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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. Germplsams with high saponin content could be used for further multiplication in research progamme 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, anti-filarial, 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 Aspargaceae 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

root of *A. adscendens* [16].

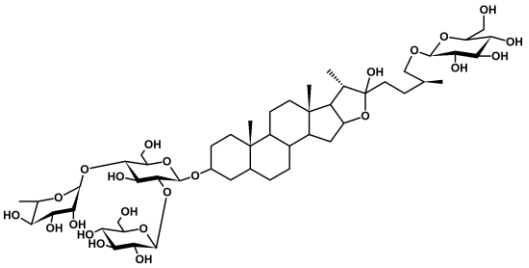

Fig. 1A. Chemical structure of shatavarin I

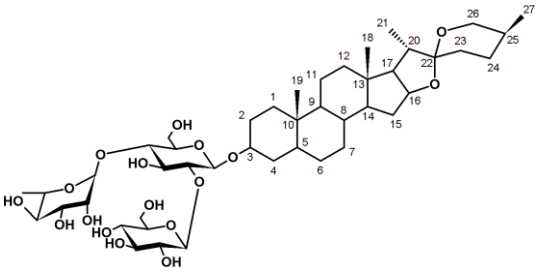

Fig. 1B. Chemical structure of shatavarin IV

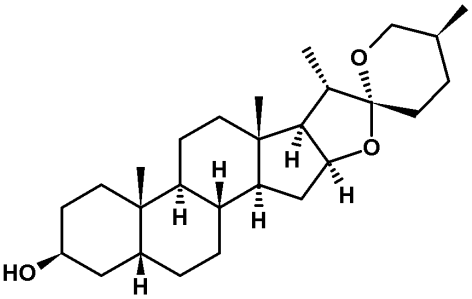

Fig. 1C. Chemical structure of sarsasapogenin

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 germplasms 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, Banglore, 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 ºC 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	32º 08'54.5"	76º 32'32.4"
2.	DAA-2	Harbag	Mandi	Himachal Pradesh	Whole Plant	1451	31º58'44.5"	76º 50'11.6"
3.	DAA-3	Nouni	Solan	Himachal Pradesh	Whole Plant	1371	30º 51'51.2"	77º 08'47.8"
4.	DAA-4	Bramnapukhar	Bilaspur	Himachal Pradesh	Whole Plant	1073	31º 15'19.8"	76º 50'44.7"
5.	DAA-5	Chambhaghat	Solan	Himachal Pradesh	Whole Plant	1499	30º 55'15.8"	77º 06'04.8"
6.	DAA-7	Fagvbad (kotlu)	Hamirpur	Himachal Pradesh	Whole Plant	1059	31º 30'00.6"	76º 55'53.6"
7.	DAA-8	Koila matha Temple	Mandi	Himachal Pradesh	Whole Plant	1112	31º 36.01.5'	76º 58'57.0'
8.	DAA-9	Koila matha Temple	Mandi	Himachal Pradesh	Whole Plant	850	31º 36'05.2"	76º 25'21.0"
9.	DAA-13	Nr. Gurdwara	Mandi	Himachal Pradesh	Whole Plant	826	31º 44'45.3"	76º 56'29.1"
10.	DAA-14	Nadaun (Jalari)	Hamirpur	Himachal Pradesh	Whole Plant	532	31º 45'43.5"	76º 21'55.6"
11.	DAA-16	Jungala village	Shimla	Himachal Pradesh	Whole Plant	1677	31º 09'25.4"	77º 04'06.4"
12.	DAA-18	Nr. Bhimatal	Nainital	Uttarakhand	Whole Plant	1658	29º 23'16.1"	79º 31'08.1"
13.	DAA-19	Nainital	Nainital	Uttarakhand	Whole Plant	1846	29º 23'00.1"	79º 26'23.2"
14.	DAA-20	Meri Daseran	Solan	Himachal Pradesh	Whole Plant	1002	31º 14'37.4"	76º 54'14.8"
15.	DAA-21	Nr. Someshwar	Ranikhet	Uttarakhand	Whole Plant	1568	29º 47'30.0"	79º 32'05.3"
16.	DAA-22	Garud	Bageshwar	Uttarakhand	Whole Plant	1501	29º 52'12.0"	79º 36'04.6"
17.	DAA-23	Nr. Bageshwar	Bageshwar	Uttarakhand	Whole Plant	1781	29º 50'13.4"	79º 53'44.3"
18.	DAA-25	Nr. Barinag	Pithoragarh	Uttarakhand	Whole Plant	1092	29º 45'28.7"	79º 59'41.8"
19.	DAA-26	Nr. Bhowali	Nainital	Uttarakhand	Whole Plant	929	29º 33'00.0"	79º 35'32.9"
20.	DAA-28	Bhowali	Nainital	Himachal Pradesh	Whole Plant	1441	29º 37'31.2"	79º 43'36.9"
21.	DAA-29	Tibafion Temple river side	Mandi	Himachal Pradesh	Whole Plant	1284	31º 36'05.4"	76º 49'39.6"
22.	DAA-32	Farmers field	Bilaspur	Himachal Pradesh	Whole Plant	1271	31º 36'05.2"	76º 49'59.2"
23.	DAA-34	Nanawan	Bilaspur	Himachal Pradesh	Whole Plant	1338	31º 59'34.0"	76º 48'27.8"
24.	DAA-41	Chamba	Chamba	Himachal Pradesh	Whole Plant	3704	32º 28'49.8"	75º 55'21.7"
25.	DAA-44	Nr. Someshwar	Ranikhet	Uttarakhand	Whole Plant	1341	29º 41'04.9"	79º 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 ºC, 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 conypododiol from the chloroform fraction of methanolic extract of *A. adscendens* based on bioactivity guided isolation. Conypododiol 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 week 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. lycopodineus*, *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*.

