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ORIGINAL ARTICLE

Assessment of variation in Shatavarin IV content in *Asparagus racemosus* through HPTLC analysis and identification of elite germplasm from Eastern India

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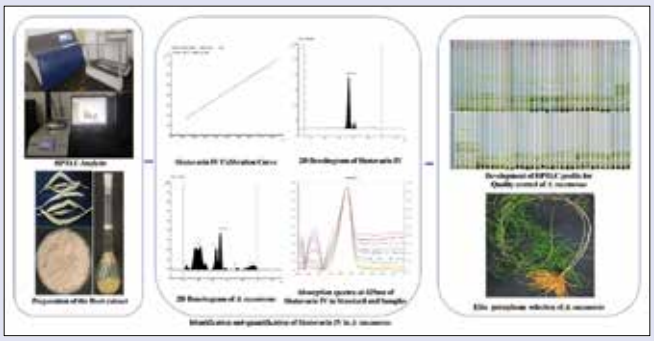
ABSTRACT

Background: *Asparagus racemosus* has been attaining its importance due to its high medicinal and pharmaceutical value. This plant resulted in the depletion of its wild population in India. This plant has been placed in the nearly threatened and endangered category of the International Union for Conservation of Nature and Natural Resources (IUCN) in the states of Chhattisgarh, Madhya Pradesh, and West Bengal. The over-demand also poses the problem of adulteration in several ways. **Objectives:** Selection of elite germplasm for commercial cultivation was done by studying the variation of Shatavarin IV from different geographical regions. The development of simple and reliable high-performance thin layer chromatography (HPTLC) method and chromatographic profile was done for quality control. **Materials and Methods:** A total of 103 *Asparagus racemosus* root samples were collected from different agro-climatic zones of Odisha. The methanol root extracts were analyzed qualitatively and quantitatively using HPTLC. The pre-coated silica gel 60 F_{254} plates were used for the development of chromatograms with ethyl acetate–methanol-water (7.5:1.5:1, v/v/v) as a mobile phase. **Results:** The Shatavarin IV was detected at R_f of 0.40 ± 0.05 and showed maximum absorption at 425 nm. The method was validated in terms of linearity, limit of detection (LOD), quantification (LOQ), precision, stability, and recovery test. The amount of Shatavarin IV varied between 0.01-0.40% among different regions. **Conclusion:** The elite germplasms were identified at Ramagiri Hill sides of Gajapati district, Odisha, having Shatavarin IV content >0.39%. The HPTLC method used here was found to be reliable, precise, and can be used for the quality control assessment of *Asparagus racemosus*.

Key words: *Asparagus racemosus*, elite germplasm and quality control, HPTLC profile, Shatavarin IV

SUMMARY

- The objective of the study was to select an elite germplasm of *Asparagus racemosus* by studying the samples from different agroclimatic zones of Odisha for commercial cultivation in order to meet the high demand and conservation of elite germplasm for future use because it has already been placed in nearly threatened and endangered category of Conservation of Nature and Natural Resources (IUCN) in the states of Chhattisgarh, Madhya Pradesh and West Bengal. The objective was also to develop chromatographic profile through a validated HPTLC method for quality control and checking the adulteration. The study revealed that there is a great variation in the Shatavarin IV content among the samples. The elite germplasms were selected among 103 samples with high content of Shatavarin IV (>0.39%) from the Ramagiri hills of Gajapati district, Odisha, India. The chromatographic profile was developed for all the 103 accession and it was consistent for all with negligible variations.



Abbreviations used:
V: Volume; R_f: Retardation factor; Nm: Nanometer; m: Meter; µm: Micrometer; g: Gram; hr: Hour; ml: Milliliter; cm: Centimeter; µL: Microliter; mm: Millimeter; UV: Ultra violet; min: Minutes; ng: Nanogram; 2D: Two dimensional; AR: Asparagus racemosus.

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INTRODUCTION

Asparagus racemosus is an important medicinal plant widely used in Indian and British Pharmacopoeias as well as in traditional Ayurveda, Siddha, and Unani systems of medicine. It has been placed in one of the Rasayana herbs in the Ayurveda system of medicine due to its tremendous medicinal value against an array of stresses.[1] In the Indian system of medicine, the root paste and juice are used for treating various ailments and as a health tonic.[2] It's a woody climber attaining a height of 1-3 m. The leaves are small, needle-like and the flowers are white with some spikes. It belongs to the family Asparagaceae and is generally found in low altitude shade

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prevalent regions of Asia, Australia, and Africa. Among all Asparagaceae species, *A. racemosus* is mainly used as indigenous medicine in India.[3]

