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Original Article

Isolation and Characterization of Saponins from *Asparagus racemosus*: A HPTLC and LC-MS Study

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ABSTRACT

Background: Shatavari *or Asparagus racemosus* Wild. Family Liliaceae is an important therapeutic herb in the health care system. It contains a sapogenin content which is a precursor of many pharmacologically active steroids. This plant is used to induce menstruation and as a cosmetic tonic. **Objectives:** The objective of the present study was to carry out a pharmacognostic and phytochemical evaluation of *Asparagus racemosus* Willd., with particular emphasis on the isolation, identification, and characterization of saponin glycosides using advanced analytical techniques such as HPTLC and LC-MS. **Materials and Methods:** The roots of *Asparagus racemosus* were authenticated and extracted using a hydro-alcoholic solvent. Pharmacognostic, physicochemical, and preliminary phytochemical evaluations were performed using standard methods. HPTLC and LC-MS analysis were carried out to identify and characterize saponin glycosides, with particular emphasis on Shatavarin IV. **Results and Discussion:** The evaluation revealed both external and internal diagnostic features of the root. Phytochemical analysis confirmed the presence of carbohydrates, glycosides, saponins, tannins, and steroids, with total saponin content of 20% w/w. Physicochemical parameters showed total ash (2.50% w/w), water-soluble ash (1.20% w/w), acid-insoluble ash (0.35% w/w), hydro-alcoholic extractive value (31.50% w/w), and loss on drying (4.73% w/w). HPTLC analysis revealed fifteen peaks (R_f 0.01-0.96) and confirmed the presence of Shatavarin IV. LC-MS analysis further identified saponin glycosides, with Shatavarin IV as the major compound at a retention time of 2.7 min. **Conclusion:** The study establishes standardized pharmacognostic and physicochemical parameters for *Asparagus racemosus* and confirms the presence of bioactive phytoconstituents, particularly saponin glycosides. The detection and characterization of Shatavarin IV through HPTLC and LC-MS validate the authenticity and quality of the plant material. These findings support the therapeutic potential of *A. racemosus* and provide a scientific basis for its standardization, and pharmacological investigations.

Keywords: HPTLC, Isolation, LC/MS, Pharmacognosy, Phytochemistry, Shatavarin-IV.

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INTRODUCTION

The Indian System of Medicine (ISM) relies heavily on plant-based medicine and has been practiced for thousands of years. It encompasses several traditional systems including Ayurveda, Unani, Siddha, Homeopathy, Yoga and Naturopathy. Ayurveda is one of the oldest and most purified system of medicine. There are some prominent ayurvedic herb which has unique property and health benefits. One of the most important plants in this tradition is Shatavari (*Asparagus racemosus*) which is widely used especially in Ayurveda (Selvaraj *et al*., 2019). In Ayurvedic medicine, Shatavari is classified as a Rasayana, a rejuvenating tonic that promotes longevity and vitality (Akhtar *et al*., 2024).

Shatavari (*Asparagus racemosus*) is a therapeutic herb which belongs to the family Liliaceae (Arya *et al*., 2018). It is an important monocot that grows in tropical as well as subtropical regions of Asia, Sri Lanka and India (Prabhakaran *et al*., 2015). It is highly valued in Ayurveda for its medicinal properties, particularly in supporting female reproductive health. Its various medicinal properties are reported in various Pharmacopoeias (Kumar *et al*., 2020). The roots of the plant are used in different formulations (Raval *et al*., 2012). The pharmacological properties depend on the phyto-active constituent present in the plant (Raval *et al*., 2012; Prabhakaran *et al*., 2015). It has been used as a medicine in lactating mothers, bleeding through urethra, epilepsy and cough condition. It is rich in phytochemicals like steroidal saponin (Shatavarin-1 to 6) (Selvaraj *et al*., 2019), carboxylic acid, isoflavones, polycyclic hydrocarbons, furan compound, flavonoids, carbohydrates, a trace of minerals, sterols, kaempferol

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and some miscellaneous constituents (Pandiyan *et al.*, 2022). It is valued for its diabetes, jaundice and immunomodulatory properties (Akhtar *et al.*, 2024). A major objective of the study was to assess the Hydroalcoholic root extract of *Asparagus racemosus* by phytochemical screening using different methods such as HPTLC and LCMS (Vijayalakshmi *et al.*, 2015).

