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Research Article

Pharmacognostical and Phytochemical Studies of Root Tubers of *Asparagus gonoclados* Baker.

*Tijare R D[1], Beknal A B[2], Mahurkar N[2], Chandy V[1]

*[1]*T. John college of Pharmacy, Bangalore- 83*
[2]HKE college of pharmacy, Gulbarga -05

ABSTRACT

Asparagus gonoclados Baker., commonly known as 'Shatavari' belonging to family Liliaceae, comprises of 100 species of wide spread distribution. Alcohol and aqueous extract of root tubers of the same was proved for antioxidant activity. Literature survey indicates that no pharmacognostical and phytochemical studies were carried out, hence present study was undertaken for the same. Root tubers of *Asparagus gonoclados* were collected, authenticated by botanist and powdered. Pharmacognostical investigation of selected part was carried out to study its macro and microscopical characters. Transverse section, fresh drug maceration and powder characters were studied. Secular outline T. S. showed exodermis, hypodermis, raphide bundles, endodermis, pericycle, proto and meta xylem and sap containing cells. Maceration and powder microscopy showed the presence of parenchyma, vessels, fibers and tracheids. Alcohol and water extracts were obtained using maceration with 70% ethanol and chloroform water respectively. Phytochemical evaluation showed the 03.98% w/w total ash, 01.033% w/w acid insoluble ash, 01.40% w/w water soluble ash, 41.09% w/w alcohol soluble extractives, 31.26% w/w water soluble extractives and 10.91 % loss on drying. Qualitative evaluation of successive solvent extracts showed the presence of alkaloid, carbohydrates, tannins, saponins, phenolic compounds, proteins and flavonoids. Histochemical studies showed the presence of starch, tannins and lignin. HPTLC studies were carried out for finger printing of Shatavarin IV a steroidal saponin. Chromatographic analysis was performed on Camag HPTLC system, Switzerland, using standard Shatavarin IV, alcohol and aqueous extracts of root tubers on silica gel G 60 F_{254} plates, using a solvent system ethyl acetate: methanol: water (75: 15: 10 v/v/v). Detection was done by densitometric scanning at wavelength of 425 nm after post derivatization with anisaldehyde sulphuric acid reagent. The R_f of standard Shatavarin IV was 0.35 and has not shown fluorescence at 366 but showed yellow colour under visible light. Alcohol and aqueous extract also showed the prominent peak at R_f 0.34, corresponding to Standard Shatavarin IV in both extracts, which was further confirmed by overlaying spectra of Standard Shatavarin IV with spectra of alcohol and aqueous extract. Current study reveals the presence of various constituents that helps in screening the same drug for various activities.

Key words: *Asparagus gonoclados,* Shatavarin IV. Root tubers, Medicinal herbs.

INTRODUCTION

The Folk medicine in various countries gives rise to traditional system of medicine such as Ayurveda, Siddha, Unani. From folk medicine and traditional system the medicinal plants were adopted in to modern system of medicines after they have been found as effective drugs through chemical and pharmacological screening [1]**.** Plants have been used for medicinal application ever since man began caring for his body and health. For centuries, the world has been dependent on the properties of plants as a source of healing. Ayurveda, Siddha, Unani and Homoeopathy continue to depend predominantly on medicinal plants as raw material for formulation of crude drugs. Central Council for research in Ayurveda and Siddha (CCRAS) have been conducting research on medicinal plants mentioned in classical text of Ayurveda for the last 3 decades [2].

The present study includes the Pharmacognostical and Phytochemical studies on the root tubers of *Asparagus gonoclados* Baker. "Shatavari" **(***Asparagus racemosus* Willds) is an important Ayurvedic drug employed in several preparations.

Asparagus gonoclados, a branched subscandent armed undershrub[3] is reported as a substitute for *Asparagus racemosus* Willd[4] which possess diuretic, galactogogue[5,] antiulcer[6] and antioxidant activity[7]. Hence the root tubers of *A. gonoclados* are investigated for pharmacognostical and phytochemical studies which may help in future for finding the new drug.

MATERIALS AND METHODS

The root tubers of plant was collected and preserved in 70 % alcohol to keep the specimens in fresh condition. Further identification of collected plant material was done and authenticated by Dr. S.N. Yoganarasimhan. The taxonomic identification was carried out following Gamble, Keshavamurthy and Yoganarasimhan.The specimen of root tubers is stored in the laboratory.

Pharmacognostical studies: The preserved material was used for macroscopical observations which was carried out as per Wallis[8]. The microscopical investigations,

Author for correspondence: E–mail: ranajit_m786@rediffmail.com

Fig. 1, 2 & 3. T. S. of root tubers of *Asparagus gonoclados* Baker.

