Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 272-275
Received: 07-07-2019
Accepted: 09-08-2019

Kishor K
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Sahni YP
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Sharma RK
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Shrman K
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Singh RP
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Dinodia N
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Singh P
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Correspondence
Kishor K
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur, Madhya Pradesh, India

Characterization of Shatavarin-IV in root extracts of *Asparagus racemosus* by UHPLC

Kishor K, Sahni YP, Sharma RK, Shrman K, Singh RP, Dinodia N and Singh P

Abstract
The herb *Asparagus racemosus* is known as "Shatavari" or "Queen of herbs". In this study, methanolic extracts from roots of *Asparagus racemosus* were analyzed by Ultra High-Performance Liquid Chromatography (UHPLC) with photodiode array (PDA) detector on C_{18} column utilizing methanol and HPLC water (80:20 v/v) as mobile phase under isocratic elution at 205 nm. *Asparagus racemosus* depicted the presence of Shatavarin-IV at retention time of 3.7 minutes with concentration of 0.08 mg/g.

Keywords: UHPLC, *Asparagus racemosus*, Shatavarin-IV, queen of herbs

Introduction
Asparagus racemosus belongs to the family Aspragaceae traditionally used as anthelmintic, antiseptic, antidiarrheal, antidysenteric, antioxidant (Velavan *et al*., 2007; Kamat *et al*., 2000) [14, 5], immunostimulant, antihepatotoxic (Muruganandan *et al*., 2001) [9], antibacterial (Mandal *et al*., 2000) [8], antioxytocic (Sekine *et al*., 1997) [12] and reproductive agent (Arora *et al*., 2005) [1] mainly due to the presence of steroidal saponins and sapogenins. This plant is recommended in Ayurveda for prevention and treatment of gastric ulcers, dyspepsia and as a galactogogue. *Asparagus racemosus* has been successfully employed by some ayurvedic practitioners for inflammation, nervous disorder, liver diseases and certain infectious diseases (Sinha and Biswas 2011) [13]. It is conceivable that herbal agents could serve as safer alternatives as growth promoters due to their suitability and preference, lower cost of production and reduced toxicity (Mahmood *et al*., 2009) [7].

Material and Methods
Plant material
Roots of *Asparagus racemosus* were procured from the Department of Aromatic and Medicinal Plants, Agriculture College, JNKVV, Jabalpur. Roots of *Asparagus racemosus* were shed dried, crushed and used for experimental purpose.

Standard and Chemicals
In this research work, standards of *Asparagus racemosus*-Shatavarin-IV (Sigma Aldrich 30151- 10mg) were procured Sigma Aldrich.
Other essential chemicals like HPLC water, Methanol and Ethanol were purchased from Hi Media laboratory Pvt. ltd, Mumbai. All the chemicals used in this study were of UHPLC grade.

UHPLC assay procedure
The concentration of Shatavarin-IV was measured by Ultra High Performance Liquid chromatography system with Photo Diode Array Detector (PDA).

Apparatus
In this study, Ultra High Performance Liquid Chromatograpy (UHPLC) apparatus of Shimadzu Corporation, Japan was used. UHPLC assembly was equipped with binary gradient solvent delivery pump (SIL-30AC) with PDA detector (SPD-M20A). Chromatographic separation was performed using C_{18} reverse phase column (Supelco Discovery Column 25cm x 4.6mm, particle size 5µ).

Extraction of Shatavarin-IV from *Asparagus racemosus* root powder
The root of *Asparagus racemosus* herbs were nicely cleaned and air dried. The dried samples were then powdered with the help of grinder. The powdered samples were kept in an air tight

container away from sun light until use. Extraction of *Asparagus racemosus* root powder was performed by Soxhlet extraction method with the help of methanol as solvent. The ratio of root powder and solvent was 1:10 g/ml. Continuous hot solvent extraction was done for 6 h at 40 ºC (Kausar *et al*., 2017) [6].