MATERIALS AND METHODS

Collection and authentication of Plant material

The fresh plant (*Asparagus racemosus*) was collected from herbal garden from Lucknow, Uttar Pradesh, India in the month of July 2023. A Herbarium Voucher Specimen of selected plant was prepared and authenticated by Dr. Sunita Garg, former head of RHMD at CSIR-NIScPR, New Delhi (NIScPR/RHMD/Consult/2023/4577-89).

Pharmacognostical studies

The technique of standardizing the crude drugs by several parameters. The parameters include morphology (colour, shape, size, fracture, odour, and taste) (Ahmad *et al.*, 2017; Brahma *et al.*, 2022) and microscopy (Transverse section of the drug) (Prabhakaran *et al.*, 2015; Unadhkat *et al.*, 2022; Singh *et al.*, 2017).

Physicochemical Parameters

This parameter includes Ash Value (Goel *et al.*, 2022; Mandal *et al.*, 2017), Extractive Value Prabhakaran *et al.*, 2015; Siraj *et al.*, 2020; Loss on Drying Tripathi *et al.*, 2015; Goel *et al.*, 2022).

Phytochemical Parameter

This Parameter is used to assess primary and secondary metabolites (Hassan *et al.*, 2022; Irawan *et al.*, 2018). A plant extract is prepared by specific extraction method (Ajuru *et al.*, 2017). The crude extracts is used to identify the presence of different types of components (Karunarathne *et al.*, 2019; Ahmad *et al.*, 2017).

Preparation of Plant Extract

The roots were washed with distilled water, shade-dried, and powdered (Sharma *et al.*, 2017). The powdered drug was extracted with a water-ethanol mixture using a Soxhlet apparatus until a clear siphon liquid was obtained (Basoudan *et al.*, 2024; Ahmad *et al.*, 2017). The filtrate was concentrated, dried, weighed, and subsequently used for phytochemical screening (Prabhakaran *et al.*, 2015; Alqethami *et al.*, 2021).

Saponin Estimation Procedure

2 g of extract in 50 mL of Petroleum ether was heated in a water bath at 40°C for 5 min with intermittent shaking. The petroleum ether layer was filtered, and the process was repeated three additional times. The petroleum ether was discarded and the marc was preserved. The obtained marc was extracted with methanol (60 mL) under mild heating, repeating the process four times. The methanolic layer was filtered and transferred into another beaker.150 mL of dry acetone was added to the methanolic layer which was combined and concentrated. Saponins formed the precipitate. Filter the saponin and calculate its percentage (Kumaran *et al.*, 2015, Ma *et al.*, 2022).

Saponin Percentage is calculated as = Residue weight/sample weight×100

Extraction of Saponin

Isolation of saponin was performed by liquid-liquid extraction. The SRF were isolated from extract. Procedure: The SRF were segmented into Aqueous phase from the organic phase using LLE (Chua *et al.*, 2019). 5 g dried extract of *Asparagus racemosus* was weighed, dissolved in 60 mL of hot water and fully extracted with 20 mL ethyl acetate. The organic layer was abstracted and 20 mL of fresh ethyl acetate was added and extracted again. The extraction was redone for six times. All the organic layers as well as aqueous layer were collaborated and evaporated up to dryness and dried in an oven at 65°C (Segaran *et al.*, 2020).