In Ayurveda, *A. racemosus* root is being used to cure a spectrum of diseases like diarrhea, nervous disorders, dysentery, dyspepsia, tumors, inflammations, neuropathy, hyperdipsia, hepatopathy, cough, hyperacidity, bronchitis, and certain infectious diseases.[4] It is used as a galactagogue and for the management of threatened abortion. The cytotoxic activity of Shatavarin IV and other Shatavarins were studied both *in vitro* and *in vivo*, showing significant anticancer activity in human cell lines.[5] The plant extract has shown significant curing ability in castor oil-induced diarrhea and PGE2-induced enteropooling in rats.[6] Numerous biological efficacies such as antisecretory and antiulcer,[7] antiinflammatory and antiarthritic,[8] anticandidal,[9] analgesic,[10] antimicrobial,[11] antioxidant and hepatoprotective,[12] cytotoxic,[13] antibacterial,[14] antiepileptic,[15] and antiapoptotic activity,[16] have been reported in the root extract of *A. racemosus*. The main bioactive compounds of *A. racemosus* are the steroidal saponins. The steroidal saponins includes Shatavarin I to Shatavarin IV. Shatavarin IV (44%) is the major steroidal saponin found in the roots of *A. racemosus*.[17] The primary chemical compounds of the plant include essential oils, asparagine, arginine, tyrosine, flavonoids, resin, and tannin.[18] Shatavarins (I-IV) are the principal constituents responsible for the medicinal properties of the plant and are extracted from the roots of *A. racemosus*.[19] Shatavarin IV possess potent anticancer activity on several human cell lines such as MCF-7, HT-29, and A-498.[4] Shatavarin IV has been shown to increase the life span and reduce aging in *Caenorhabditis elegans*. It also increases the level of stress response mRNA molecules by increasing the stress response genes.[20] In the cell-free assay, the core Golgi enzymes transferase was inhibited by Shatavarin IV and in immunocompromised animals, it shows immunomodulation activity against T-dependent antigens.[21] Therefore, it can be inferred that Shatavarin IV is the major bioactive constituent responsible for the pharmacological activities of *A. racemosus*. The production and accumulation of secondary metabolites are being affected by several environmental factors.[22] The genetic, morphogenetic, ontogenic, and environmental factors are largely responsible for the biosynthesis and accumulation of secondary metabolites in plants.[23] Previously, variation in secondary metabolites has also been reported from the plants of different geographical locations.[24]

Therefore, identification of elite germplasm of *A. racemosus* among various populations from different geographical locations concerning Shatavarin IV content holds significant importance. Selection of elite germplasm would help in conserving and propagating *A. racemosus* which is now endangered in its natural habitats.[25,26] The high demand has led to adulteration of the herbal drugs of *A. racemosus*. Several analytical chromatographic methods such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography (GC) are being used for the analysis of phytoconstituents in medicinal plants etc.[27] In the last few decades, HPTLC is widely used for the qualitative and quantitative phytochemical analysis of herbal drugs.[28] Thus, considering the importance of Shatavarin IV content, the investigation was made to identify the elite germplasm and investigate the variation in Shatavarin IV content among various populations of *A. racemosus* from Odisha.

MATERIALS AND METHODS

Plant sample

The fresh roots of mature plants were collected and identified by a taxonomist, Prof. PC Panda, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha. The root samples of *A. racemosus* were shade dried and pulverized in a mechanical grinder to obtain coarse powder (60 mesh). The powdered samples were used for the HPTLC analysis.

Chemicals and reagents

The reference standard Shatavarin IV (99% purity) was procured from Natural Remedies Private Limited, Bangalore, India. The chemical structure of Shatavarin IV is given in Figure 1. Ethyl acetate, Methanol, Sulphuric acid, and Water (HPLC grade) were obtained from Merk Life Science Private Limited, Mumbai, India. p-Anisaldehyde was purchased from HiMedia Laboratories Pvt Ltd., Mumbai, India. Acetone and Acetic acid glacial were purchased from Merck Specialities Private Limited, Mumbai, India. The HPTLC silica gel 60 F_{254} plates were procured from Merck KGaA, Darmstadt, Germany.

Extraction procedure for Shatavarin IV and standard solution preparation

The powdered plant samples were extracted using methanol. 0.80 g of powdered root sample was added to 10 mL of methanol and placed in a Water bath (MS Stirring Water Bath) at 60°C for 1 hr. It was then filtered using a syringe filter (0.22 µm). The filtered solution was used for HPTLC analysis. The standard Shatavarin IV was prepared by dissolving in methanol.

HPTLC chromatographic conditions

The chromatographic analysis was done by applying the extracted solution of roots of *A. racemosus* and standard Shatavarin IV on precoated silica gel 60 F_{254} (10 × 20 cm) HPTLC plates. The samples and standards were applied to the plates using a CAMAG Linomat V sample applicator equipped with a 100-µL syringe. The volume of application of the sample and standard was 6 µL per band. The band length of application was 8 mm. The separation between the bands was 12 mm. A linear ascending development was carried out using mobile phase ethyl acetate-methanol-water (7.5:1.5:1, v/v/v) in a 20 × 10 cm twin-trough glass chamber (CAMAG) saturated for 20 min at room temperature. The volume of the mobile phase was 20 mL and the plate was developed up to 70 mm. The developed plate was then airdried with the help of an air-dryer. The plates were visualized in a CAMAG UV cabinet at 254 nm and 366 nm light. Then, it was derivatized by dipping the plate in anisaldehyde–sulfuric acid reagent and subsequent heating at 110°C for 5 min in a hot-air oven. The plates were then viewed at 366 nm in a CAMAG UV cabinet and the photos were taken in the fluorescence light after derivatization. The plate was scanned using CAMAG TLC scanner 4 equipped with winCATS Software (Slit dimension 6.00 × 0.45). The scanning was done at 425 nm and the amount of Shatavarin IV present in the samples was quantified with the help of a calibration curve. The spectra matching was carried out by overlaying the spectra of the sample and standard Shatavarin IV in the range of 300-700 nm.