Sample preparation for Shatavarin-IV extract

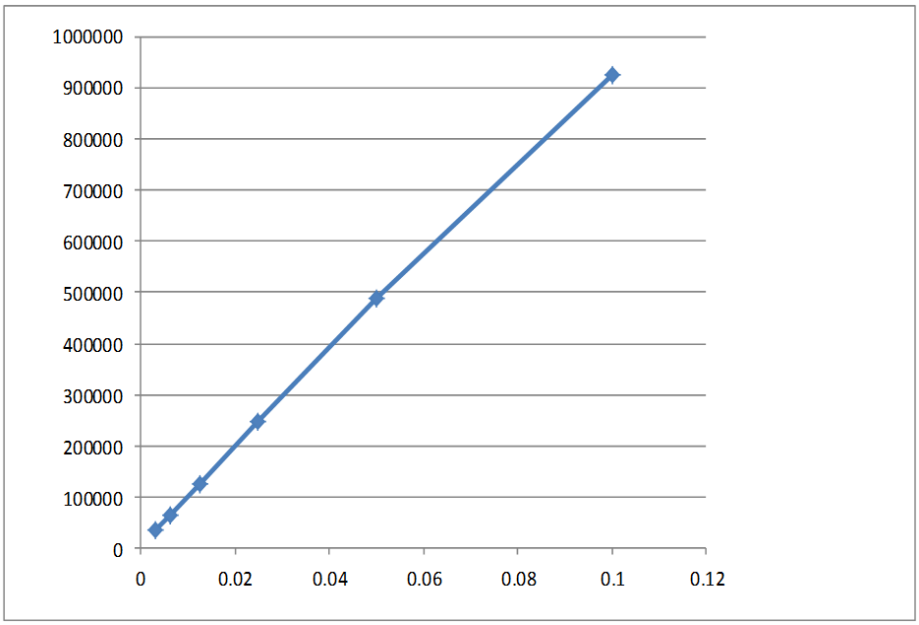
Different concentrations of Shatavarin extract were prepared by diluting with methanol and run under above chromatographic condition. All prepared samples were purified using nylon syringe filter having pore size of 0.22 µm.

Chromatographic condition for Shatavarin-IV

Methanol: HPLC Water (80:20 V/V) was used as mobile phase for Sharavarin-IV. The mobile phase was filtered by 0.22µ nylon syringe filter before use. Flow rate for the mobile phase was 1ml.min^{-1} and run time was 10 minutes. The temperature of column was 25±0.5 ºC. The effluent was monitored at 205nm wavelength according to Negi *et al*., 2011 [10].

Table 1: Concentrations of Shatavarin-IV for preparation of standard calibration curve

Concentration (mg. ml^{-1})	Peak Area (mAu)
0.1	926472
0.05	489463
0.025	246629
0.0125	125367
0.00625	69647
0.003125	34823



