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Study of Anatomical Biomarkers for the Standardization of *Asparagus Racemosus* Willd (Liliaceae)

Rupali Subhashrao Kaikade
Research Student
Dept. of Botany
Bar. RDIK and NKD College
Sant Gadge Baba Amravati University
Badnera, Amravati, Maharashtra, India.
rupalik.kaikade@rediffmail.com

Shubhangi Nagorao Ingole
Associate Professor
Head, Dept. of Botany
Bar. RDIK and NKD College
Sant Gadge Baba Amravati University
Badnera, Amravati, Maharashtra, India.
shubhaingole@gmail.com

Abstract: *Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. Anatomical biomarkers of different plant parts have been used as aids in the taxonomical recognition of species. In this study we selected a widely available perennial climber Asparagus racemosus Willd (Liliaceae). Microscopic evaluation revealed the presence of anomocytic and diacytic stomata on stem and cladode; non-glandular uniseriate filliform trichomes. Transverse section of stem showed rounded outline with deeps and furrows and zigzag margin, distinguished hypodermis, wide parenchymatous cortex, scattered vascular bundles, vessel elements and stomatal number were also determined. These microscopic identification can be used as a rapid inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of a plant.*

Keywords: *Asparagus racemosus, Anatomical biomarkers, Trichomes, Vessel elements*

1. INTRODUCTION

Plant materials are used throughout the world as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market. It is therefore important to establish their quality because adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. Anatomical biomarkers of different plant parts have been used as aids in the taxonomical recognition of species. The importance of micromorphological features (anatomical biomarkers) for the taxonomic consideration of angiosperms is now well established (Ramayya, 1972 [1], Tomlinson, 1979 [2]; Ogundipe and Akinrindale, 1998 [3] and Parveen et al., 2000 [4]). The first summary of the systematically more useful anatomical characters with an evaluation of their importance was provided by Fritsch (1903) [5]. Metcalfe and Chalk (1950 [6], 1967 [7] and 1983 [8]) have enumerated a large number of anatomical characters of diagnostic value for different families.

In this study we selected a widely available perennial climber *Asparagus racemosus* Willd belonging to family Liliaceae, grows all over India in Tropical areas and in Himalayas upto altitude of 1300-1400m. Traditionally roots are used mainly to promote milk secretion and demulcent, diuretic, aphrodisiac, tonic and antidiarrhoeal (Goyal et al., 2003 [9]). From the reviewed literature it has been seen that very scanty or no work has been done on the anatomical biomarkers of *Asparagus racemosus*. Hence the present work is carried out with an objective to study its anatomical biomarkers.

2. MATERIALS AND METHODS

2.1. Plant Collection and Authentication

The plant materials for the present study were collected from the college garden of Bar. RDIK and NKD College, badnera, Amravati. The collection was made in accordance with the flowering

seasons to enable the collection of the flowering materials and proper diagnosis. Herbarium specimens of collected plants were made and macro characters were studied in the field. The investigatory vegetative and floral study of the species was carried out in the laboratory by using dissecting and binocular microscope. The plant was identified with the help of the standard floras (Naik, 1998 [10]).

2.2. Morphological Analysis

Macroscopic observations of the plants were done. The shape, size, surface characters, texture, colour, odour, taste was noted.

2.3. Anatomical Analysis

For the anatomical studies, the fresh as well as fixed material in F.A.A. was used. Present study is based mostly on free hand sections, macerations and peelings of fresh and preserved materials. All the sections were stained in saffranin and dehydrated following the usual method of Johansen (1940) [11] and mounted in Canada balsam. The details of anatomical characters were studied under Labomed binocular research microscope. To study the stomatal complex from various vegetative parts, epidermal peeling of all vegetative parts was directly done mechanically by forcep or by scrapping with the help of razor blade. The hard and difficult materials were peeled off after treating with 5% aqueous sodium hydroxide. Cleared parts were washed with distilled water and treated with 2% acetic acid for 1-2 hrs to neutralize residual sodium hydroxide in the material. Finally the prepared peels were stained with 1% aqueous saffranin followed by mounting in 50% glycerine.

For each species, the quantitative analysis of stomatal complex was made by calculating stomatal frequency, size of stomata and epidermal cells from random sampling of 5- different peels. Other parameters include stomatal shape, distribution and orientation. Stomatal classification is based on the morphological classification recorded by Baranova (1992) [12].