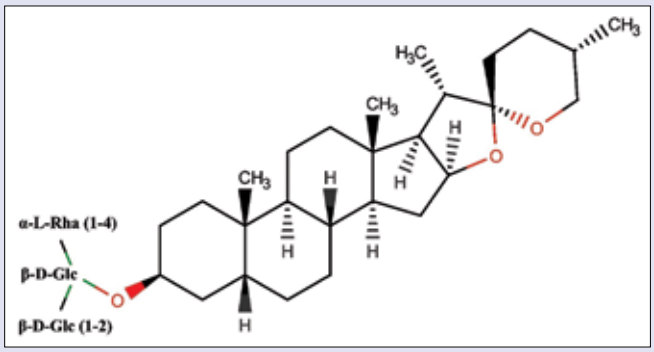

Figure 1: Chemical Structure of Shatavarin IV

Method validation

The developed HPTLC method was validated in terms of linearity, stability, precision, and recovery. These assessments were done by the International Conference on Harmonization (ICH) guidelines.[29]

The calibration curve was done by applying different concentrations of Shatavarin IV. The limit of detection (LOD) is the minimum concentration of an analyte at which the peak area of the signal is at least three times greater than a signal to noise ratio (S/N ≥ 3). The limit of quantification (LOQ) is the concentration of an analyte at which the peak area is at least ten times greater than the signal to noise ratio (S/N ≥ 10). The precision was determined by injecting the replicate solution of standard three times within a day (Intra-day stability). The stability (Inter-day) test of the standard was determined by injecting the same solution of standard for three consecutive days (0, 24, 48 hr). The recovery test was determined by the method of standard addition, which was done by spiking the sample with a known amount of amount Shatavarin IV.

RESULTS

Optimization of extraction condition

The extraction condition of the plant root extract was optimized by taking different parameters. Herein, a different combinations of solvents (methanol, acetone, and water), temperature (30, 40, 50, and 60°C), and extraction time (20, 40, 60, and 80 min) were analyzed and maximum Shatavarin IV was found in methanol at 60°C for 60 min.

Method validation

The validation must reveal the characteristic feature of the performance alongside the fitness of the method for the intended purpose.[30] The methods need to be validated for quality control as per the quality and accreditation standards of ICH, Food and Drug Administration (FDA) and Environmental Protection Agency (EPA), United States Pharmacopeia (USP) etc.[31] The quantification of Shatavarin IV was done with the help of calibration curve of standard Shatavarin IV. The visual confirmation of Shatavarin IV in the sample chromatograms was made by comparing the retardation factor (R_f) reference standard Shatavarin IV. The R_f of Shatavarin IV was found to be 0.40 ± 0.05 in HPTLC chromatographic analysis.

The calibration plot was linear over the range of 72-432 ng/spot, and the correlation coefficient (R^2) of 0.9968 (Shatavarin IV) revealed a good linear relationship between peak area and concentration [Figure 2]. The calibration curve was determined by the linear regression equation $y = 14.25x + 779.45$ (where x is the concentration of Shatavarin IV and y is the peak area). The limit of detection (LOD) and limit of quantification (LOQ) was determined using signal-to-noise ratio. The LOD of Shatavarin IV was found to be 24 ng and LOQ of shatavarin IV was found to be 72 ng, respectively [Table 1]. The method of quantitative analysis showed satisfactory results in precision, stability and recovery tests. The relative standard deviation (RSD) of precision (intra-day) and stability (inter-day) was found to be 1.63% and 1.69%, respectively, which were in the acceptable range [Table 2]. The recovery percentage of Shatavarin IV was found to be more than 97% with RSD value of 0.97% [Table 3]. The specificity of method was determined by overlaying spectra of peak of Shatavarin IV in standard and *A. racemosus* root extract [Figure 3]. The absorption maxima were observed at 425 nm in both sample and standard for Shatavarin IV. The comprehensive chromatographic profile was given in the Figure 4. The chromatographic profile developed in this study could be used for quality control and authentic identification of *A. racemosus*. The 2D densitogram of Shatavarin IV standard and a representative sample is given in Figures 5 and 6, respectively.

Elite germplasm identification

The quantity of Shatavarin IV varied between 0.01% to 0.40% among 103 samples collected from different phytogeographical regions of Odisha [Table 4]. The germplasm having the highest percentage of Shatavarin IV i.e., 0.40% was selected as the elite germplasm for commercial and conservational purposes.

