

Variation of Saponin Content in *Asparagus Adscendens* Germplasms from Western Himalayan Region of India using High Performance Liquid Chromatography-Evaporative Light Scattering Detector

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Abstract: *Asparagus adscendens* Roxb. (Asparagaceae) also known as Shatavar Bhed in Ayurvedic medicine, is used for the treatment of female disorders. Traditionally, it is also used in diarrhea, dysentery, leucorrhoea and general debility. Saponins and stigmasterol glycosides were reported from *A. adscendens*. It is distributed in the Western Himalayas regions of India. Twenty five germplasms of *A. adscendens* were collected from Himachal Pradesh and Uttarakhand in the year 2008 and were multiplied in the experimental field of ICAR-DMAPR, Anand. Saponin content expressed as shatavarin IV was estimated in methanolic extract of harvested roots of *A. adscendens* using HPLC- ELSD. The wide range of variation (0.45 – 5.29%) were recorded in the 25 accessions of *A. adscendens*. DAA5 (5.29%), DAA25 (5.11%), DAA26 (4.74%), DAA2 (4.35%) and DAA28 (3.70%) were identified as accessions with high saponin content which could be exploited for their potentialities. Germplasm with high saponin content could be used for further multiplication in research programme for development of agrotechniques for ensuring raw material of standardized content and quality.

Keywords: *Asparagus*, saponin, shatavarin IV, sarsasapogenin, HPLC-ELSD

1 Introduction

Asparagaceae is a monogeneric family [1]. The genus *Asparagus* has more than 300 species growing widely in eastern Asia including India, China, Korea and Japan [2]. *Asparagus* species are widely used in traditional medicines and reputed to be a tonic and geriatric. *Asparagus adscendens* Roxb. (Asparagaceae) is an extremely branching plant found in the Himalayas up to an altitude of 5000 feet and is known for its medicinal utility [3, 4]. *A. adscendens* is also distributed widely in the Punjab plains and sub-mountainous region of Pakistan. It is characterized by relatively thick cuticle and epidermis as well as thick endodermis [5]. It is useful as nutritive, tonic, galactagogue and demulcent [6]. Bioactivities such as anti-cancer, antifilarial, anti-stress and anti-inflammatory were also reported for *A. adscendens* [7, 8]. *A. adscendens* is a major constituent of 'Geri forte', herbal formulation widely studied for stimulation of insulin secretion [9]. Inhibitory effects on starch digestion, presence of insulinotropic and insulin-enhancing activity and of *A. adscendens* were reported. Its aqueous extract was shown to induce a

significant non-toxic increase (19–248%) in glucose-dependent insulinotropic actions in the clonal pancreatic β cell line, BRIN-BD11. Also, its extract produced an increase (81%) in glucose uptake in 3T3-L1 adipocytes and produced a decrease (21%) in starch digestion *in vitro*. The former actions are suggested to be dependent on the active principle(s) in the intact plant being absorbed. Shatavarins such as shatavarin I, II, III and IV derived from steroidal aglycones were isolated from *Asparagus* species [10]. Steroidal saponins with a majority containing a sarsasapogenin aglycone are commonly found in Asparagaceae family. Isolation of saponins from different parts such as fruits, roots and leaves of *A. adscendens* were reported by Sharma et al. [11–13]. Tondon et al. [3] reported isolation of stigmasterol glycosides from roots of *A. adscendens*. Jadhav and Bhutani [14] reported new sarsasapogenin glycosides along with spirostanol glycosides (asparanin C and D) and furostanol glycosides (asparosides C and D) from the roots of *A. adscendens*. Shatavarin I and IV (Fig. 1A and 1B), were isolated from different species of *Asparagus* [15]. A saponin with sarsasapogenin as aglycone (Fig. 1C) was reported from the

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root of *A. adscendens* [16].