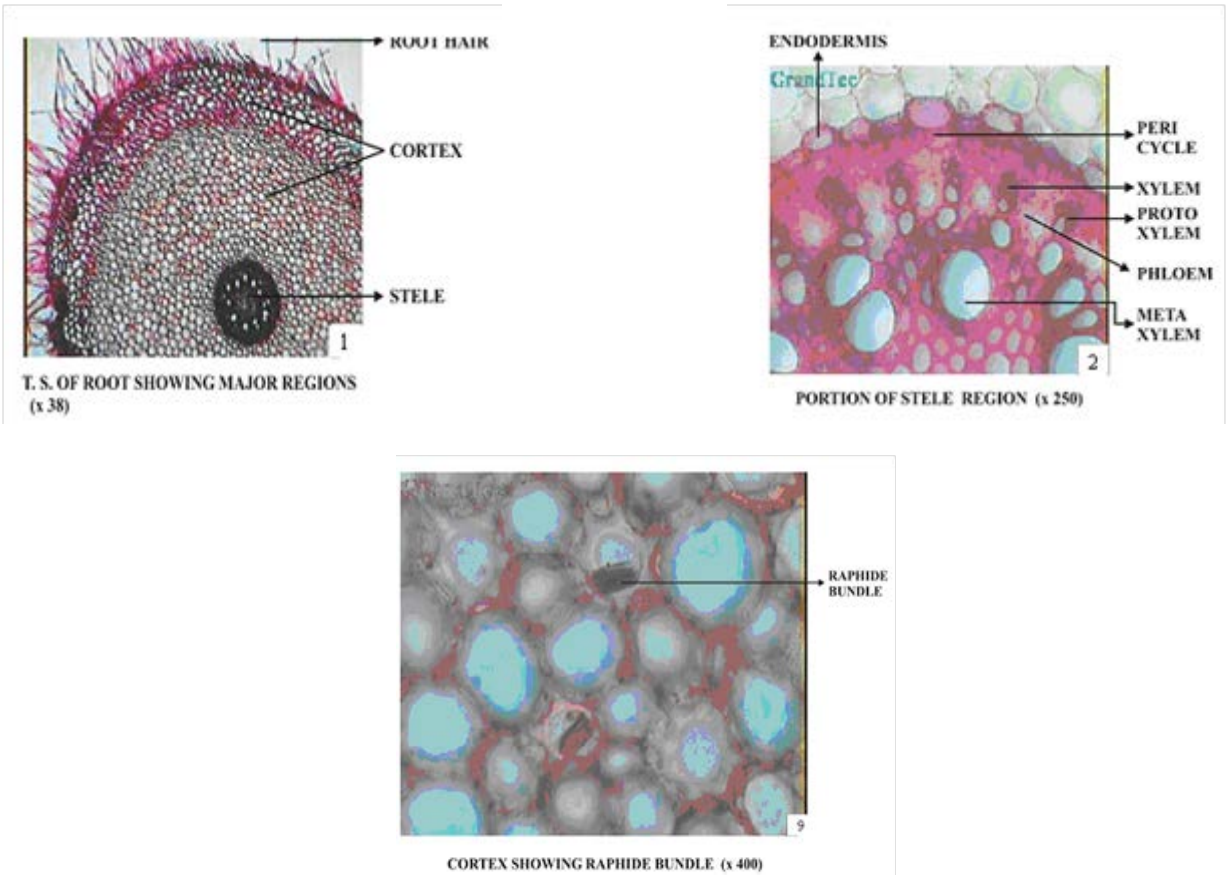


Fig. 4 & 5. Macerate of root tubers of *Asparagus gonoclados* Baker.