Table 1: Sensitive Analysis and calibration curve of shatavarin IV

Component	linear range (ng/band)	Regression equations	R^2	LOD (ng)	LOQ (ng)
Shatavarin IV	72-432	y=14.25x+779.45	0.9968	24	72

LOD: Limit of detection; LOQ: Limit of quantification

Table 2: Intra-day and inter-day precision of Shatavarin IV by HPTLC method

Component	Precision	Amount applied (ng)	Amount found (ng)	Mean	RSD (%)
Shatavarin IV	Intra-day	705	703	701	1.63
			699		
			701		
	Inter-day	705	703	702.33	1.69
			700		
			704		

RSD: Relative standard deviation

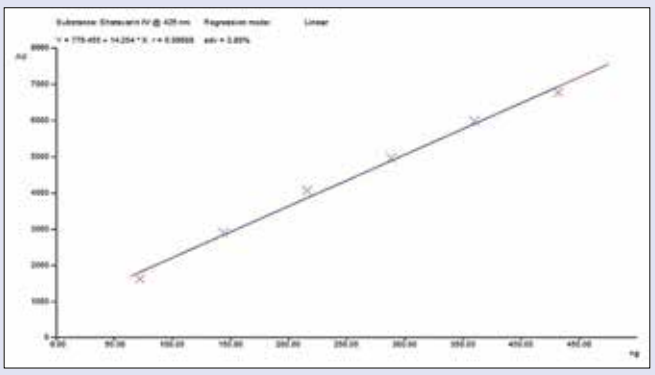

Figure 2: Calibration curve of Shatavarin IV for quantification though HPTLC analysis

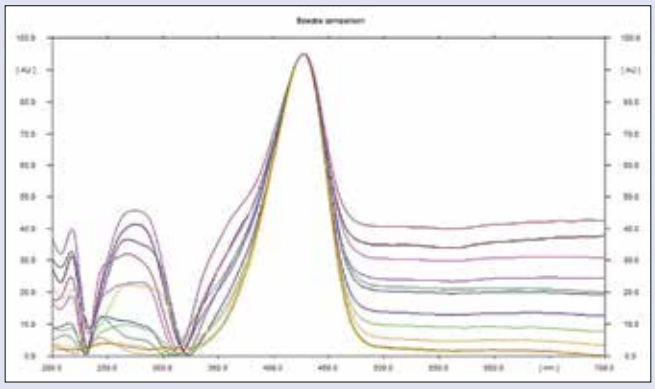

Figure 3: UV spectra of standard and samples of *Asparagus racemosus* for Shatavarin IV

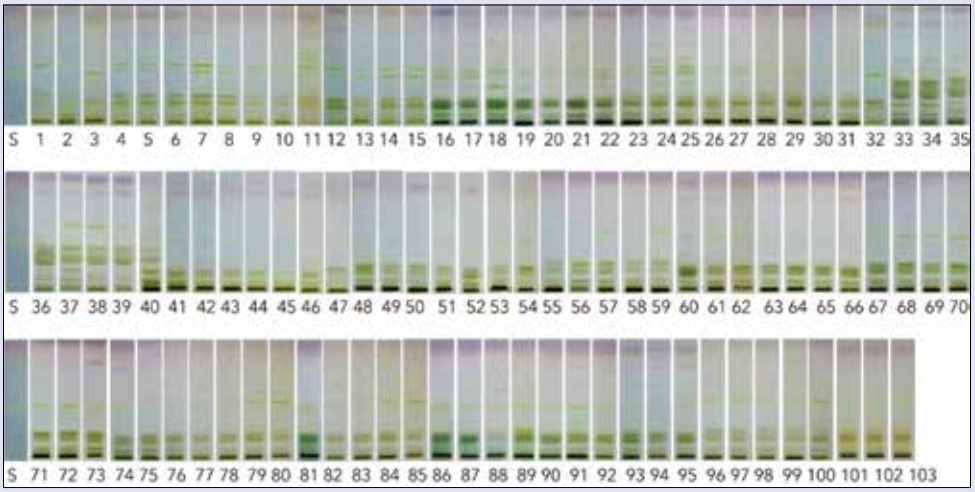

Figure 4: HPTLC chromatogram profile of Asparagus racemosus for Shatavarin IV

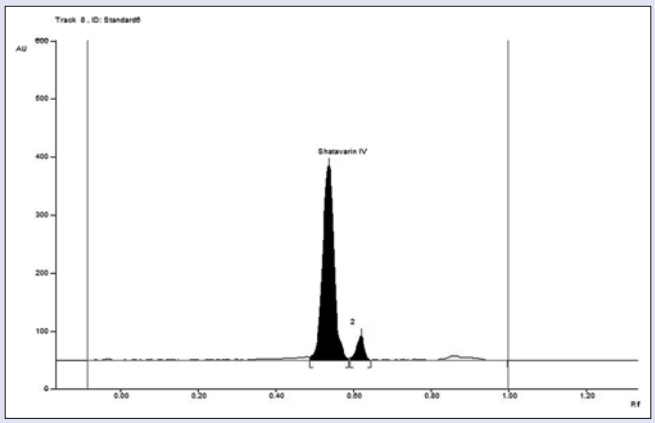

Figure 5: 2D HPTLC densitogram of Shatavarin IV standard

Table 3: Recovery study of Shatavarin IV by HPTLC method

Component	Original amount (ng)	Added Amount (ng)	Found (ng)	Recovery rate (%)	Average recovery (%)	RSD (%)
Shatavarin IV	725	200	888.56	96.06	97.17	0.96
			897.78	97.05		
			910.32	98.41		
		300	1005.45	98.09	97.57	0.39
			995.67	97.13		
			999.43	97.50		
		400	1102.22	97.97	97.55	0.43
			1099.37	97.72		
			1090.78	96.95		