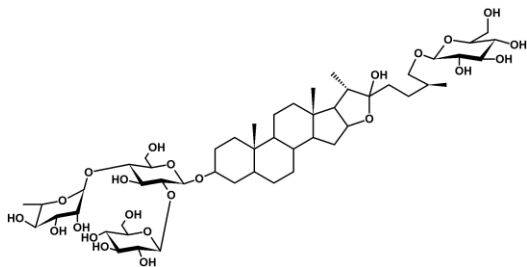


Fig. 1A. Chemical structure of shatavarin I

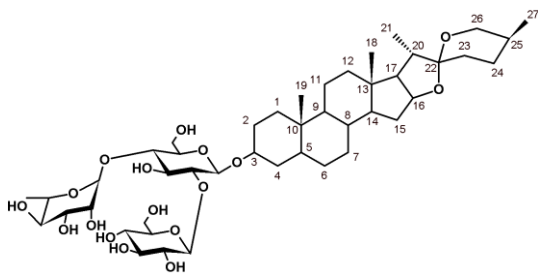


Fig. 1B. Chemical structure of shatavarin IV

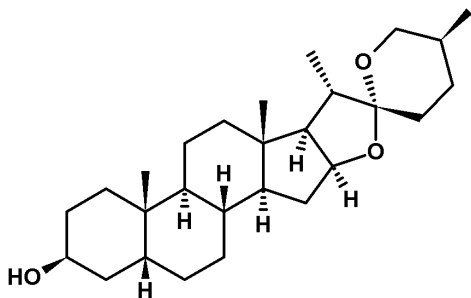


Fig. 1C. Chemical structure of sarsasapogenin

In the present investigation, twenty five accessions of *A. adscendens* collected from the Himalayan regions spread over two states of India namely Himachal Pradesh and Uttarakhand were screened for saponin content expressed as shatavarin IV using high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD). To the best of information available in literature, this is the first report for saponin content variation in germplasm of *A. adscendens* collected from the Himalayan region of India.

2 Experimental

2.1 Collection of Accessions and Maintenance

Exploration trips were undertaken during year 2008 in the

natural habitats of the species to collect the available natural diversity of *A. adscendens* from locations spread over two Western Himalayan states of India (Table 1). Latitude and longitude of each location were recorded using global positioning system. From each location, five plants of each accession at vegetative stage were collected.

Whole plants of the twenty five identified accessions were brought from the respective collection points and planted in the experimental fields of the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India under open conditions.

The cultivation/maintenance was done by following standard package of practices. The experimental plot represented the subtropical area of India (22.556°N, 72.951°E) at an altitude of 40.63 m above mean sea level with average rainfall of 800 mm. The minimum and maximum temperature ranged between 12.7–42°C. Fresh tuberous roots were collected from field, cleaned and dried in shade and fine powder was prepared.

2.2 Chemicals and Standard

The HPLC grade solvents acetonitrile and methanol were purchased from Merck, Mumbai, India. Deionized water used throughout the experiment was obtained from a Millipore water purification system (Millipore, Milli Q gradient A10, France). Chloroform and hydrochloric acid were of analytical grade and purchased from Sisco Research Laboratory (SRL), Mumbai, India. Standard shatavarin IV and sarsasapogenin were purchased from Natural Remedies, Bangalore, India and Chromadex, USA, respectively.

2.3 Preparation of Methanolic Extract and Acid Hydrolysis of Extract

Powdered plant material was used for preparation of methanolic extract by refluxing with methanol. Dried methanolic extracts was hydrolyzed according to method described by Wang et al. [17]. Briefly, extracts were hydrolyzed with hydrochloric acid (2M) for 3 hours. After hydrolysis, samples were cooled and neutralized with sodium hydroxide (40%). Hydrolysis product was further extracted with chloroform. Aglycone fraction extracted in chloroform was concentrated at 60 °C using rotary evaporator. The residue obtained was dissolved in methanol to make appropriate concentration solution for HPLC-ELSD analysis.

