Pharmacognostical studies on the root tubers of *Asparagus gonoclados* Baker –Alternate source for the Ayurvedic drug Shatavari

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*Asparagus gonoclados* Baker is an important medicinal plant belonging to the family Liliaceae (*sensu lato*). Many species of *Asparagus* Linn. including *A. gonoclados* are used as substitute of the well known Ayurvedic drug, *Shatavari*. Root tubers of *Shatavari* possesses adaptogenic, antioxidant, cooling, emollient, diuretic, galactagogue, nervine tonic, rejuvenating, and stomachic properties; they are useful in treatment of diseases like diarrhoea, dysentery, dyspepsia, epilepsy, fatigue, inflammations, nervous disorders, tumours and tuberculosis. The present study provides taxonomy of the species, pharmacognostical and physico-chemical details of the root tubers of *A. gonoclados*. This helps in laying down standardisation and pharmacopoeial parameters. Presence of Shatavarin IV in the alcohol and aqueous extracts is reported in this species for the first time.

Keywords: *Asparagus gonoclados*, HPTLC, Pharmacognosy, Physico-chemical analysis, Root tubers, Shatavarin IV.

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**Introduction**

*Shatavari* is a well known Ayurvedic drug1. The accepted botanical source2 of *Shatavari* is *Asparagus racemosus* Linn. However, many other species of *Asparagus* including *A. gonoclados* Baker are used as *Shatavari*. Fasciculated tuberous roots of *Shatavari* is considered as one of the *Rasayana* (adaptingenic) drugs, having cooling, diuretic, emollient, galactagogue, nervine tonic, rejuvenating and stomachic properties3; they are useful in treatment of diseases like nervous disorders, dyspepsia, diarrhoea, dysentery, tumours, inflammations, tuberculosis, epilepsy and fatigue to mention a few6, 7. The drug *Shatavari* is used in nearly 67 Ayurvedic preparations like Anuthaila, Brahma rasayana, Dhanwanthararishtha, Mahathikhtha kashaya, Narayana thaila, Rasnadi kashaya, Sahacharadi thaila, Saraswatharishtha, Shatavari panaka, Shatavari ghritha, Shatamulyadi lehya, Vasishtha rasayana and Vidaryadi ghritha to mention a few8,9. The genus *Asparagus* Linn. (Family — Liliaceae *sensu lato*) consists of 300 species all over the world, out of which 20 are found in India10. *A. gonoclados* contains apigenin, kaempferol, rutin and chalcone glycoside; flowers contain anthocyanin, malvin and asparagines4, 5; and aerial parts contain 4,4',6-trihydroxy-auronone11, 12. Pharmacognostical investigation with macerate and powder study details on the root tubers of *A. gonoclados* which help in identification of crude drug besides reporting of Shatavarin IV, in this species is not available13-16 and hence the present study was taken up by us.

**Materials and Methods**

Fresh root tubers were collected from forests situated in between Madikeri and Sakaleshpura, Kodagu district, Karnataka during March, 2006, preserved in 70% ethyl alcohol for histological studies. Botanical identification was carried out using local floras17, 18, identified by Dr S N Yoganarasimhan, Plant Taxonomist, authenticated at the herbarium of Regional Research Institute (Ayurveda), Bangalore. Voucher herbarium specimen19 (Anil Kumar Tijare 009) was prepared and preserved along with crude drug sample at the herbarium of M S Ramaiah College of Pharmacy, Bangalore. Pharmacognostical evaluation like histochemical and macerate studies were carried out by taking free hand sections following Johansen10, Wallis21 and powder studies following Evans22. Photomicrographs were obtained by observing free hand sections of drug under compound binocular

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microscope (Olympus-CH20i model) with built in analogue camera (CMOF, 1.4 mega pixel). Computer images were captured using AV-Digitaliser having Grand VCD 2000 – Capture Guard. Measurements of cells and tissues were carried out using Micro Image Lite Image Analysis Software (Cybernetics, Maryland, USA). Physico-chemical constants, organic analysis, ultra-violet analysis and chromatographic studies were carried out from shade-dried powder following prescribed methods\textsuperscript{23-27}. HPTLC studies were carried out on aqueous and alcohol extracts using Camag HPTLC system equipped with Linomat V sample applicator, Camag TLC scanner 3 and CATS 4 software for interpretation of data. An aluminium plate (5 × 10 cm) precoated with silica gel 60F\textsubscript{254} (E Merck) was used as adsorbent. The plates were developed using ethyl acetate: methanol: water (75:15:10) using anisaldehyde sulphuric acid as postderivatasion reagent and scanned at 425 nm. Shatavarin IV standard was obtained from Natural Remedies, Bangalore.