RSD: Relative standard deviation

DISCUSSION

Extraction conditions and mobile phase

The most important step in the phytochemical analysis is the extraction of desired bioactive compound. This is based on the principle to elute maximum-desired bioactive compound from the complex plant matrix without destroying the chemical nature of the compound.[32] Alcohols are commonly used for the extraction of saponins with methanol being widely used for better extraction yield.[33] According to literature, methanol is the most effective solvent to extract higher amount of saponins from the plant matrix as compared to other solvents.[34] Previously, methanol was used to extract saponins from *Momordica cochinchinensis* Spreng.[33] and *Salicornia herbacea*.[24] The saponin extraction from the Asparagus species were also done using methanol as extraction solvent for chromatographic analysis.[35,36] The major bioactive compound Shatavarin IV was determined in the methanolic extracts of *A. racemosus* by a number of researchers.[28,37,38] Therefore, the use of methanol in appropriate temperature and duration for the quantitative determination of Shatavarin IV is justified. The temperature parameter of the phytochemical extraction plays an important role in extracting the desired bioactive compound and chemical stability of the compound. In our study, by increasing the extraction temperature up to 60°C increases the extraction yield but gradual small increase in temperature beyond that the yield was declined and solvents started to evaporate (after ~65°C). The surface tension and viscosity of the extraction solvent decreases at high temperature and at this condition the solvent can weaken the cell wall membrane of the plant cell in order to elute maximum desired phytochemicals.[39] The decline in Shatavarin IV yield when exposed to higher temperature for long time might be due to degradation of phytochemicals in the plant cells.[40] The mobile phase (ethyl acetate-methanol-water, 7.5:1.5:1, v/v/v) used in our study efficiently separate the bands on the chromatographic plates. The mobile phase gave bands of good resolution with stable peaks. The mobile phase used in our study was standardized by earlier researchers for better separation of bands in chromatographic determination of Shatavarin IV.[38,41] The twin-trough glass chamber was saturated with chromatographic paper and mobile phase for 20 min in order to get uniform distribution of mobile phase.

Elite germplasm selection of *A. racemosus*

The *A. racemosus* samples were collected in a single growing season with similar growth texture to avoid seasonal and developmental variation of secondary metabolites. The shatavarin IV content varied greatly among the samples from different regions of Odisha. The intraspecific chemical variation in plants is attributed to plant plant ontogeny, environmental and genetic factors.[42] The plant secondary metabolites are also known to exhibit geographical variations.[43] In our study, the seasonal and developmental variations might not have played a role as the samples were collected in a single growing season with similar growth stage. The variation in Shatavarin IV in the present study might be attributed

Table 4: Quantitative estimation of Shatavarin IV (%) in *Asparagus racemosus* samples collected from different geographical location of Odisha, India