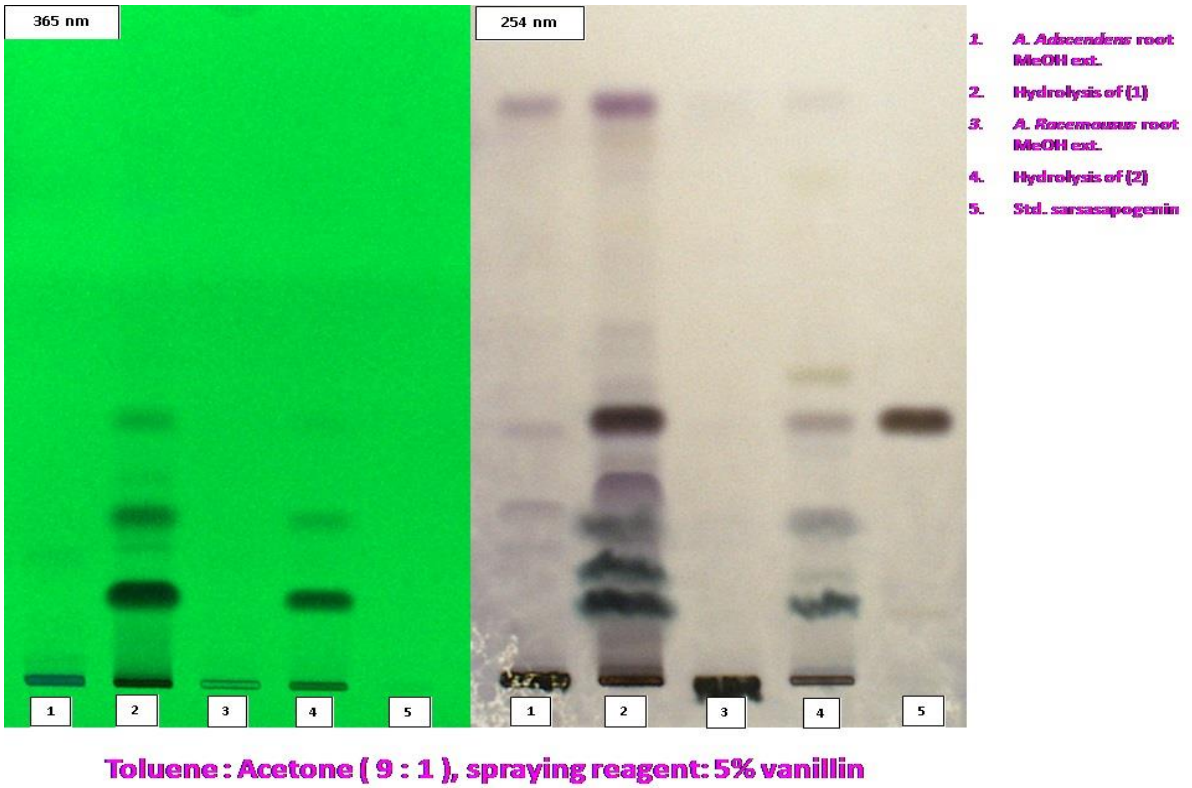

Fig. 2A. TLC profile of methanolic extract and hydrolyzed product of *A. adscendens* (1) 365 nm (2) 254 nm