2.4 HPLC-ELSD Analysis

Chromatographic analysis was performed on a HPLC system (Shimadzu LC-10AD, Kyoto, Japan) equipped with

RP18 column (4.6 X 150 mm, 5 µm, SunFire, Waters,

Table 1. Location details of germplasms collection sites

| Sl. No. | Accession No. | Village | District | State | Type of material | Altitude (m) | Latitude | Longitude |
|---------|---------------|----------------------------|-------------|------------------|------------------|--------------|--------------|--------------|
| 1. | DAA-1 | Tapovan | Kangda | Himachal Pradesh | Whole Plant | 1089 | 32° 08'54.5" | 76° 32'32.4" |
| 2. | DAA-2 | Harbag | Mandi | Himachal Pradesh | Whole Plant | 1451 | 31°58'44.5" | 76° 50'11.6" |
| 3. | DAA-3 | Nouni | Solan | Himachal Pradesh | Whole Plant | 1371 | 30° 51'51.2" | 77° 08'47.8" |
| 4. | DAA-4 | Bramnapukhar | Bilaspur | Himachal Pradesh | Whole Plant | 1073 | 31° 15'19.8" | 76° 50'44.7" |
| 5. | DAA-5 | Chambhaghat | Solan | Himachal Pradesh | Whole Plant | 1499 | 30° 55'15.8" | 77° 06'04.8" |
| 6. | DAA-7 | Fagvbad (kotlu) | Hamirpur | Himachal Pradesh | Whole Plant | 1059 | 31° 30'00.6" | 76° 55'53.6" |
| 7. | DAA-8 | Koila matha Temple | Mandi | Himachal Pradesh | Whole Plant | 1112 | 31° 36.01.5' | 76° 58'57.0' |
| 8. | DAA-9 | Koila matha Temple | Mandi | Himachal Pradesh | Whole Plant | 850 | 31° 36'05.2" | 76° 25'21.0" |
| 9. | DAA-13 | Nr. Gurdwara | Mandi | Himachal Pradesh | Whole Plant | 826 | 31° 44'45.3" | 76° 56'29.1" |
| 10. | DAA-14 | Nadaun (Jalari) | Hamirpur | Himachal Pradesh | Whole Plant | 532 | 31° 45'43.5" | 76° 21'55.6" |
| 11. | DAA-16 | Jungala village | Shimla | Himachal Pradesh | Whole Plant | 1677 | 31° 09'25.4" | 77° 04'06.4" |
| 12. | DAA-18 | Nr. Bhimatal | Nainital | Uttarakhand | Whole Plant | 1658 | 29° 23'16.1" | 79° 31'08.1" |
| 13. | DAA-19 | Nainital | Nainital | Uttarakhand | Whole Plant | 1846 | 29° 23'00.1" | 79° 26'23.2" |
| 14. | DAA-20 | Meri Daseran | Solan | Himachal Pradesh | Whole Plant | 1002 | 31° 14'37.4" | 76° 54'14.8" |
| 15. | DAA-21 | Nr. Someshwar | Ranikhet | Uttarakhand | Whole Plant | 1568 | 29° 47'30.0" | 79° 32'05.3" |
| 16. | DAA-22 | Garud | Bageshwar | Uttarakhand | Whole Plant | 1501 | 29° 52'12.0" | 79° 36'04.6" |
| 17. | DAA-23 | Nr. Bageshwar | Bageshwar | Uttarakhand | Whole Plant | 1781 | 29° 50'13.4" | 79° 53'44.3" |
| 18. | DAA-25 | Nr. Barinag | Pithoragarh | Uttarakhand | Whole Plant | 1092 | 29° 45'28.7" | 79° 59'41.8" |
| 19. | DAA-26 | Nr. Bhowali | Nainital | Uttarakhand | Whole Plant | 929 | 29° 33'00.0" | 79° 35'32.9" |
| 20. | DAA-28 | Bhowali | Nainital | Himachal Pradesh | Whole Plant | 1441 | 29° 37'31.2" | 79° 43'36.9" |
| 21. | DAA-29 | Tibafion Temple river side | Mandi | Himachal Pradesh | Whole Plant | 1284 | 31° 36'05.4" | 76° 49'39.6" |
| 22. | DAA-32 | Farmers field | Bilaspur | Himachal Pradesh | Whole Plant | 1271 | 31° 36'05.2" | 76° 49'59.2" |
| 23. | DAA-34 | Nanawan | Bilaspur | Himachal Pradesh | Whole Plant | 1338 | 31° 59'34.0" | 76° 48'27.8" |
| 24. | DAA-41 | Chamba | Chamba | Himachal Pradesh | Whole Plant | 3704 | 32° 28'49.8" | 75° 55'21.7" |
| 25. | DAA-44 | Nr. Someshwar | Ranikhet | Uttarakhand | Whole Plant | 1341 | 29° 41'04.9" | 79° 28'05.3" |