Code	Locality	District, State	Latitude	Longitude	Altitude (m)	Shatavarin IV %
AR-1	Nabaghanpur	Nayagargh, Odisha	20° 06' 44.10" N	85° 05' 15.18" E	131	0.05
AR-2	Baigunia	Nayagargh, Odisha	20° 15' 31.32" N	85° 12' 39.60" E	64	0.16
AR-3	Nabaghanpur	Nayagargh, Odisha	20° 06' 44.10" N	85° 05' 15.18" E	131	0.33
AR-4	Tangi	Khordha, Odisha	19° 56' 44.34" N	85° 27' 19.08" E	18	0.22
AR-5	Tangi	Khordha, Odisha	19° 56' 44.34" N	85° 27' 19.08" E	19	0.21
AR-6	Tangi	Khordha, Odisha	19° 56' 44.34" N	85° 27' 19.08" E	20	0.23
AR-7	Sankesh	Rayagada, Odisha	19° 11' 12.12" N	83° 18' 48.60" E	448	0.33
AR-8	Sibapadar	Rayagada, Odisha	19° 42' 15.98" N	83° 27' 52.56" E	388	0.22
AR-9	Therubali	Rayagada, Odisha	19° 17' 56.90" N	83° 26' 29.76" E	249	0.19
AR-10	Therubali	Rayagada, Odisha	19° 26' 44.34" N	83° 27' 19.08" E	415	0.16
AR-11	Sibapadar	Rayagada, Odisha	19° 43' 43.68" N	83° 29' 13.20" E	360	0.17
AR-12	Budisila	Gajapati, Odisha	19° 07' 45.12" N	84° 08' 0.60" E	658	0.27
AR-13	Budisila	Gajapati, Odisha	19° 07' 45.12" N	84° 08' 0.60" E	658	0.24
AR-14	Budisila	Gajapati, Odisha	19° 07' 45.12" N	84° 08' 0.60" E	658	0.39
AR-15	Patrabasa	Gajapati, Odisha	19° 09' 40.60" N	84° 14' 52.08" E	827	0.31
AR-16	Mahendragiri	Gajapati, Odisha	18° 58' 16.32" N	84° 21' 12.24" E	1089	0.24
AR-17	Mahendragiri	Gajapati, Odisha	18° 58' 20.64" N	84° 21' 12.60" E	1089	0.22
AR-18	Mahendragiri	Gajapati, Odisha	18° 58' 18.84" N	84° 21' 10.80" E	1089	0.09
AR-19	Badamasingha	Gajapati, Odisha	18° 57' 6.12" N	84° 20' 47.40" E	723	0.08
AR-20	Badamasingha	Gajapati, Odisha	18° 56' 34.08" N	84° 20' 27.24" E	723	0.09
AR-21	Kainpur	Gajapati, Odisha	18° 56' 19.68" N	84° 17' 40.20" E	572	0.04
AR-22	Ramagiri	Gajapati, Odisha	19° 05' 33.00" N	84° 16' 30.36" E	741	0.16
AR-23	Ramagiri	Gajapati, Odisha	19° 05' 32.28" N	84° 16' 26.40" E	741	0.10
AR-24	Ramagiri	Gajapati, Odisha	19° 05' 33.07" N	84° 16' 26.04" E	741	0.40
AR-25	Ramagiri	Gajapati, Odisha	19° 05' 33.18" N	84° 16' 24.60" E	741	0.39
AR-26	Gandahati	Gajapati, Odisha	18° 52' 17.04" N	84° 12' 26.64" E	76	0.14
AR-27	Gandahati	Gajapati, Odisha	18° 52' 06.96" N	84° 13' 29.64" E	94	0.24
AR-28	Guma	Gajapati, Odisha	18° 51' 40.32" N	84° 02' 39.84" E	100	0.25
AR-29	Guma	Gajapati, Odisha	18° 56' 48.84" N	84° 01' 24.60" E	476	0.10
AR-30	Guma	Gajapati, Odisha	18° 56' 48.84" N	84° 01' 24.60" E	476	0.10
AR-31	Guma	Gajapati, Odisha	18° 56' 48.84" N	84° 01' 24.60" E	476	0.04
AR-32	Kamakhyanagar	Dhenkanal, Odisha	20° 40' 47.60" N	85° 43' 25.93" E	116	0.21
AR-33	Kamakhyanagar	Dhenkanal, Odisha	20° 40' 15.20" N	85° 43' 25.93" E	116	0.04
AR-34	Kamakhyanagar	Dhenkanal, Odisha	20° 40' 15.20" N	85° 43' 25.93" E	117	0.05
AR-35	Tarava	Angul, Odisha	20° 42' 5.11" N	84° 51' 13.89" E	257	0.11
AR-36	Tarava	Angul, Odisha	20° 42' 5.11" N	84° 51' 13.89" E	258	0.11
AR-37	Purunakot	Angul, Odisha	20° 38' 29.18" N	84° 51' 53.46" E	182	0.04
AR-38	Purunakot	Angul, Odisha	20° 38' 29.18" N	84° 51' 53.46" E	182	0.00
AR-39	Purunakot	Angul, Odisha	20° 38' 29.18" N	84° 51' 53.46" E	182	0.02
AR-40	Balukhanda Area	Puri, Odisha	19° 51' 29.88" N	86° 02' 27.6" E	8	0.03
AR-41	Balukhanda Area	Puri, Odisha	19° 51' 29.88" N	86° 02' 27.6" E	8	0.15
AR-42	Balukhanda Area	Puri, Odisha	19° 51' 27.00" N	86° 2' 11.76" E	8	0.12
AR-43	Balukhanda Area	Puri, Odisha	19° 51' 27.00" N	86° 02' 11.76" E	8	0.08
AR-44	Balukhanda Area	Puri, Odisha	19° 51' 21.96" N	86° 02' 11.4" E	8	0.08
AR-45	Balukhanda Area	Puri, Odisha	19° 51' 21.96" N	86° 02' 11.4" E	8	0.06
AR-46	Balukhanda Area	Puri, Odisha	19° 51' 27.00" N	86° 02' 11.76" E	8	0.12
AR-47	Sidhamatha hill	Keonjhar, Odisha	21° 37' 05.80" N	85° 34' 16.68" E	634	0.11
AR-48	Sidhamatha hill	Keonjhar, Odisha	21° 37' 06.74" N	85° 34' 15.60" E	634	0.04
AR-49	Sidhamatha hill	Keonjhar, Odisha	21° 37' 07.06" N	85° 34' 15.24" E	634	0.02
AR-50	Sidhamatha hill	Keonjhar, Odisha	21° 37' 07.28" N	85° 34' 14.88" E	634	0.03
AR-51	Sidhamatha hill	Keonjhar, Odisha	21° 37' 06.13" N	85° 34' 16.68" E	634	0.06
AR-52	Badaghagra hill	Keonjhar, Odisha	21° 36' 59.32" N	85° 33' 08.64" E	634	0.03
AR-53	Badaghagra hill	Keonjhar, Odisha	21° 37' 31.94" N	85° 33' 05.76" E	511	0.12
AR-54	Badaghagra hill	Keonjhar, Odisha	21° 37' 20.89" N	85° 33' 03.24" E	634	0.21
AR-55	Badaghagra hill	Keonjhar, Odisha	21° 37' 20.96" N	85° 33' 03.24" E	634	0.10
AR-56	Badaghagra hill	Keonjhar, Odisha	21° 37' 20.13" N	85° 33' 02.52" E	634	0.04
AR-57	Badaghagra hill	Keonjhar, Odisha	21° 37' 20.02" N	85° 33' 02.88" E	634	0.26
AR-58	Gonasika hill area	Keonjhar, Odisha	21° 31' 42.88" N	85° 30' 37.80" E	688	0.06
AR-59	Gonasika hill area	Keonjhar, Odisha	21° 31' 41.88" N	85° 30' 38.52" E	688	0.30
AR-60	Mahapadasala	Keonjhar, Odisha	21° 13' 35.40" N	86° 15' 19.44" E	94	0.01
AR-61	Tagamana nala	Keonjhar, Odisha	21° 14' 33.72" N	86° 14' 55.68" E	86	0.12
AR-62	Belachuti	Keonjhar, Odisha	21° 14' 41.28" N	86° 15' 02.88" E	94	0.02
AR-63	Bahali	Cuttack, Odisha	20° 29' 36.24" N	85° 10' 22.08" E	128	0.16
AR-64	Bahali	Cuttack, Odisha	20° 29' 36.24" N	85° 10' 22.08" E	128	0.09
AR-65	Bahali	Cuttack, Odisha	20° 29' 36.24" N	85° 10' 22.08" E	128	0.21
AR-66	Patrabhaga	Cuttack, Odisha	20° 30' 38.52" N	85° 09' 20.52" E	244	0.14