Chromatographic Analysis

HPTLC: It is a modern form of TLC that provides accuracy and reproducibility. HPTLC is an advance technology based on the principle of TLC which requires less and provide better resolution. It is widely used for the qualitative as well as quantitative analysis of complex mixtures in food, herbal products, forensics and pharmaceuticals (Vyas *et al.*, 2023). The study provides TLC fingerprint profiles and estimation study of biomarkers (Mehta *et al.*, 2021). The major advantage of HPTLC is that it can examine samples together using small amount of mobile phase (Champati *et al.*, 2022). Procedure: The HPTLC was performed on a 10×10 cm pre-activated HPTLC silica gel 60 F254 plate (Hazra *et al.*, 2019). Samples are applied on the plate with an automatic TLC applicator (Mohan *et al.*, 2024). The plates were developed using ethyl acetate, ethanol, and water at a ratio of 8:2:1.2 as a solvent system. The scanning was done on CAMAG scanner II (Bhargava *et al.*, 2021; Abraham *et al.*, 2020). The chromatographic finger print was developed for detection of Phytoconstituents and R_f values were calculated (Prabhakaran *et al.*, 2015).

LCMS Analysis

LCMS is an analytical tool for the identification of wide variety of non-volatile or volatile organic compounds. (Swati *et al.*, 2023; Vijaylakshmi *et al.*, 2024). It is highly sophisticated instrument used for the detection of low and high molecular weight compounds (Patil *et al.*, 2014). The Phyto-active components present in the SRF of the roots of *Asparagus racemosus* were identified by LCMS method. The analysis was performed using TOF/Q-TOF Mass Spectrometer system which is equipped with a Dual AJS ESI source (Sawada *et al.*, 2013). The gradient elution

was operated for 30 min (stop time) at a flow rate of 100.000 mL/min[2]. The full scan mass spectra were obtained within the range of m/z, amu 100-3000 at 1.00 scan rate (Irawan *et al.*, 2018). The valve switch time was 1 which was enabled with 10 µL injection volume (Marr *et al.*, 2021).

The mobile phase consists of Channel A and Channel B. Channel A contains water with 0.1% formic acid, while Channel B comprises Acetonitrile (ACN) with 10% water and formic acid. Both solvents were employed in the analysis to ensure effective separation and ionization of analytes.

Ethical Statement

The present study involved phytochemical, isolation and analytical characterization of saponins from *Asparagus racemosus* roots using HPTLC and LC-MS techniques. No human participants or animals were used in the above study. All procedures were performed in accordance with standard laboratory practices and institutional guidelines.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± SD. HPTLC densitometric data were analyzed using appropriate chromatographic software. LC-MS data were evaluated based on molecular ion intensity and fragmentation patterns for qualitative confirmation.

RESULTS

Pharmacogenetic studies

Morphological Characters

The morphological characters were examined by organoleptic method using fresh roots of *Asparagus racemosus* Willd (Table 1).

Microscopical Characters

The T.S. of Asparagus racemosus (Shatavari) (Figure 1) root shows an epidermis with unicellular root hairs, followed by an outer cortex of 6-7 layers of thick-walled polygonal cells and an inner cortex of 16-22 layers of thin-walled oval to polygonal cells. A single-layered thin-walled endodermis surrounds the centrally located, radially arranged vascular bundles (xylem and phloem) and a large pith.

Physico-chemical Parameters

All the Physico-chemical parameters and their values are mentioned below (Table 2).

Preliminary Phytochemical Screening

The Phytochemical screening of the hydro-alcoholic extract of the roots of *Asparagus racemosus* (Table 3).

Saponin Estimation

The percentage of Saponin content:

= 0.40/2×100

= 20%

Table 1: Organoleptic properties of roots of *A. racemosus* Willd.

