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Phytochemical evaluation of medicinal plant *Asparagus racemosus*

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Abstract
Asparagus racemosus is an indigenous medicinal plant of the family Liliaceae; is important for its saponin content and antioxidant activity. It was found that a callus culture derived from the root explants produces more alkaloids compared to nodal callus cultures and maximum accumulation was found to be 10.38 ± 0.14 mg/g after 60 days of inoculation. Total alkaloids from the nodal calli were found to be 7.69 ± 0.136 mg/g of callus. Recent developments in transgenic research have opened up the possibility of the metabolic engineering of biosynthetic pathways to produce these high value secondary metabolites. The high performance liquid chromatography (HPLC) chromatogram of the *in vitro* culture and the natural plant root extract were compared and it was found that all the major peaks were present in the *in vitro* extract and the overall alkaloids profile was similar to the natural root extract. The present review is a pragmatic approach to accrue the findings on this very important herb.

Keywords: *Asparagus racemosus*, anti-oxidant activity, flavonoids, HPLC, saponin

Introduction
Asparagus racemosus is also known as Shatavari, which belongs to family Liliaceae. The roots are cylindrical, fleshy and tuberous. The roots are 30-100 cm in length, 1-2 cm in thickness and yellowish-cream in colour. The roots contain long needle shaped structure known as pith which is meant for the conduction of water. The plant enjoys considerable reputation in Indian system of medicine. Traditionally, the plant has been in use as a galactagogue which stimulates the secretion of breast milk. The other uses of plant are in aphrodisiacs, demulcent, rheumatism, diarrhoea, dysentry,tuberculosis, diabetes, antioxidant, antitussive, nervous disorders, hyperacidity, general debility, habitual abortion and safe delivery. *Asparagus racemosus* is also considered to be an Ayurvedic rejuvenating tonic for overall health and vitality in female. The reputed adaptogenic properties of the plant are attributed to the presence of high concentrations of saponins, known as Shatavarins. Roots of *A. racemosus* were found to possess antioxidant and anti-ADH activity (Kamat *et al*., 2000; Wiboonpun *et al*., 2004) [4], anti-tumour and anticancer activity (Senna *et al*., 1993; Shao *et al*., 1996; Diwanay *et al*., 2004), anti-ulcerogenic activity (Datta *et al*., 2002), anti-inflammatory activity (Mandal *et al*., 1998) and antimicrobial activity (Mandal *et al*., 2000).
The objective of the present study is to assess alkaloids profile of *A. racemosus* to provide total alkaloid content in callus culture and inhibition pattern. High performance liquid chromatography (HPLC) is a powerful tool in analysis the production process of pharmaceutical and biological products.

Materials and Methods
Callus cultures derived from the nodal and root explants were screened for the presence and accumulation of alkaloids at various growth phases. The results have been graphically represented in Figure 1. It was found that a callus culture derived from the root explants produces more alkaloids compared to nodal callus cultures and maximum accumulation was found to be 10.38±0.14 mg/g after 60 days of inoculation. Total alkaloids from the nodal calli were found to be 7.69 ± 0.136 mg/g of callus. In the wild type roots Shatavarin IV is generally found to be 0.05 to 0.08% where as in our cultures it was found to be 1.1% which indicates that there is approximately 20 fold increase in the alkaloids content in the in vitro cultures. The high performance liquid chromatography (HPLC) chromatogram of the in vitro culture and the natural plant root extract were compared and it was found that all the major peaks were present in the in vitro extract and the overall alkaloids profile was similar to the natural root extract (Figures 2 and 3). Total phenolic contents (Table-1) were determined by Folin Ciocalteu

reagent (McDonald *et al*., 2001). Determination of Total flavonoids was assessed by Aluminum chloride colorimetric method (Table 2). Free Radical scavenging activity of the sample extracts were measured by colorimetric assay using 2,2-diphenyl picryl hydrazyl radical (DPPH, a stable free radical)) as a source of free radical in accordance with the method of Blois (1958).

Plant	Method (DPPH EC_{50})
Asparagus racemosus	344.96 ±0.76

Results and Discussion

Alkaloid was extracted from the cultures as per the previously published methods (Mathur *et al*., 1994). Briefly, cells wereextracted separately with methanol (1:2) overnight and procedure was repeated 4 times. All the extracts were pooled and concentrated at 60°C on a rotary evaporator to dryness. The residue was redissolved in 10 ml of H_2O and further extracted with n- butanol. The n- butanol fraction was finally concentrated to dryness on a rotary evaporator under reduced pressure and redissolved in 5 ml of methanol and stored and analyzed using standard Shatavarin IV as the marker. These extracts were also compared with standard alkaloids (Sigma chemicals), which is a precursor of Shatavarins. For the preparation of standard solutions, Shatavarin IV was dissolved in methanol and was diluted to get a final concentration range of 100 to 500 gml^{-1}The solutions were filtered through a 0.2 µmacrodisc. Evaluation of each point was repeated 3 times at 220 nm and the calibration curve was fitted by linear regression. This calibration curve was utilized for the estimation of total Shatavarin present in the methanolic extracts from the wild type plant and *in vitro* extracts.