Results and Discussion

\textit{A. gonoclados} is an armed scandent climber; branches triquetrous; spines 3-4 mm long. Cladodes 2 to 6 per node, 0.5-2 mm, falcate, acute. Flowers white, in 2-2.5 cm long axillary racemes; bracts 0.5 mm long, scarious, keeled; pedicels up to 1 mm long, joined above the middle. Perianth lobes 6, biseriate, outer perianth is longer and inner linear-pathulate. Stamens 6, filiform. Stigmas 3 (Fig. 1). It is known as by following vernacular names: \textit{Abheerupatri} (Sanskrit); \textit{Shakakul} (Hindi); \textit{Kadu majjige gida} (Kannada); \textit{Shathavalli} (Malayalam); \textit{Shatavarimool} (Marathi); \textit{Kilavari, Thanneervittan kizhangu} (Tamil); and \textit{Challagadalu, Pillipichara} (Telugu).

Macroscopical characters of Root tuber

The root tubers arise adventitiously from a short root stock, cylindrical, 20-25 cm long, creamy white, without characteristic odour, and with a mild sweetish taste, smooth, tapering at both ends and bulged in the middle, with a few rootlets, skin is soft and can be easily scraped. In fresh condition when cut, it exudes a white sap. A few transverse striations are also seen on the surface. A cut surface of the fresh root shows a broader light yellow region and a narrower silvery-white stelar region (Fig. 2).

Microscopical characters

Transverse section of root tuber is circular in outline. The outermost layer is light yellow, which is the piliferous layer found at the distal end and consisting of rectangular cells, many extending as root hairs and measuring 38-94-130µ (Fig. 3). Next to this layer lies 6-8-layered velamen-like exodermis consisting of slightly radially elongated and comparatively thick walled cells measuring 7-9-15µ which is followed by a large cortex made up of thin walled parenchymatous cells with intercellular spaces (Fig. 4). Raphide bundle of calcium oxalate crystals are found sparsely distributed in the cortical cells (Fig. 7). Each raphide bundle consists of large number of acicular crystals, measuring 14-13-14µ; some cortical cells contain milky sap content (Fig. 8); sap cells measure 21-24-30µ. Endodermis is single layered, consisting of narrow rectangular thick walled cells. Next to endodermis lies single layered pericycle made up of thin walled rectangular cells. The stelar region is made up of radially arranged vascular bundles consisting of 20 to 25 xylem groups; xylem exarch, measure 2-5-8µ and phloem groups alternate with xylem and measure 3-6-7µ; a large central parenchymatous pith is present (Figs. 5, 6).

Macerate of root tuber exhibits the following elements (Figs. 9-23):

1. Peel showing numerous root hairs.
2. Unicellular root hairs which are long, broad at base.
3. Parenchyma cells of different size and shape measuring 88-105-119µ.
4. Pitted parenchyma with numerous oblique pits, measuring 94-118-156µ.
5. Fibres with tapering ends, narrow lumen, aseptate or septate, measuring 321-319-515µ.
7. Vessels of different size and shape, cylindrical or barrel shaped, with reticulate and pitted thickenings, measuring 242-247-252µ.

The plant and root tubers of \textit{Asparagus gonoclados} are distinguished by presence of:

(i) Short, falcate cladodes; (ii) Velamen-like exodermis; (iii) Raphide bundle; (iv) White sap content; and (v) Septate and aseptate fibres.

Histochemical Tests

The sections of root tubers when treated with iodine solution did not turn blue showing absence of starch; with phloroglucinol turned pink showing the presence of lignin; with Ferric chloride and Dragendorff reagent did not show any change
MADHAVAN et al: PHARMACOGNOSTICAL STUDIES ASPARAGUS GONOCLADUS ROOT TUBERS


indicating absence of tannin and alkaloids, respectively; when sections were shaken with water a froth like layer was observed indicating the presence of saponins.

**Powder Analysis**

The powder is pale yellow, possesses a pleasant odour and sweet taste. When treated with chloralhydrate, phloroglucinol and HCl and stained with safranin, fibres which are long and with tapering ends, tracheids with blunt ends, unicellular root hairs and parenchymatous cells were observed.

**Physico-chemical studies**

The percentage of moisture content was 6.67, total ash 5.07, acid insoluble ash 0.57, water soluble ash 0.65, alcohol soluble extractive 32.63 and water soluble extractive 26.85%; the colour, consistency and successive extractive values (%) were petroleum ether-dark green, sticky mass, 2.39 (60-80°C); benzene- pale yellow, semi solid, 01.9; chloroform-dark brown, sticky mass, 02.70; ethanol-reddish brown, sticky mass, 44.74 and water-light brown, semi solid, 18.00.