Sl. No.	Character	Result
1.	Shape	Fleshy Tuberous
2.	Size	8-25 cm in length and 0.5-1 cm width
3.	Colour	Cream- Coloured
4.	Taste	Bitter and slightly sweet
5.	Odour	Characteristic

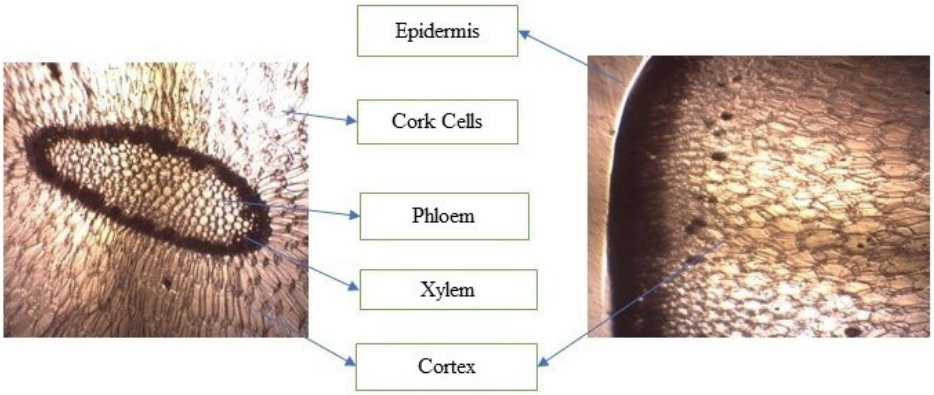


Figure 1: Transverse section of root of *Asparagus racemosus* Willd.

Table 2: Physico-chemical parameters of Hydro-alcoholic extract of roots of *Asparagus racemosus* Willd.

Sl. No.	Parameter	Result
1.	Total ash	2.50%w/w
2.	Water soluble ash	1.20%w/w
3.	Acid insoluble ash	0.35%w/w
4.	Extractive value	31.50%w/w
5.	Loss on Drying (LOD)	5.25%w/w

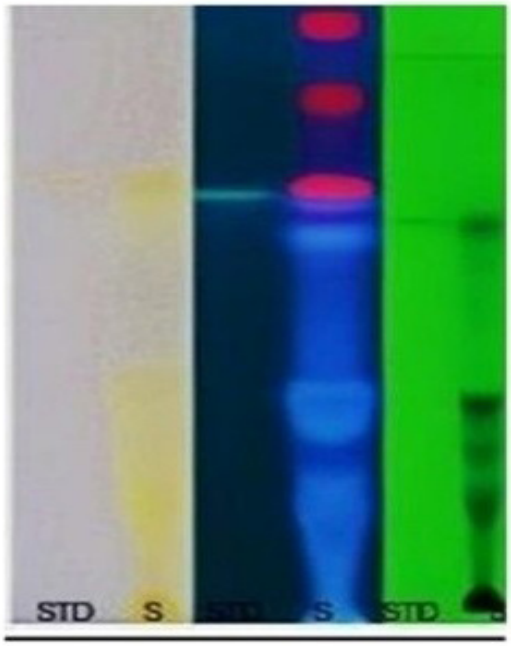


Figure 2: HPTLC profiles of the saponin-rich fractions of roots of *Asparagus racemosus* and Standard Shatavarin-IV.

High-Performance Thin Layer Chromatography

The HPTLC profile of the samples revealed the presence of Seventeen peaks. The chromatogram showed the presence of Shatavarin IV in both the standard and sample tracks, as shown by the violet zones observed during daylight mode (Figure 2). The mobile phase consisted of a mixture of ethyl acetate, ethanol, and water at a ratio of 8:2:1.2 provided satisfactory separation with a distinct R_f value. The bands that were separated based on their chemical properties were seen in the range of R_f 0.01 to 0.96 in the extract. Among these bands, the one with the R_f value of 0.60 indicated the existence of a specific compound, Shatavarin IV as shown in Figures 3.

Figure 3 (a) shows a single, sharp peak at a R_f value of approximately 0.60 for standard Shatavarin-IV which confirms its purity, specificity, and reproducibility under the selected chromatographic conditions.

Figure 3 (b) reveals multiple well-resolved peaks across the R_f range of 0.05-0.90 in the saponin-rich extract, reflecting the complex saponin composition of *A. racemosus* roots. Notably, a prominent peak appears at a R_f value close to that of the Shatavarin-IV standard (~0.60), which indicates the presence of Shatavarin-IV.