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

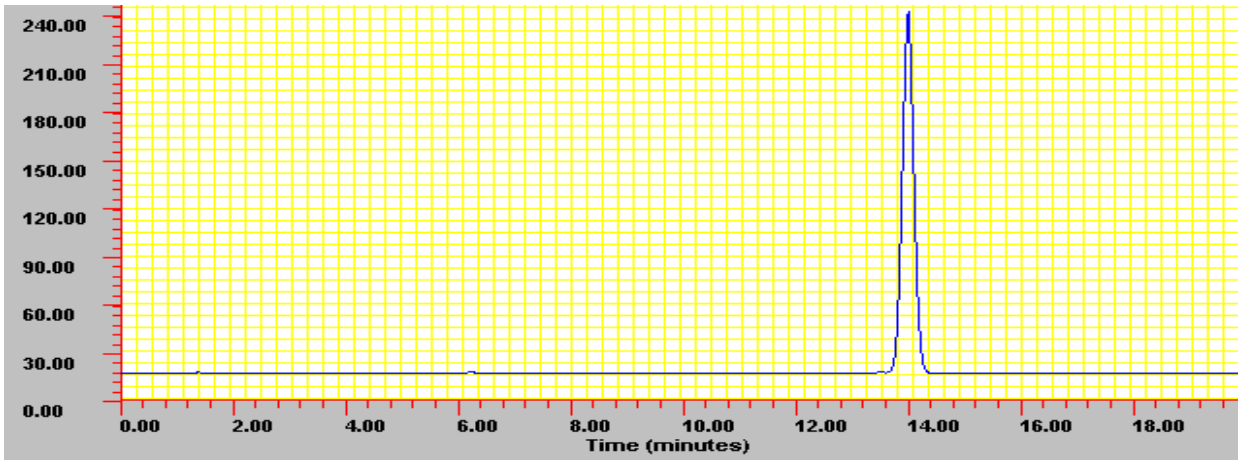

Fig. 2B. HPLC-ELSD Chromatogram of standard sarsasapogenin.

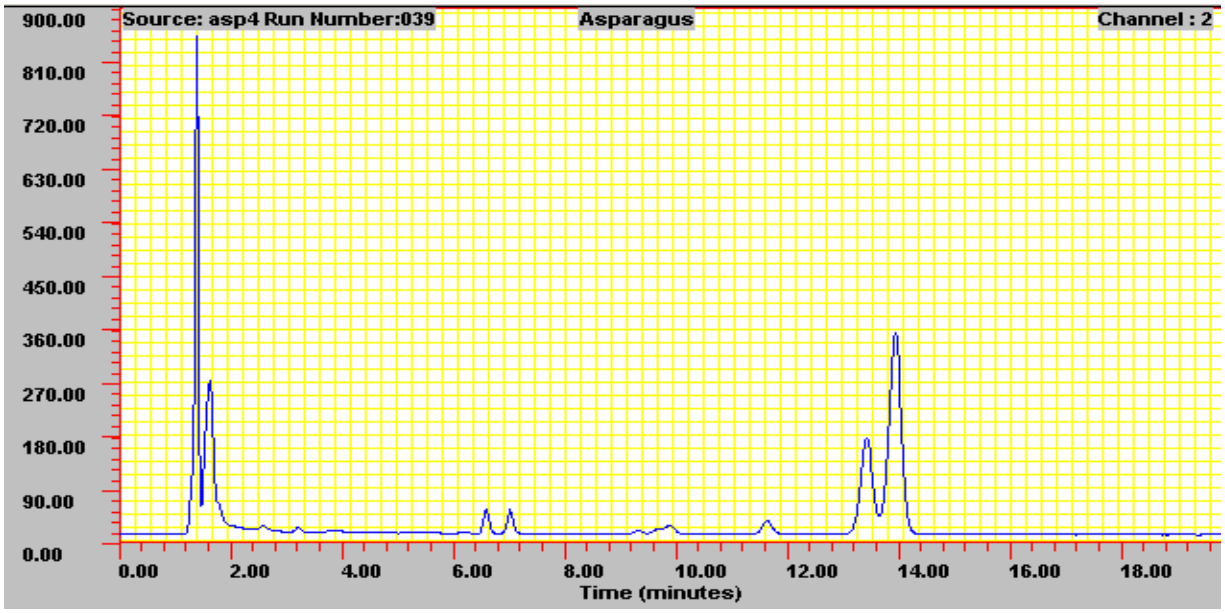

Fig. 2C. HPLC-ELSD Chromatogram of extract of *A. adscendens*

4 Conclusions

The collected germplasms of *A. adscendens* showed wide variation in saponin content. Germplsams with high saponin content could be used for further multiplication in research progamme 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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