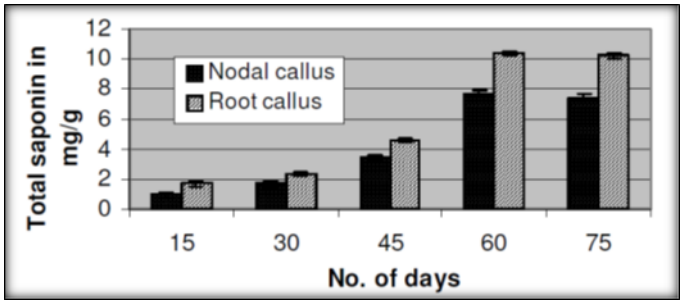

Fig: 1 Total Alkaloid content in callus culture of *Asparagus racemosus*

HPLC analysis of the alkaloidsamples were carried out using a Knauer smart line manager- 5000 system (Germany) fitted with a C18 column (4 um, 150 mm x 3.9 mm I.D.), UV detector and 20_L injection loop. Acetonitrile and 30% aqueous methanol were used as the mobile phase with gradients from 8 to 100% of Acetonitrile in 60 min. The volume of sample injection was 20 _l in all the cases. The peaks of Shatavarin were identified by comparing the retention time of the peaks with those of the reference compounds eluted under same conditions.

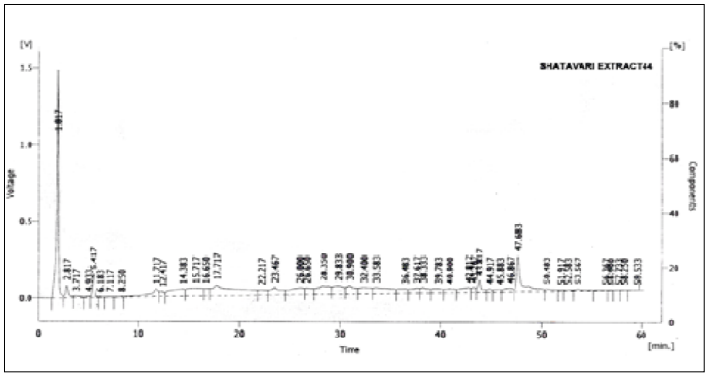

Fig: 2 HPLC profile of Natural root extract

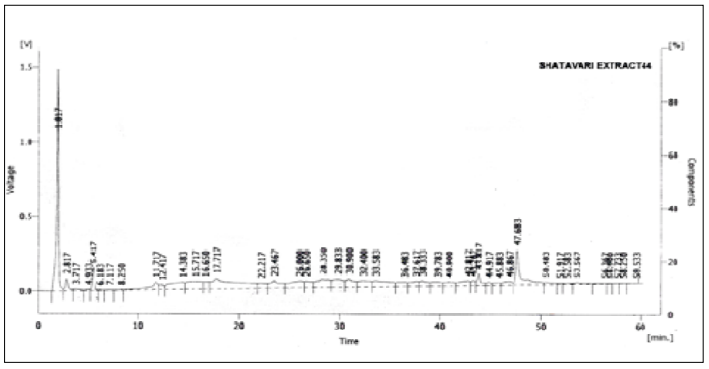

Fig: 3 HPLC profile of Nodal callus extract

Total phenolic contents were determined by Folin Ciocalteu reagent (McDonald *et al*., 2001). A dilute extract of each crude extracts (0.5 ml of 1:10g ml –l) or garlic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms

of gallic acid equivalent (mg g-l of dry mass), which is a common reference compound.

Table 1: Total phenolic contents determined by Folin Ciocalteu reagent (McDonald *et al*., 2001).

Total phenols content		
Methanol		Aqueous
*N	1214.23 ± 2.42	211.30 ± 1.10
*C	1432.45 ± 2.54	254.32 ± 1.12

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Each extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml -1 in methanol.

Table: 2 Total flavonoids contents determined by Aluminum chloride colorimetric method.

Total flavonoids content		
Methanol		Aqueous
*N	321.17± 2.10	120.09 ± 0.20
*C	423.12± 3.12	143.19 ± 0.21

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