Contd...

Table 4: Contd...

Code	Locality	District, State	Latitude	Longitude	Altitude (m)	Shatavarin IV %
AR-67	Patrabhaga	Cuttack, Odisha	20° 30' 38.52" N	85° 09' 20.52" E	244	0.27
AR-68	Patrabhaga	Cuttack, Odisha	20° 30' 38.52" N	85° 09' 20.52" E	244	0.16
AR-69	Kandhabadabhuin	Cuttack, Odisha	20° 30' 11.88" N	85° 09' 57.60" E	244	0.18
AR-70	Kandhabadabhuin	Cuttack, Odisha	20° 30' 11.88" N	85° 09' 57.60" E	244	0.14
AR-71	Kandhabadabhuin	Cuttack, Odisha	20° 30' 11.88" N	85° 09' 57.60" E	244	0.03
AR-72	Jemadeipur	Cuttack, Odisha	20° 29' 51.72" N	85° 09' 52.20" E	81	0.15
AR-73	Jemadeipur	Cuttack, Odisha	20° 29' 51.72" N	85° 09' 52.20" E	81	0.20
AR-74	Jemadeipur	Cuttack, Odisha	20° 29' 51.72" N	85° 09' 52.20" E	81	0.11
AR-75	Khajuria	Cuttack, Odisha	20° 29' 09.24" N	85° 09' 45.72" E	81	0.24
AR-76	Khajuria	Cuttack, Odisha	20° 29' 09.24" N	85° 09' 45.72" E	81	0.19
AR-77	Khajuria	Cuttack, Odisha	20° 29' 09.24" N	85° 09' 45.72" E	81	0.30
AR-78	Purukutia	Cuttack, Odisha	20° 29' 0.24" N	85° 10' 50.52" E	124	0.13
AR-79	Purukutia	Cuttack, Odisha	20° 29' 0.24" N	85° 10' 50.52" E	124	0.07
AR-80	Purukutia	Cuttack, Odisha	20° 29' 0.24" N	85° 10' 50.52" E	124	0.04
AR-81	Purukutia	Cuttack, Odisha	20° 29' 0.24" N	85° 10' 50.52" E	124	0.04
AR-82	Panchu pandav hills	Bargargh, Odisha	20° 53' 33.36" N	82° 49' 54.12" E	296	0.02
AR-83	Panchu pandav hills	Bargargh, Odisha	20° 53' 33.36" N	82° 49' 54.12" E	296	0.09
AR-84	Panchu pandav hills	Bargargh, Odisha	20° 53' 38.76" N	82° 49' 48.72" E	296	0.28
AR-85	Nrusinghanath	Bargargh, Odisha	20° 53' 58.92" N	82° 49' 04.44" E	296	0.32
AR-86	Nrusinghanath	Bargargh, Odisha	20° 53' 45.60" N	82° 48' 49.68" E	296	0.19
AR-87	Nrusinghanath	Bargargh, Odisha	20° 53' 45.60" N	82° 48' 49.68" E	296	0.13
AR-88	Nrusinghanath	Bargargh, Odisha	20° 53' 47.04" N	82° 48' 48.24" E	296	0.08
AR-89	Nrusinghanath	Bargargh, Odisha	20° 53' 43.08" N	82° 48' 43.20" E	296	0.16
AR-90	Gorunda	Bargargh, Odisha	20° 53' 53.16" N	82° 48' 32.76" E	296	0.14
AR-91	Zero point	Sambalpur, Odisha	21° 29' 42.36" N	83° 51' 11.52" E	188	0.23
AR-92	Debrigargh	Sambalpur, Odisha	21° 29' 57.12" N	83° 46' 15.60" E	181	0.11
AR-93	Debrigargh	Sambalpur, Odisha	21° 29' 56.04" N	83° 46' 20.28" E	181	0.12
AR-94	Debrigargh	Sambalpur, Odisha	21° 29' 56.04" N	83° 46' 20.28" E	181	0.06
AR-95	Debrigargh	Sambalpur, Odisha	21° 29' 56.04" N	83° 46' 20.28" E	181	0.16
AR-96	Karanjia	Mayurbhanj, Odisha	21° 37' 29.64" N	86° 06' 59.40" E	340	0.28
AR-97	Similipala	Mayurbhanj, Odisha	21° 59' 06.36" N	86° 17' 32.64" E	798	0.21
AR-98	Similipala	Mayurbhanj, Odisha	21° 58' 13.44" N	86° 20' 21.48" E	693	0.21
AR-99	Similipala	Mayurbhanj, Odisha	21° 58' 13.8" N	86° 20' 24.36" E	693	0.29
AR-100	Similipala	Mayurbhanj, Odisha	21° 58' 13.80" N	86° 22' 28.20" E	763	0.30
AR-101	Narayani Khola	Ganjam, Odisha	19° 41' 52.44" N	85° 09' 13.32" E	43	0.18
AR-102	Narayani Khola	Ganjam, Odisha	19° 41' 52.44" N	85° 09' 13.32" E	44	0.19
AR-103	Narayani Khola	Ganjam, Odisha	19° 41' 52.44" N	85° 09' 13.32" E	45	0.18