Table 3: Preliminary Phyto-chemical screening of Hydro-alcoholic extract of roots of *Asparagus racemosus* Willd.

Sl. No.	Test	Hydro-alcoholic extract
1.	Carbohydrate	Positive
2.	Proteins and Amino acids	Negative
3.	Alkaloids	Negative
4.	Glycosides	Positive
5.	Saponins	Positive
6.	Flavonoids	Positive
7.	Tannins and Phenolics	Positive
8.	Steroids	Positive
9.	Fixed oils	Negative

Liquid Chromatogram Mass Spectroscopy

Data Interpretation and Identification of Compounds

The chromatograms and mass spectra were processed by MZ mine 2.5 software by Agilent. The continuous data which was acquired in positive mode was processed. KEGG was the online data base search method which is used to identify the compounds. Other data base such as MoNA and PubChem were used for search of compounds. The fragments of accurate masses were obtained in the same ionization mode, which is matched with the database by setting a tolerance of 5 ppm. The identified metabolites were then exported in a sheet containing the active molecule name, molecular mass, retention time, peak area, peak height. and *m/z* ratio. After database matching the complex and adduct search were performed, followed by the peak list which is directly exported from MZ mine.

LCMS Reports

The LC-MS Chromatogram obtained from the Saponin rich fractions of Hydroalcoholic extracts of roots of *Asparagus racemosus* Willd was identified and mass spectrum of Shatavarin-IV was shown in Figure 4. It was noticed that different peaks were obtained at different retention times. The retention time of the major compound was mentioned in Table 4.

DISCUSSION

The study successfully established a systematic approach for the isolation and characterization of saponins from *A. racemosus* roots. The Morphological and physicochemical evaluations confirmed the authenticity and quality of the plant material,

Table 4: Important compound identified from the Hydroalcoholic extract of the roots of *Asparagus racemosus*.

Sl. No.	Retention Time (min)	Component Name	Chemical formula	Molecular Weight
1.	2.2	Sarasapogenin	$C_{27}H_{44}0_3$	416.64
2.	2.78	Asparanin B	$C_{45}H_{74}O_{17}$	887.06
3.	6.15	Kaempferol-3-o-pentoside	$C_{20}H_{18}O_{10}$	418.3
4.	23.78	Scutellarein	$C_{15}H_{10}O_6$	286.24
5.	24.33	Isorhamnetin	$C_{16}H_{12}O_7$	316.26
6.	24.70	Quercetin	$C_{15}H_{10}O_7$	302.23

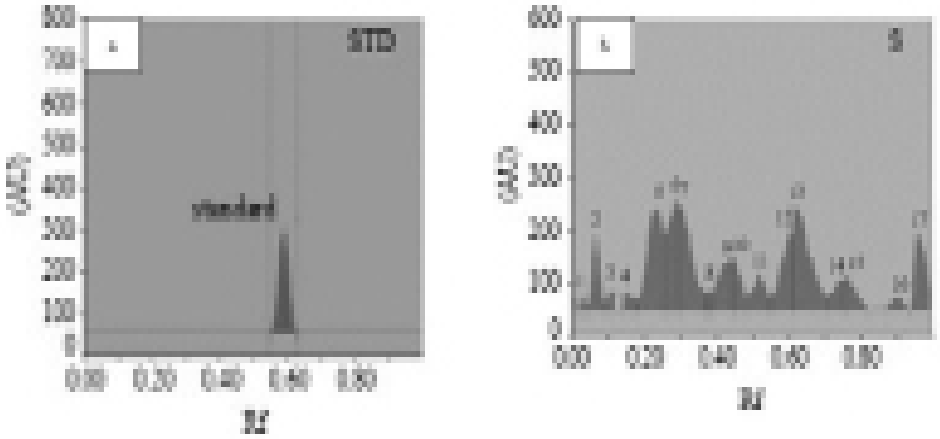


Figure 3: HPTLC chromatogram of standard Shatavarin IV (a) and SRF (b) from hydro-alcoholic root extracts of *Asparagus racemosus* Willd.

