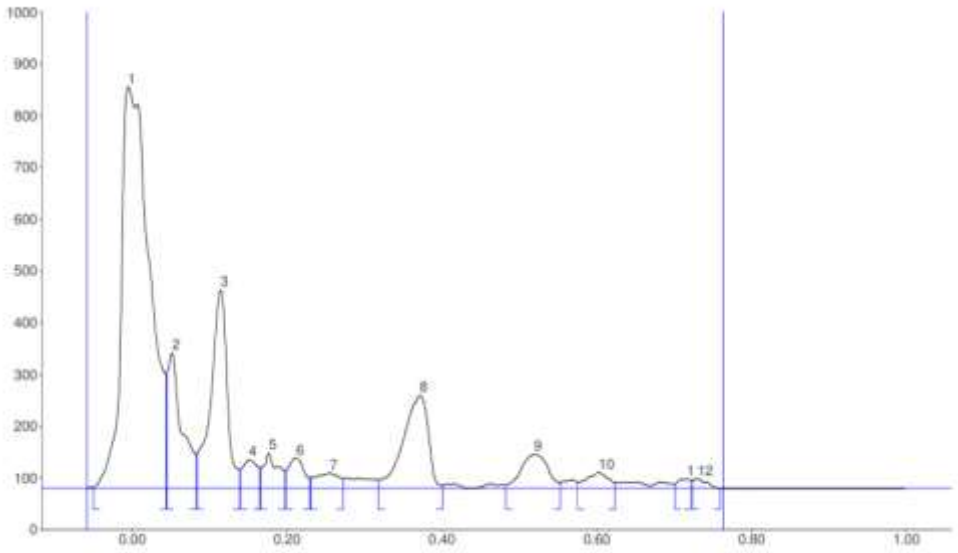

Figure 3. Overview graph of methanolic extract of *Shatavari choorna* (powder of dried tuberous roots of *Asparagus racemosus* Willd.) at 425nm.

Table No 1. Peak and area of methanolic extract of *Shatavari choorna* (powder of dried tuberous roots of *Asparagus racemosus* Willd.) at 425 nm

Peak No	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.05	-0.01	0.04	28026.4	51.18
2	0.04	0.05	0.08	4811.7	8.79
3	0.08	0.11	0.14	8036.6	14.68
4	0.14	0.15	0.16	1046.3	1.91
5	0.17	0.18	0.20	1274.6	2.33
6	0.20	0.21	0.23	1158.5	2.12
7	0.23	0.26	0.27	913.2	1.67
8	0.32	0.37	0.40	5730.4	10.46
9	0.48	0.52	0.55	2260.8	4.13
10	0.58	0.60	0.62	866.9	1.58
11	0.70	0.72	0.72	300.9	0.55
12	0.72	0.73	0.76	331.5	0.61