to soil condition, climatic variation and level of gene expression.[44-46] Chaudhary and Dantu, 2012 reported Shatavarin IV content in the range of 0.25-0.31% in thin and thick roots of *A. racemosus* through HPTLC analysis.[47] Though few reports are available on the determination of Shatavarin IV content using HPTLC analysis,[48-50] hardly any report is available on the extent of Shatavarin IV variation in *A. racemosus* from different natural populations.

In the present investigation, out of collected 103 accessions, 3 accessions showed Shatavarin IV content more than 0.37%. These accessions (AR-14, AR-24, and AR-25) were from Budisila and Ramagiri hills of Gajapati district of Odisha. Thus, Gajapati district of Odisha could be selected as potential site for obtaining high quality of *A. racemosus* with respect to Shatavarin IV content. Previously, the potential germplasm of *A. racemosus* was identified on the basis of root yield and Shatavarin IV content from different regions of Gujarat, India. Among the twelve selected accessions, one accession having 0.015% of Shatavarin IV content was selected as the elite germplasm by previous researchers.[25]

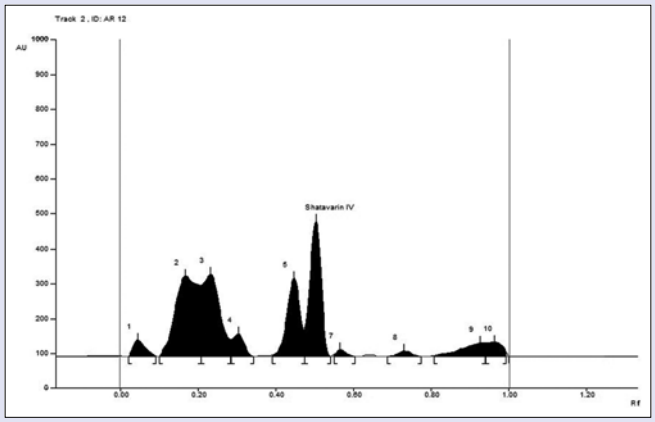

Figure 6: 2D HPTLC densitogram of Shatavarin IV in *Asparagus racemosus* root extract

CONCLUSION

The optimized HPTLC method developed here could be used for qualitative and quantitative analysis of steroidal saponin Shatavarin IV in *A. racemosus* and its herbal products. The remarkable variation of Shatavarin IV content in *A. racemosus* determined in the present study might be due to the difference in the soil condition and level of gene expression which needs to be explored further. The potential elite accessions (AR-14, AR-24, and AR-25) of *A. racemosus* were identified based on high content of shatavarin IV from Budisila and Ramagiri hill sides of Gajapati, Odisha would be helpful in conservation and commercial cultivation of this highly valued medicinal plant facing the danger of depletion in the natural population.

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Conflicts of interest

There are no conflicts of interest.

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