USA), an evaporative light scattering detector (Varian 385-LC, Agilent Technologies, USA), a Rheodyne 7725i injector (Rheodyne Ind, USA) with a 20 µL stainless steel loop and WINACT software (Shimadzu). The chromatographic separation was carried out in an isocratic mode using acetonitrile and water (9 : 1) as a mobile phase. Flow rate was 1.2 mL/min. The injection volume was 20µL. The conditions for HPLC-ELSD analysis of sarsasapogenin were as follows: nebulization temperature:

60 °C, gas flow rate (air):1.2 L/min and the gain factor: 2.

3 Results and Discussion

A. adscendens is traditionally used as a nerve tonic and remedy for memory impairments. Khan et al. [18] isolated conyopododiol from the chloroform fraction of methanolic extract of *A. adscendens* based on bioactivity guided isolation. Conyopododiol exhibited significant inhibition of

both acetyl cholinesterase and butyryl cholinesterase. In the present study, methanolic extract of dried roots of *A. adscendens* was prepared. Methanolic extract was further hydrolysed for identification and quantification of saponins. Hydrolysis was confirmed by thin layer chromatographic analysis with vanillin-sulphuric acid spray (Fig. 2A).

Extraction yield of *A. adscendens* roots was summarized in Table 2. A wide variation in methanolic extract yield was found and it was in the range of 11.51-38.12 percent. Similarly, hydrolysis yield was in the range of 3.52-26.91 percent. As most of the aglycone of steroidal saponins have no or weak absorption in ultraviolet-visible region, ELSD coupled with HPLC was employed for identification and quantification of saponins in present study. Shatavarin IV and sarsasapogenin, the commercially available saponin and sapogenins were selected for characterization of *A. adscendens* germplasms.

Several combinations of mobile phase were examined for base line separation of peaks. The best base line separation of peaks was recorded with a combination of acetonitrile – water (9 : 1) as mobile phase. Operating conditions of ELSD such as nebulization temperature, evaporation temperature, gas flow rate (air) and the gain factor were optimized in order to have high signal-noise ratio. Representative HPLC chromatogram of standard sarsasapogenin and hydrolysed extract samples of *A.*

adscendens are shown in Fig. 2B and Fig. 2C, respectively. Peak of sarsasapogenin was eluted at retention time 14.00 minute. A calibration curve was constructed by linear regression of the natural logarithm of the detector response of analyte (peak area) against the natural logarithm of its concentration. Based on the molecular structure of shatavarin IV, a conversion factor was used to get shatavarin IV content in extract from the value of sarsasapogenin concentration. Sarsasapogenin content also showed variation and its concentration was in the range of 0.21-2.48%. Its concentration was highest in accession DAA 5 (2.48) followed by accessions DAA 25 (2.40), DAA26 (2.22%), DAA2 (2.04%) and DAA 28 (1.74%).