To get an integrated picture of the trichome types and their organographic distribution varied temporary microprepartions viz. epidermal peels, mounts of cleared whole organs or their portions, scrapping and transections were used. The observation was made directly under compound microscope and camera lucida sketches were made.

For studying vessel elements, wood samples from thick woody branches (of 5-7cm in diameter) in cases of woody and thickest portion of stem in herbaceous species were collected from plants. First the dried material of wood from stem was cut into small longitudinal pieces. The pieces were further cut into small thin slices and processed for maceration by Jefrey's method: In plant Microtechnique by Johansen, 1940, using a mixture of equal parts of 2% aq. nitric acid and 5% aq. chromic acid. The softening time for wood varied according to material but in general, material was put into macerating mixture for about 24-72hrs. Softened material crushed very gently with the use of thick glass rod with rounded end. Separated elements thoroughly washed with water to remove the acid and stained with aq. safranin (1%). Vessel elements were selected dehydrated and mounted in glycerin or Canada balsam. Observations have been confined to the late metaxylem elements. All measurements and camera lucida drawing were taken at 10x and 40x magnifications.

3. RESULTS AND DISCUSSION

3.1. Morphological Analysis

Asparagus racemosus is a perennial climber climbs upto 1-3 m high. It is an extensively scandent spinous, much branched undershrub. Roots- numerous fusiform, succulent and tuberous with a diameter of 0.5 to 1.5 cm arises as a cluster from the basal end of the stem. Stem- woody, sparsely covered with recurved spines. Leaves- reduced to small scales called as cladode which is in tufts of 2-6 in a node, finely accuminate, falcate. Inflorescence- branched raceme. Flowers - white, fragrant, bracteates, pedicilate, actinomorphic, bisexual, hypogynous and trimerous. Perianth- 6, arranged in two whorls of three each(3+3), membranous, gamophyllous, valvate. Androecium- 6 stamens in two whorls of 3 each, opposite the sepals (3+3); anther- epiphyllous, dithicous, introrse, basifixed, versatile. Filaments are usually free. Gynoecium- tricarpellary, syncrpous, superior, trilocular, two ovules in each locule, axile placentation. Stigma- trifid. Fruit- Berry.

3.2. Anatomical Analysis

3.2.1. Epidermal Features of Stem in Surface View

Epidermal cells: much elongated, longer than broad, 48x16µ; orientation- longitudinal to long axis of organ; wall- thin, straight to curve; surface- smooth; needle shaped raphides present; cuticular stiations absent.

Stomatal complex: surface stomatiferous; stomatal distribution- mostly in between two epidermal cells; orientation- longitudinal to long axis of organ; shape- rounded; guard cells almost equal; inner wall- thin; distribution pattern- 1-2- celled apart; type- anomocytic; frequency- 2/ unit area.

3.2.2. Trichome Complex

I. Nonglandular Uniseriate Filliform

1. Uniseriate Conical

- Body- falcate- conical; 60x24µ; acutely pointed at apex; base- flat; content- granulated, not obliterated; wall- thin, curved; surface- smooth; lumen- moderate broad; seating on single epidermal cell.
- Body- conical; bend at apex; 72x28µ to 84x32µ

2. Multicellular Conical

- Body- 2- celled; basal cell smaller than terminal cell; 56x32µ; obtusely pointed at apex; base- sub- rounded; lateral wall and cross wall- thin and concave; surface- smooth; lumen- broad; seating upon four adjoining distinguished epidermal cells.

3.2.3. Transverse Section of Stem

Transectional outline through middle region roundish with deeps and furrows and zigzag margin. Adaxial, abaxial and lateral epidermis- 1- layered; cells- squarish to slightly rounded; wall- outer and inner- angular with thick cuticle. Trichomes present.

Hypodermis- distinguished, continuous, chlorenchyma- 2-3- layered in deep regions and 4-5- layered in furrows adaxially, abaxially and laterally; cells- predominantly rounded and few horizontally elongated; followed by collenchyma, 6-7- layered adaxially and 5-6- layered abaxially and laterally.

Cortex- wide, parenchymatous; cells- rounded, small to large, isodiametric, compactly arranged.