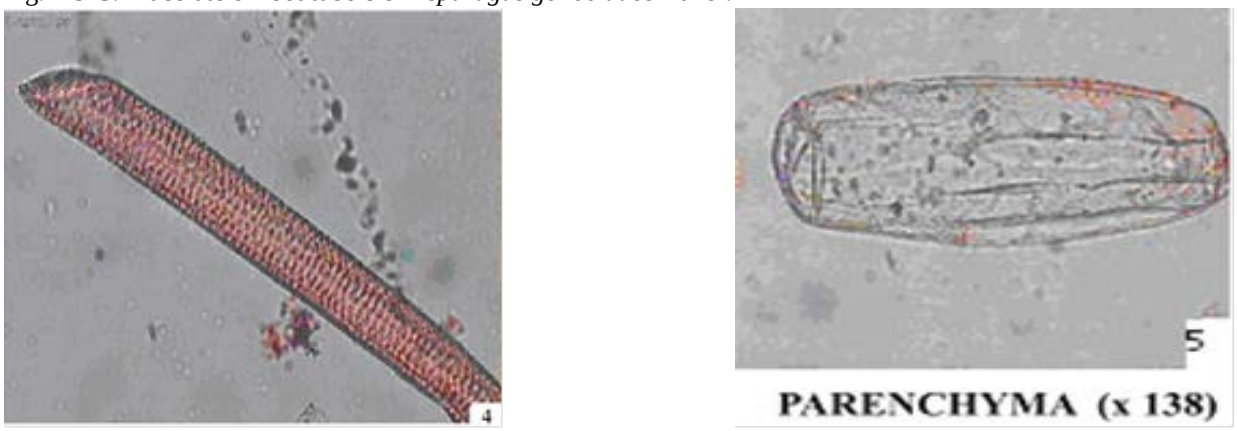


Table 1. For Physical constants of *Asparagus gonoclados*

Sl. No.	Parameter Evaluated	Result (in %w/w)
01	Total ash	03.98
02	Acid insoluble ash	01.033
03	Water soluble ash	01.40
04	Water soluble extract	31.26
05	Alcohol soluble extract	41.09
06	Loss on drying	10.915

Fig.6. HPTLC of Shatavarin IV

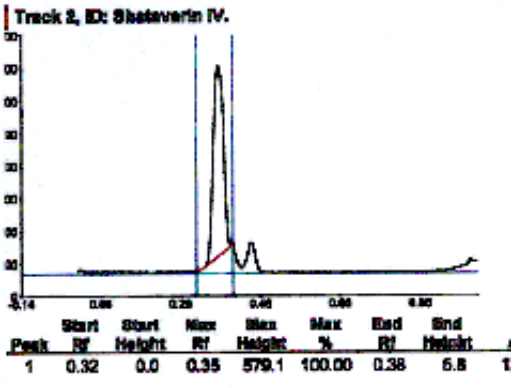
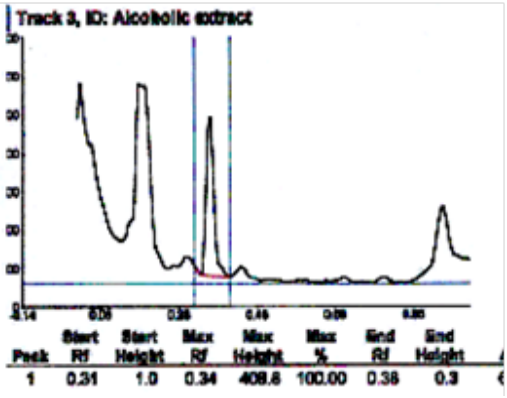
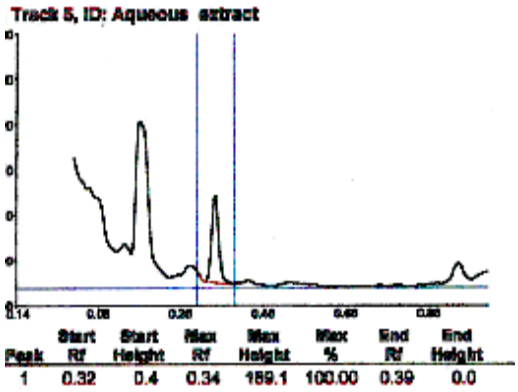


Fig. 7 & 8. Shatavarin IV in Alcohol and aqueous extracts





histochemical tests, and maceration were carried out following Trease and Evans[9].

Phytochemical studies: Successive solvent extraction was carried out by using different solvents according to order of polarity an extracts obtained were tested for different phytoconstituent. Different physical constants were determined following Indian Pharmacopoeia, and the phytochemical tests were carried out following, Brain and Turner[11], Kokate[12] and Harborne[13]. Chromatographic studies were carried out following Krebs Wagner[14] et al and Harborne, For fluorescence studies, powder was sieved through 60 mesh and observations made following Chase and Pratt and Ushakumari[15] *et al*. The different

physicochemical parameters such as ash value, extractive value and moisture content were determined by following Indian Pharmacopoeia[10]