A strong relation of descriptive features with plant anatomy was recorded for *Asparagus* species from Pakistan by Nawaz et al. [5]. Also, comparative anatomy of root and stem of some native and exotic *Asparagus* L. species were investigated. *A. densiflorus* and *A. setaceus* had thicker roots, larger parenchymatous cells, and well developed vascular tissue than the other species and cultivars. Shrestha et al. [19] reported morphological features and extract values of *A. curillus*, *A. lycopodiinus*, *A. penicillatus* and *A. racemosus* from Nepal. Morphological variation of roots was found significant. Extractive values ranged from 48.74 - 80.74 %. It was minimum for *A. penicillatus* and maximum for *A. curillus*.

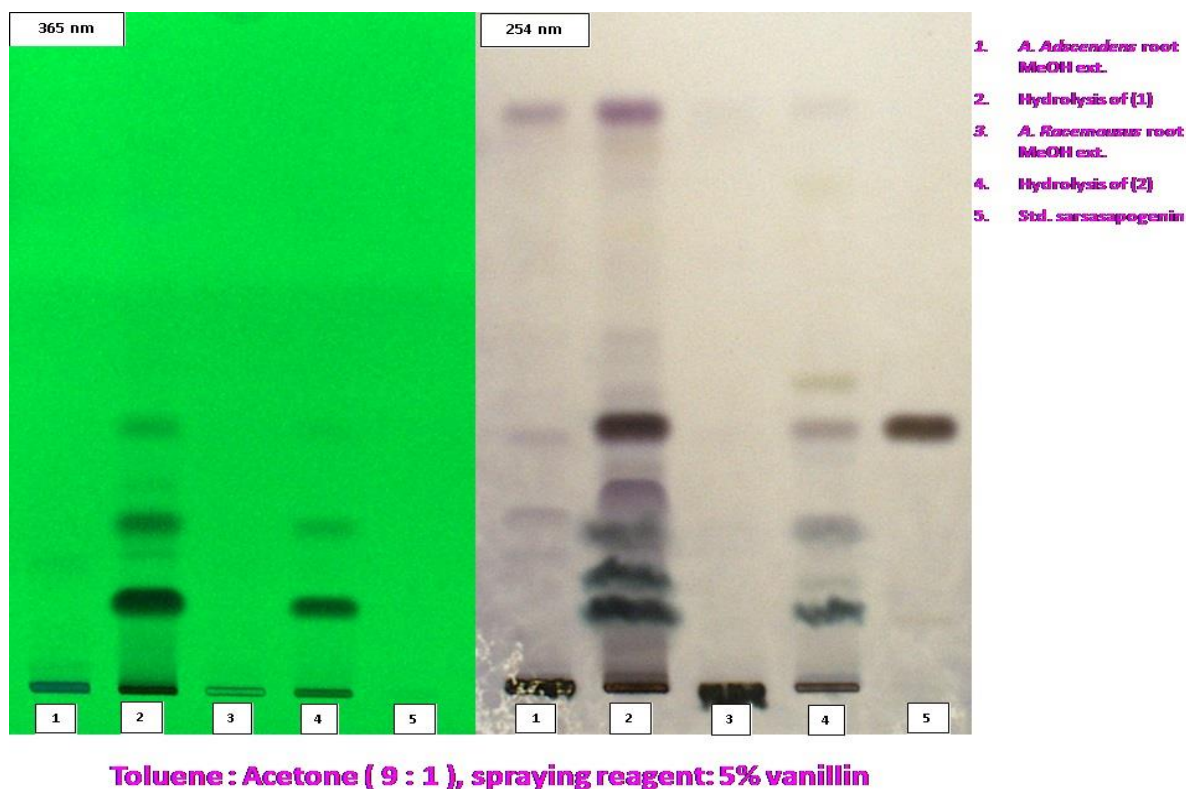


Fig. 2A. TLC profile of methanolic extract and hydrolyzed product of *A. adscendens* (1) 365 nm (2) 254 nm

