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Evaluation of Shatavarin IV Compound from Methanolic Extract of *Asparagus racemosus* by High Performance Thin Layer Chromatography

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ABSTRACT

Asparagus racemosus is an herb of *Asparagus* or *Liliaceae* family. It is commonly utilized in human and animal husbandry care management. *Asparagus racemosus* has powerful antioxidant anti-inflammatory uterine tonic antimicrobial, anti-cancer, and galactagogue properties. Shatavarin is a steroidal saponin found in *Asparagus racemosus* roots. In this experiment, roots of *Asparagus racemosus* were collected from Herbal Garden, Ethnos Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu. Shatavarin IV was identified and quantified by a simple precise and reliable High Performance Thin Layer Chromatography technique.

Keywords: *Asparagus racemosus*, Shatavarin, Steroidal saponin, High Performance Thin Layer Chromatography.

INTRODUCTION

India includes 15 different agro-climatic zones and 17000-18000 flowering plant species, of which 6000-7000 have been determined to have medicinal use in people and archived health-care systems from Ayurveda, Siddha, and Unani [1].

Traditional herbs and plants are used in Indian medicine to treat a variety of ailments. Due to their extraordinary adaptability and proven efficacy, traditional plant-based medications have recently received a boost in research and development in modern medicine [2]. Traditional and alternative medicinal systems are attracting traditional and alternative medicinal systems in our communities due to the high cost and adverse effects of modern treatments [3].

Shatavari is the popular name for *Asparagus racemosus* Willd (Liliaceae). It's a branching spinose undershrub that grows wild in India's tropical and subtropical regions [4]. *Asparagus* exists in around 300 different species around the world. Because steroidal saponins and sapogenins are discovered in several portions of the plant, the genus is regarded medicinally valuable [5]. *Asparagus racemosus* has been used medically in Indian and British pharmacopoeias, as well as in indigenous medical systems [5].

Asparagus racemosus is recommended as a cooling agent and uterine tonic in Ayurveda. An anticancer drug asparagine present in *Asparagus* that can benefit with leukaemia treatment. The saponins, present in the asparagus is having antioxytocic and antispasmodic properties which is specific to uterine musculature contraction. It helps dairy animals produce more milk. Its mixture with milk aids nursing women in secretion of more milk [6].

The most active components in the roots of *Asparagus racemosus* are steroidal saponins (Shatavarins I-IV). Shatavarin IV is a glycoside having two rhamnose molecules and one glucose group that belongs to the sarsasapogenin family. In cell free assays, Shatavarin IV has been shown to have considerable inhibitory action against Core 2 GlcNAc transferase, as well as immunomodulation activity against particular T-dependent antigens in immuno deficient animals [7].

To identify and analyse shatavarin IV from *Asparagus racemosus* root samples growing at the Ethno Veterinary Herbal Product Research and Development Centre's herbal garden, a reliable, easy, and precise High performance thin layer chromatography (HPTLC) method was developed. Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Orathanadu, Thanjavur District, Tamil Nadu.

MATERIALS AND METHODS

Plant Source

Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, TANUVAS, Orathanadu, Thanjavur District, Tamil Nadu was used to collect raw roots of *Asparagus racemosus*. For additional analysis, the roots were rinsed, shade-dried, and pulverised.

Methanolic Extract Preparation

In a Soxhlet system, 30 g of *Asparagus racemosus* powder was extracted for 12 cycles with 500 ml of methanol. The extracts were subjected to Rotary evaporator (R-300, Büchi, Switzerland) in a vacuum, the solvent was removed to dryness. (50°C and 218 mbar). Sigma Aldrich made available the standard Shatavarin IV (USA). The solvents utilised in the experiment were all HPLC grade.

Detection of Shatavarin IV from *Asparagus racemosus* with HPTLC

A silica gel 60 F254 Thin Layer Chromatographic (TLC) plate was used for the chromatographic separation (Merck). With help of the sample applicator (CAMAG Linomat 5), seven different volumes of standard shatavarin IV solution (1 mg/mL) and replicates of 1,2,3, and 4 L of *Asparagus racemosus* samples (100 mg/l) were loaded to TLC plates. The type of application was banding, which was done on the Y axis of the plate with an 8.0 mm spacing and a length of 8.00 mm. The plate was then developed at room temperature in a twin-trough vertical development chamber (CAMAG) (20x10) with the mobile phase of ethyl acetate–methyl alcohol–water (7.5:1.5:1, v/v) which had been saturated for twenty minutes. The front position of the solvent was maintained at 70 mm.

After development, TLC plates were dried for 5 minutes with the use of an air-dryer. Then, the derivatization of TLC plate was done by derivatizer (CAMAG) with anisaldehydesulfuric acid solution, and then five minutes in a hot-air oven at 105°C. The plate was scanned with a CAMAG visualizer2 at a scanning speed of 20 mm/s with a slit dimension of 6.0 mm x 0.45 mm by utilising tungsten and Deuterium lamp light sources at wavelengths of R White, 254 nm, and 366 nm. On the HPTLC plates, the presence of shatavarin IV in *Asparagus* samples was detected as bands with identical Retardation factor (Rf) values to the shatavarin IV standard. For both the standard and sample areas, the retardation factor (Rf) was measured, which is the ratio of the compound's distance moved to the solvent's distance moved in a given time.

Quantification of Shatavarin in *Asparagus racemosus* Samples

At R White, the area calibration for substance shatavarin IV was measured using a scanner (CAMAG S/N: 250410). For the analysis of shatavarin IV from *Asparagus racemosus* samples, a standard curve was created by using seven reference Standard samples and four *Asparagus* samples.