HPTLC studies: Different concentrations of standard Shatavarin IV, alcohol and aqueous extract solutions were prepared in ethanol, methanol and ethanol respectively for fingerprinting of the Shatavarin IV in both alcohol and aqueous extract of *A. gonoclados* Baker. TLC aluminium sheet precoated with silica gel G 60 $F_{254,}$ (10 X 10 cm) was used for development of chromatogram of standard shatavarin IV and both extracts solutions, in solvent system ethyl acetate: methanol: water (75:15:10) after activation at 115^0 C for 30 min. 2 µl each of standard Shatavarin IV solution, alcohol and aqueous extract solutions were applied in duplicate on a precoated silica gel G 60 F_{254} TLC plate, as tracks 1-6, band width of 8 mm, with Linomat V applicator using a Hamilton syringe. Detection of spot was done under 425 nm. Chamber saturation time was 1 h. The TLC plates were kept for development to a migration distance of 74 mm. The developed plates were dried with hot air, and post derivatised by dipping in Anisaldehyde sulphuric acid reagent, dried in hot air and scanned at 425 nm, band width 6mm, slit dimension 6.00 x 3.00 nm, scanning speed 20nm/sec and source of radiation was Tungsten lamp. The R_f and peak area of the standard and the extracts were interpreted by using the software. The developed plates were photodocumented using Camag Reprostar 3 and photographs taken using 366 nm and visible light.

RESULTS AND DISCUSSION

Identification of selected plant and drug has been done by taxonomical, macro and microscopical characters. The root tubers are cylindrical, creamy white, smooth, with a few rootlets. The skin of tubers can be easily peeled. Roots are tapering at both ends and bulged in the middle. The diagnostic morphological character of the plant is the presence of flat, short, curved cladodes.

Fig. 1 & 2 indicate the microscopical examination of root tubers thats shows exodermis, which is light yellow in colour. The outermost layer consist of the rectangular cells, many cells are extended in root hairs. Next to this 6-8 layers of hypodermis and large cortex made of

polygonal cells are present which also showed the presence of raphide bundle in cortical cells which is a diagnostic character for the identification of *A. gonoclados* Baker. from *A. racemosus* Wild.

Single layered endodermis with narrow rectangular thick walled cells next to which single layer pericycle with thin walls is observed. Vascular bundle shows 20 – 25 xylem and phloem groups arranged in alternate radii in a circle surrounding large central pith. The microscopical examination also shows the presence of proto and meta xylem. The diagnostic microscopical chatacters are raphide bundle, mucilaginous sap, scattered vascular bundle and pyliferous layer with numerous root hairs. Macerate of root tuber (Fig. 3 & 4) is a key to identify the different cells present in different parts of root tubers. Macerate shows the presence of the root hairs, parenchyma, and pitted parenchyma. Fibers are with tapering end, narrow lumen, septate and aseptate fibers. Tracheids are with simple pits and vessel with different size and shape.

The fluorescence analysis was also carried out to observe the behavior of powder with different reagents. The physicochemical constants like moisture content, ash values such as total ash, acid insoluble ash, water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value, were determined which are represented in Table 1. These help in maintaining pharmacopoeial standards for the drug.

The phytochemical investigation of root tubers of selected plant was done by using the different extracts, obtained through successive solvent extraction with petroleum ether, benzene, chloroform, ethanol and water. The petroleum ether, benzene, chloroform, acetone, alcohol and aqueous extract were dark pale yellow, pale yellow, pale yellow, reddish brown and light brown color respectively. The yield of alcoholic extract (44.37%) was more and benzene extract was less (1.91%). Phytochemical investigation also shows the presence of carbohydrate, glycoside, saponins, phenolic compounds, flavonoids, tannins and gums. It was observed that maximum number of constituents were present in alcohol and aqueous extract.

Saponins such as Shatavarin I – IV were present in *Asparagus racemosus.* Identification of active constituents in extract of drugs was carried out by HPTLC as it can resolve the various constituents present in the extract into individual components as distinct bands. The plant was reported for the presence of apigenin, kempferol, rutin, chalcone glycoside and saponins. Based on the chemical constituents of *Asparagus racemosus* the present study, HPTLC fingerprinting of Shatavarin IV in the alcohol and aqueous extract of *Asparagus gonoclados* was done by using a solvent system ethyl acetate: methanol: water (7.5:1.5:1). Spectrum analysis was carried out to determine the wavelength of Shatavarin IV which was found to be 425 nm, after post derivatisation with anisaldehyde sulphuric acid reagent. The prominent peak at Rf 0.34 in both alcohol and aqueous extracts (Fig.7 & 8) shows the presence of Shatavarin IV which is corresponding to Standard Rf 0.35 (Fig. 6).

CONCLUSION

The current studies may help in identification of *Asparagus gonoclados* Baker among the other species of Asparagus based on diagnostic characters such as presence of raphide bundle, sap cells, pitted parenchyma and tapered end fibers. HPTLC studies help to know the chromatographic profile of Shatavarin IV and its Identification.

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