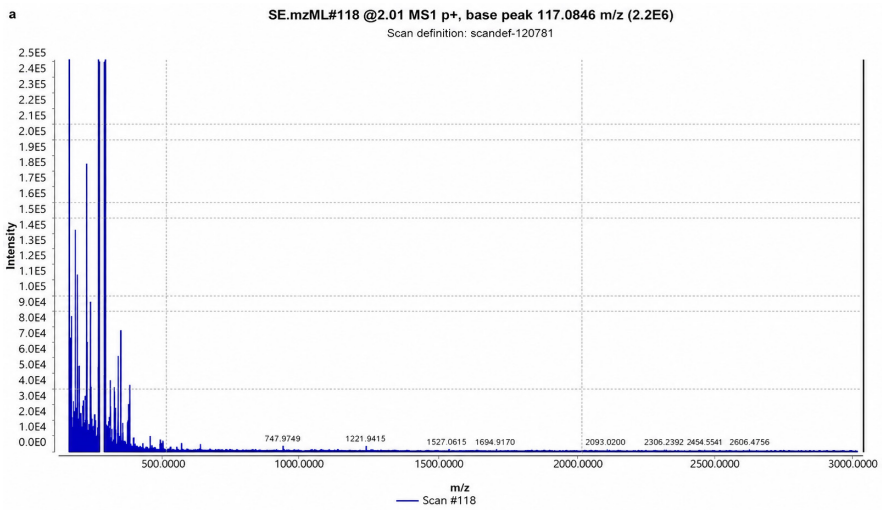


Figure 4: Mass Spectrum obtained at retention time 2.7.

while HPTLC and LC-MS analyses enabled reliable identification of saponins, particularly Shatavarin-IV.

CONCLUSION

The present study was assigned to evaluate different Pharmacognostic parameters and physicochemical properties of crude drug. The phytochemical screening of plant metabolites were performed which helped in the Authentication of *A. racemosus* Willd. Various chemical components were identified via HPTLC and LCMS methods. The HPLC and LC-MS analysis reported details of the separation and identification of the Compounds within a sample. The HPLC data signifies Retention time and the LC-MS data signifies mass-to-charge ratio (MS data). The results of the study revealed the presence of saponin compounds like Sarasapogenin, Asparanin B, and Kaempferol-3-o-pentoside.

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ABBREVIATIONS

T.S: Transverse section; **SRF:** Saponin rich fractions; **HPTLC:** High-Performance Thin Layer Chromatography; **LC-MS:** Liquid Chromatography Mass Spectrometry; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **ISM:** Indian System of Medicine; **LOD:** Loss on drying; **LLE:** Liquid-liquid extraction; **ESI:** Electro spray ionization; **FA:** Formic Acid; **ACN:** Acetonitrile; **R_f:** Retention factor; **m/z:** Mass-to-charge ratio; **TOF/Q-TOF:** Time-of-Flight/Quadrupole Time-of-Flight; **amu:** Atomic Mass Unit; **TLC:** Thin-Layer Chromatography; **SD:** Standard deviation; **AU:** Absorbance units; **CSIR-NIScPR:** Council of Scientific and Industrial Research - National Institute of Science Communication and Policy Research; **RHMD:** Raw Herbarium Materials and Dispensary; **MoNA:** Mass Bank of North America.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

The authors collectively contributed to the conceptualization, experimental design, data acquisition, analysis, and interpretation of the study.

SUMMARY

Saponins were isolated and characterization was done using HPTLC and LC-MS techniques. HPTLC analysis provided a characteristic fingerprint profile for saponin-rich fractions, enabling qualitative assessment and standardization. Further LC-MS analysis confirmed the presence of major steroidal saponins, particularly Shatavarin derivatives, based on molecular ion peaks and fragmentation patterns. The integrated analytical approach ensures reliable identification, purity assessment, and quality control of *A. racemosus* saponins.

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