Calibration curve of Shatavarin-IV for *Asparagus racemosus*

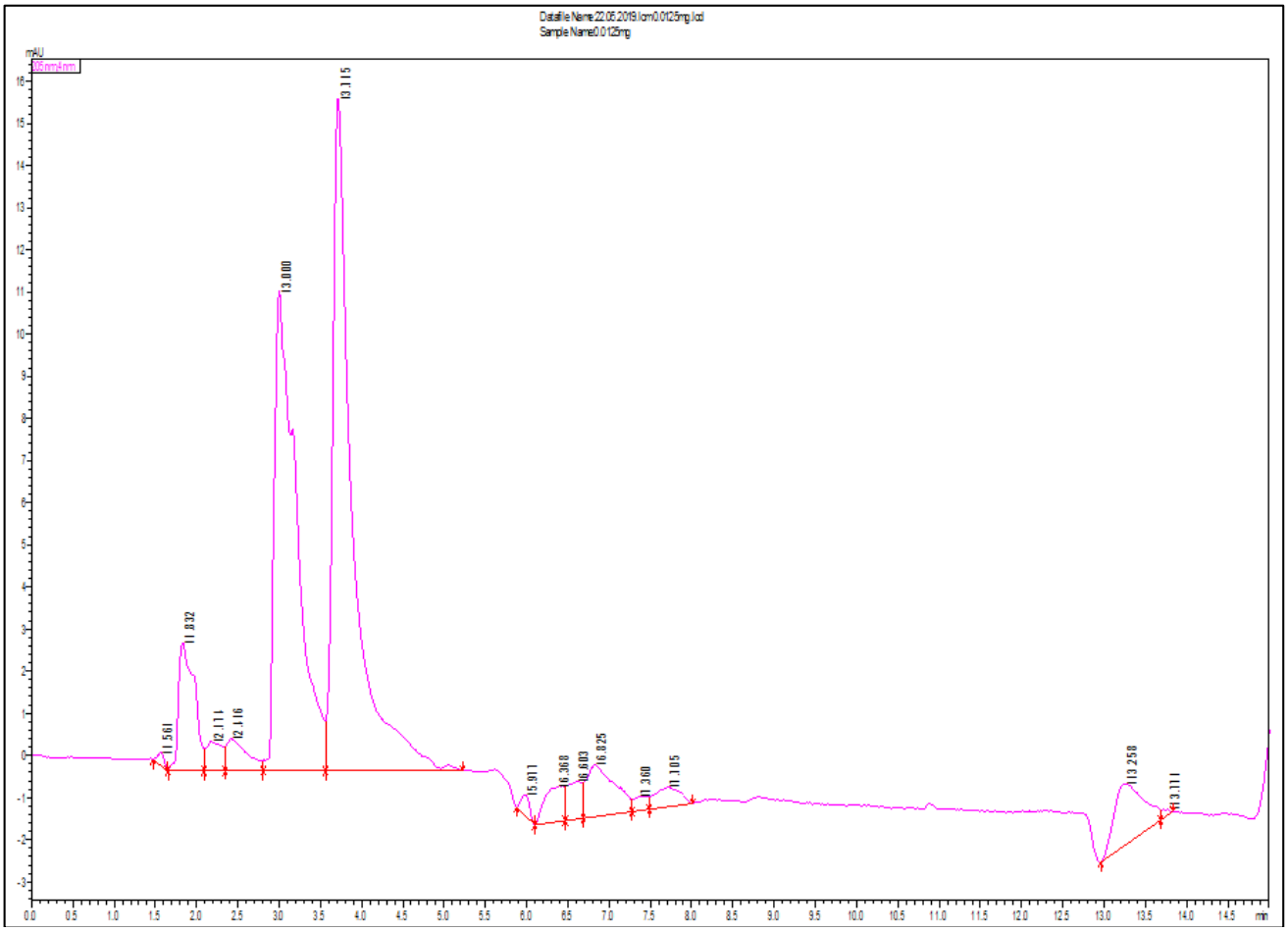
Results and Discussion

Quantification of the bioactive components in *Asparagus racemosus* using Ultra High Performance Liquid Chromatography (UHPLC).

The bioactive components of *Asparagus racemosus* were analyzed using UHPLC wherein Standard Operating Protocol (SOP) for various concentrations corresponding to retention time was developed. Representative chromatogram of standard bioactive components viz Shatavarin-IV was obtained with respect to *Asparagus racemosus.*

The Standard Operating Protocol (SOP) was developed until reproducible and distinguishable peak of standard was observed. Quantification of bioactive components of *Asparagus racemosus* was obtained by drawing a calibration curve between area and various concentration of Shatavarin-IV.

The study was undertaken to determine the concentration of Shatavarin-IV from *Asparagus racemosus* as by using UHPLC which indicated the presence of Shatavarin-IV with concentration of 0.08 mg/g at retention time of 3.7 minutes and wavelength of 205 nm. The chromatogram exhibited a sharp peak with corresponding concentration, suggesting a precise and accurate quantification of Shatavarin-IV. However Gohel *et al*. (2015) [3] was quantify the Shatavarin-IV and highest yield of Shatavarin –IV was obtained 1.60 mg/g crude powder with highest purity achieved 66 per cent of lower atmospheric temperature. Castilla *et al*. (2013) [2] detected the content of saponin in Huétor asparagus ranged from 1.09 to 2.73 mg/100 g fw. The commercial hybrids showed saponin concentrations between 0.03 and 1.22 mg/100 g fw and 90% of the samples have saponin contents lower than 1 mg/100 g fw. Patil *et al*. (2014) [11] was conducted quantitative determination of Shatavarin-IV from dietary supplements containing *Asparagus racemosus* by HPLC/Tandem mass spectrometric studies on steroidal saponins. The method showed excellent linearity (r2 > 0.998) over the concentration range of 7.5 to 254 ng/mL with LOD of 2.5 ng/mL. Precision (RSD) and accuracy (recovery) were found in the ranges of 2.00 to 5.15 and 102 to 110%, respectively. Haghi *et al*. (2013) [4] conducted HPLC study to quantify Shatavarin-IV from *Asparagus racemosus*. The above reports of various co-workers clearly validate the Standard Operating Protocol (SOP) used in the present study and further suggest that quantification of Shatavarin-IV is precise, accurate and selective quantification by using UHPLC. The chromatographic findings determined the presence of Shatavarin-IV in the sample and data obtained are in close conformity to earlier reports.