Table 2. Estimation of sarsasapogenin in *A. adscendens* germplasms

| Sl. No. | Sample | Extract yield | Hydrolysis yield | Sarsasapogenin (%) | Total saponin expressed as shatavarin-IV (%) | Total saponin expressed as shatavarin-IV in root (%) |
|---------|--------|---------------|------------------|--------------------|--|--|
| 1. | DAA1 | 19.55 | 4.31 | 0.93 | 1.99 | 0.39 |
| 2. | DAA2 | 15.81 | 4.74 | 2.04 | 4.35 | 0.69 |
| 3. | DAA3 | 25.14 | 8.07 | 0.42 | 0.90 | 0.23 |
| 4. | DAA4 | 17.71 | 8.23 | 0.76 | 1.61 | 0.29 |
| 5. | DAA5 | 32.82 | 3.58 | 2.48 | 5.29 | 1.74 |
| 6. | DAA7 | 20.16 | 9.52 | 0.39 | 0.84 | 0.17 |
| 7. | DAA8 | 25.68 | 10.93 | 0.97 | 2.07 | 0.53 |
| 8. | DAA9 | 28.42 | 8.99 | 0.92 | 1.96 | 0.56 |
| 9. | DAA13 | 18.89 | 7.81 | 0.32 | 0.69 | 0.13 |
| 10. | DAA14 | 20.17 | 22.19 | 0.44 | 0.94 | 0.19 |
| 11. | DAA16 | 34.59 | 17.89 | 0.94 | 2.00 | 0.69 |
| 12. | DAA18 | 14.90 | 18.23 | 0.39 | 0.82 | 0.12 |
| 13. | DAA19 | 29.06 | 17.36 | 0.21 | 0.45 | 0.13 |
| 14. | DAA20 | 22.34 | 15.77 | 0.79 | 1.69 | 0.38 |
| 15. | DAA21 | 38.12 | 13.44 | 0.72 | 1.53 | 0.58 |
| 16. | DAA22 | 15.75 | 22.76 | 0.63 | 1.35 | 0.21 |
| 17. | DAA23 | 16.30 | 26.91 | 0.83 | 1.78 | 0.29 |
| 18. | DAA25 | 25.17 | 16.60 | 2.40 | 5.11 | 1.29 |
| 19. | DAA26 | 26.72 | 9.61 | 2.22 | 4.74 | 1.27 |
| 20. | DAA28 | 25.42 | 6.09 | 1.74 | 3.70 | 0.94 |
| 21. | DAA29 | 14.77 | 19.77 | 0.53 | 1.13 | 0.17 |
| 22. | DAA32 | 11.51 | 23.51 | 1.20 | 2.56 | 0.29 |
| 23. | DAA34 | 25.86 | 19.96 | 0.52 | 1.10 | 0.28 |
| 24. | DAA41 | 28.89 | 20.22 | 0.46 | 0.97 | 0.28 |
| 25. | DAA44 | 30.49 | 18.87 | 0.58 | 1.24 | 0.38 |