RESULTS AND DISCUSSION

The Rf values (Table 1) for shatavarin IV standard and *Asparagus* samples were analysed by HPTLC, demonstrating the availability of shatavarin IV in the *Asparagus* extracts. Figures 1, 2, and 3 depicted the development of a TLC plate featuring remission of R white and 366 nm, as well as the detection of shatavarin IV in an *asparagus* sample using Rf value. Because of its sensitivity and precision, HPTLC has been used in a handful of studies for the identification and analysis of therapeutic phytoconstituents.

Previous studies with CAMAG HPTLC employed winCATS software, however the current study used the advanced modern software visionCATS. Furthermore, no reports on the analysis of

shatavarin IV from *Asparagus* grown in the Thanjavur area of Tamil Nadu are known.

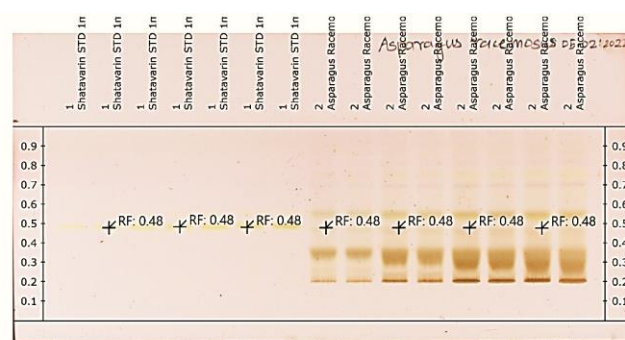


Figure 1: HPTLC plate produced over remission R White with bands of references (Tracks 1–7) and *Asparagus* (Track 8 - 5)

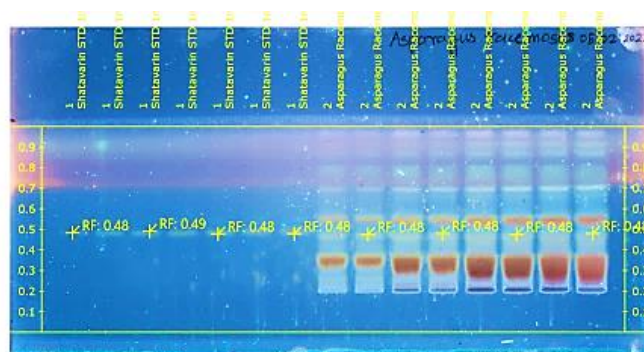


Figure 2: HPTLC Plate developed under remission 366nm showing bands of standards (Track 1 to 7) and samples (Track 8 to 15)

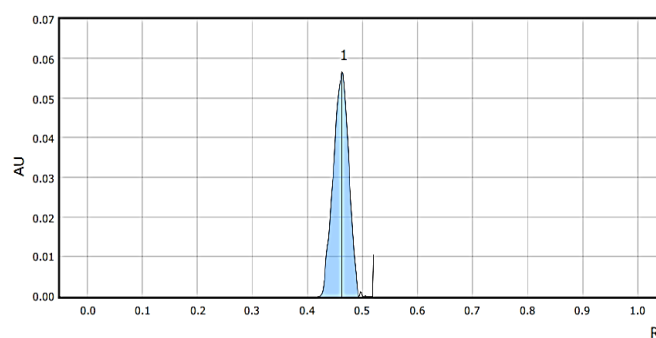


Figure 3: Shatavarin IV was identified using an HPTLC plate track peak in an *Asparagus racemosus* sample

In the CAMAG scanner, a calibration curve was created with seven standard and four *Asparagus* samples for quantification. The coefficient of variation (CV) was 3.84 percent, and the correlation coefficient was 99.39965%. Based on the calibration curve, the amount of shatavarin IV in the *Asparagus* sample was calculated to be 676.1 µg per 100 mg (0.6761 percent). The standard curve was linear in the *Asparagus* sample range of 181.9 µg/mL to 1.393 mg/mL. The standard curve for relative quantification of shatavarin IV in *Asparagus* was shown in Fig.4. Bhurat *et al.*, (2018) [8] found 0.18 % of Shatavarin IV in aqueous extract and Haldar *et al.*, (2018) [9] quantified 0.408% of Shatavarin IV in the methanolic extract or tuberous root. The level of shatavari IV was comparatively high in the sample studied.

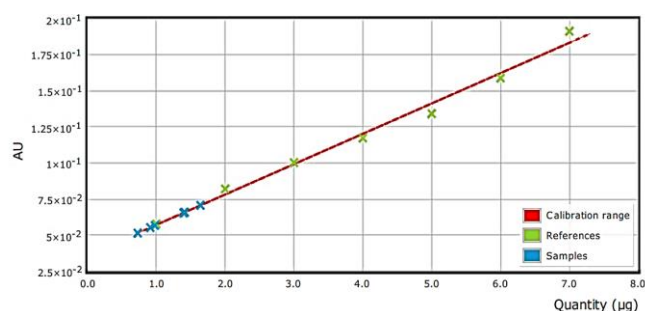


Figure 4: HPTLC standard curve for shatavarin IV determination in Asparagus sample (100mg/ml)

CONCLUSION

The results obtained in the present study suggest that the roots of *Asparagus racemosus* cultivated at herbal garden of Ethno Veterinary Herbal Product Research and Development Centre, Veterinary College and Research Institute, Orathanadu contain shatavarin IV, which has potential for use in the health care of animal kingdom.

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Conflict of Interest

None declared.

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