**Preliminary Organic Analysis**

Preliminary organic analysis revealed the presence of carbohydrates, flavonoids, glycosides, phenols and tannins in ethanol and water extracts; saponins in chloroform, ethanol and water extracts; alkaloids in ethanol, proteins, amino acids, gums and mucilage in water extract whereas volatile oils and phytosterols were absent in all extracts.

**Chromatographic Studies**

HPTLC method was developed for the fingerprinting of Shatavarin IV in alcohol and aqueous extracts of *A. gonoclados*. Alcohol extract revealed 9 phytoconstituents with *R*<sub>f</sub> 0.04, 0.17, 0.25, 0.28, 0.34, 0.42, 0.68, 0.79, and 0.93 (Fig. 24) while aqueous extract revealed 7 phytoconstituents with *R*<sub>f</sub> 0.06, 0.12, 0.16, 0.28, 0.34, 0.42 and 0.93 (Fig. 25). Band with *R*<sub>f</sub> 0.34 in both extracts correspond to that of standard Shatavarin IV (*R*<sub>f</sub> 0.35) (Fig. 26). The specificity was confirmed by overlaying the spectra of standard Shatavarin IV (*λ*<sub>max</sub> 426 nm), with the absorption spectrum obtained from the corresponding band in the track of alcohol and aqueous extracts (Fig. 27).

Shatavarin IV (standard) and both alcohol and aqueous extracts of *A. gonoclados* exhibited yellow fluorescence in visible light (Fig. 28) and at 366 nm.
exhibited light blue fluorescence (Fig. 29), whereas at 254 nm did not quench any fluorescence.

**Ultra-violet analysis**

Powdered drug under visible light and UV rays when treated with different reagents emitted various colour radiations (Table 1) which help in identifying the drug in powder form.

**Conclusion**

The macro- and microscopical characters of the root tubers of *A. gonoclados* along with detection of Shatavarin IV by HPTLC is presented. The pharmacognostical characters help in the identification of drug and also in laying down pharmacopeial standards. Due to the similarity observed in pharmacognostical characters with that of *A. racemosus* and also in the phytoconstituents, *A. gonoclados* can be a good substitute of *A. racemosus* which is not only the accepted botanical source of

<table>
<thead>
<tr>
<th>Treatment of powder</th>
<th>Visible light</th>
<th>Ultra-violet light</th>
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<tbody>
<tr>
<td>Powder as such</td>
<td>Pale cream</td>
<td>Cascade green</td>
</tr>
<tr>
<td>Powder + 50% H$_2$SO$_4$</td>
<td>Pale cream</td>
<td>Cascade green</td>
</tr>
<tr>
<td>Powder + 50% HNO$_3$</td>
<td>Pale cream</td>
<td>Mint green</td>
</tr>
<tr>
<td>Powder + 5% KOH</td>
<td>Pale cream</td>
<td>Mint green</td>
</tr>
<tr>
<td>Powder + Methanol</td>
<td>Raw silk</td>
<td>Water green</td>
</tr>
<tr>
<td>Powder + 1N HCl</td>
<td>Pale cream</td>
<td>Cascade green</td>
</tr>
<tr>
<td>Powder + 1N Methanolic NaOH</td>
<td>Mid buff</td>
<td>Mint green</td>
</tr>
<tr>
<td>Powder + Ethanol</td>
<td>Raw silk</td>
<td>Water green</td>
</tr>
<tr>
<td>Powder + 1N Ethanolic NaOH</td>
<td>Raw silk</td>
<td>Cascade green</td>
</tr>
<tr>
<td>Power + Acetone</td>
<td>Golden brown</td>
<td>Cascade green</td>
</tr>
</tbody>
</table>

Note: The colours mentioned in the table are based on the “Asian paints” premium gloss enamel, Asian paints limited, Mumbai.
Shatavari but also possesses adaptogenic, antioxidant and other important therapeutic properties. Shatavarin IV in *A. gonoclados* is detected for the first time which helps to undertake studies on the biomarker and bioactive compounds. Further spectral studies like NMR, MS, IR and UV are required in this regard.

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References
8. Warrier PK, Nambari VPK and Ganapathy PM, Some Important Medicinal Plants of the Western Ghats, India, A Profile, IDRC, MAPPA, New Delhi, 2001, p. 35.