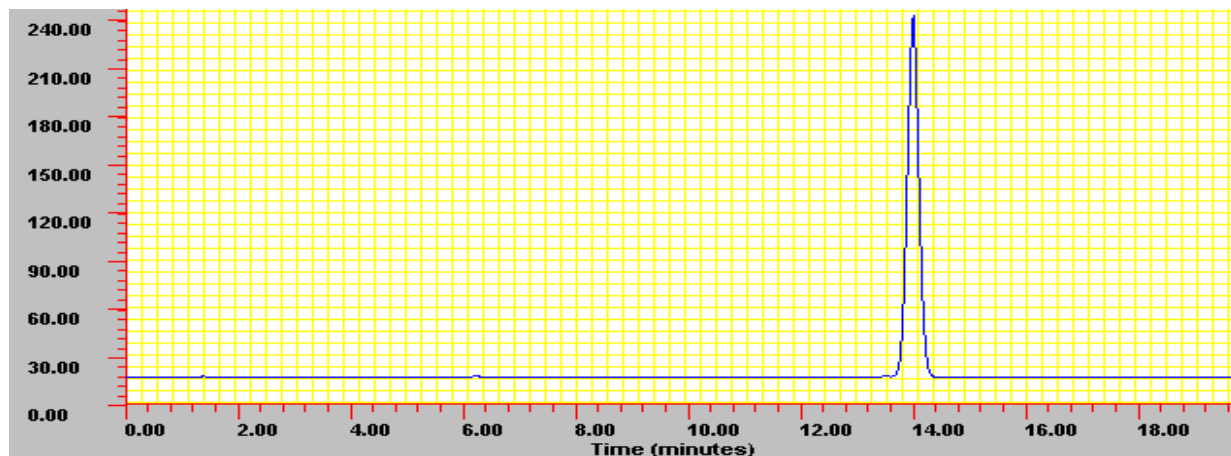


Fig. 2B. HPLC-ELSD Chromatogram of standard sarsasapogenin.

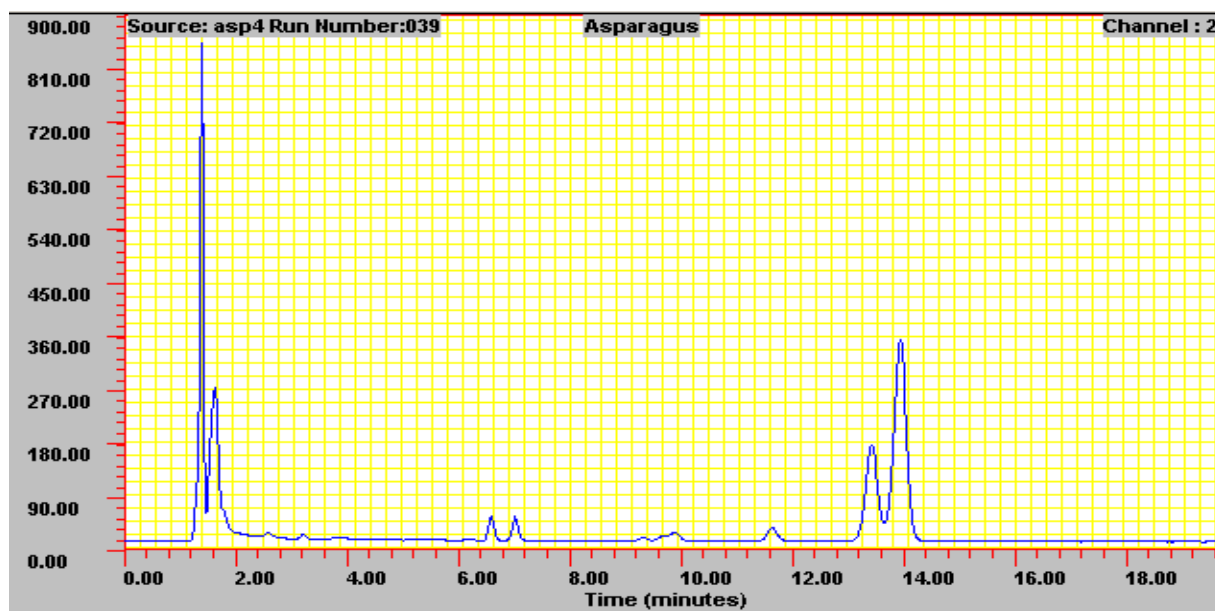


Fig. 2C. HPLC-ELSD Chromatogram of extract of *A. adscendens*

4 Conclusions

The collected germplasms of *A. adscendens* showed wide variation in saponin content. Germplasms with high saponin content could be used for further multiplication in research programme for development of agrotechniques to

ensure raw material of standardized content and quality. This will be helpful in enhancing the quality of medicinal products produced from them.

However, further more phytochemical research work is needed for identification and characterization of other saponins present in the different parts of *A. adscendens*.

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