Ultra High Performance Liquid Chromatography (UHPLC) chromatogram of standard Shatavarin- IV of *Asparagus racemosus*

Conclusions

In this study the quantification of Shatavarin-IV was performed by using UHPLC. Shatavarin-IV is responsible for various pharmacological activities attributed to the *Asparagus racemosus*. The mobile phase of chromatogram exhibited good separation of Shatavarin-IV (0.08 mg/g) from its matrix with a mean retention of 3.7 minutes at wavelength of 205 nm.

References

1. Arora R, Gupta D, Chawla R, Sharma A, Kumar R, Prasad J *et al*. Radioprotection by plant products; present status and future prospects. Phytotherapy Research. 2005; 19(1):1-22.
2. Castilla VS, Carmona JS, Alventosa FJM, Araujo JA, Arcos RR, Sacristan CP *et al*. Optimization of a method for the profiling and quantification of saponins in different green *Asparagus* genotypes. Journal of Agricultural and Food Chemistry. 2013; 61(26):6250-6258.
3. Gohel R, Solanki B, Gurav N, Patel G, Patel B. Isolation and characterization of Shatavarin-IV from root of *Asparagus racemosus* wild. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7:362-365.
4. Haghi G, Hatami A, Mehran M. Determination of Shatavarin in root extracts of *Asparagus racemosus* by HPLC-UV. Analytical Chemistry Letters 2013; 1(2):1-6.
5. Kamat JP, Boloor KK, Devasagayam TPA, Venkatachalam SR. Antioxidant properties of *Asparagus racemosus* against damage induced by gamma-radiation in rat liver mitochondria. Journal of Ethnopharmacology. 2000; 71(3):425-435.
6. Kausar H, Abidin L, Mujeeb M. Comparative assessment of extraction methods and quantitative estimation of thymoquinone in the seeds of *Nigella sativa* by HPLC. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(12):1425-1428.
7. Mahmood S, Mushtaq-Ul-Hassan M, Alam M, Ahmad F. Comparative efficacy of *Nigella sativa* and *Allium sativum* as growth promoters in broilers. International Journal of Agriculture and Biology. 2009; 11:775-778.
8. Mandal SC, Nandy A, Pal M, Saha BP. Evaluation of antibacterial activity of *Asparagus racemosus* Willd. root. Phytotherapy Research. 2000; 14(2):118-119.
9. Muruganandan S, Garg H, Lal J, Suresh C, Dinesh K. Studies on the immunostimulant and anti-hepatotoxic activities of *Asparagus racemosus* root extracts. Journal of Medicinal Aromatic Plant Science. 2001; 22(4A):49-52.
10. Negi JS, Singh P, Pant GJN, Rawat MSM. High-performance liquid chromatography analysis of plant saponins: an update. Pharmacognosy. 2011; 5(10):1900-1904.
11. Patil D, Gautam M, Gairola S, Jadhav S, Patwardhan B. HPLC/tandem mass spectrometric studies on steroidal saponins: an example of quantitative determination of Shatavarin-IV from dietary supplements containing *Asparagus racemosus*. Journal of AOAC International. 2014; 97(6):1497-1502.
12. Sekine T, Fukasawa N, Murakoshi I, Ruangrungsi N. A 9, 10- dihydrophenanthrene from *Asparagus racemosus*. Phytochemistry. 1997; 44(4):763-764.

13. Sinha SN, Biswas M. Effect of extracts from *Asparagus racemosus* wild root against pathogenic bacteria. International Journal of Applied Biology and Pharmaceutical Technology. 2011; 2(3):312-314.
14. Velavan S, Nagulendran K, Mahesh R, Hazeena Begum V. *In vitro* antioxidant activity of *Asparagus racemosus* root. Pharmacognosy Magazine. 2007; 3:26-33.