Vascular bundle- pattern of vascular configuration in middle region displaying scattered vascular bundles with large xylem vessels; vessels- paired, circular in outline with large lumen. Total vascular bundle- 12

3.2.4. Vessel Elements

Vessels angular, predominantly longer than broader; perforation plates predominantly 2 per vessels, simple and unique with side perforation plate, present on almost transverse end wall, termination- horizontal; shortly ligulated at one end to non- ligulated; sculpturing pattern on lateral wall- pitting, pits- simple, much crowded, rounded to oval and few horizontally elongated, small; arrangement- randomly irregular.

Measures- Longest vessel- 188x52µ	Shortest vessel- 76x32µ
Length (avg)- 153µ	Breadth (avg)- 46µ

3.2.5. Cladode

3.2.5.1. Epidermal Features in Surface View

Epidermal cells: elongated longitudinally, isodiametric, 44x20µ, longer than broad; orientation- longitudinal to long axis of organ; walls- thin, straight, and smooth; cuticular striations- absent.

Stomatal complex: stomatiferous, longitudinal to long axis of organ, shape- elongate; guard cells almost equal, lying at the level of epidermal cell. Distribution pattern- longitudinal to long axis of

organ, 1-2 cells apart. Type- diacytic. Size- 28×20µ.

Trichome complex: Unicellular Short papilliform: Body cylindrical- conical, 24x20µ; rounded at apex; base- broad; content- granular; wall- smooth, thick; seated upon normal ordinary cell.

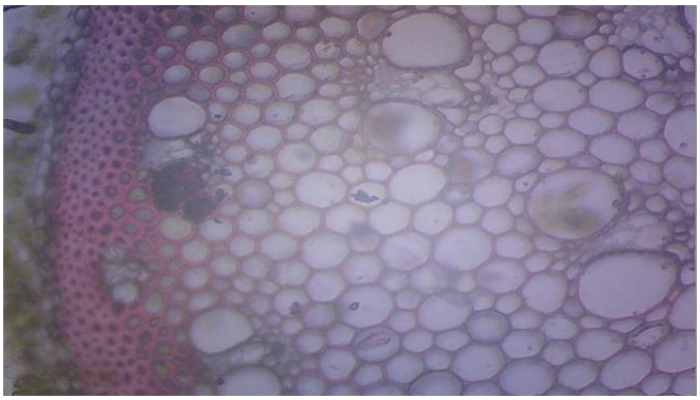

Fig11. *T.S. of Stem*

Fig12. *T.S. of stem showing scattered Vascular bundles*

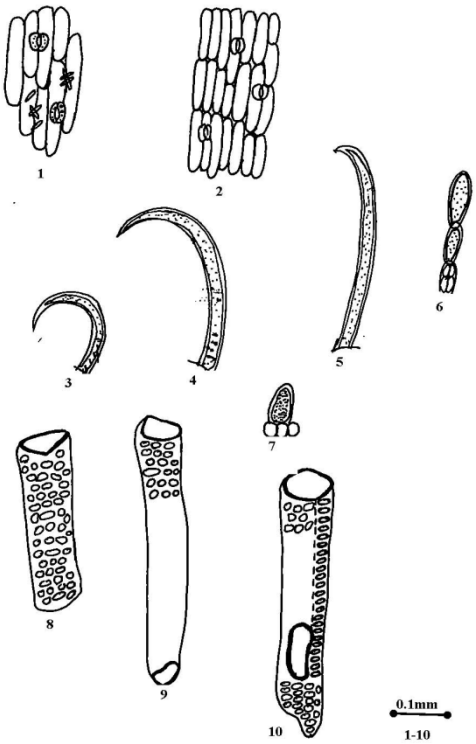

Fig1-10. *1. Epidermal features in surface view of stem, 2. Epidermal features in surface view of cladode, 3-5. Nonglandular uniseriate filliform trichome, 6- Multicellular trichome, 7- short pappiliform trichome, 8-10. Vessel elements*

The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques namely macro and microscopic identification to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs because in these cases most of the morphological diagnostic features are lost. Anomocytic and diacytic stomata on stem and cladode; non-glandular uniseriate filliform trichomes are of diagnostic value.

4. CONCLUSION

The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Study of these anatomical biomarkers can be useful to substantiate and authenticate drug.

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AUTHORS' BIOGRAPHY

Miss. Rupali Subhashrao Kaikade, is a research student of Botany. I completed my Post graduate (M.Sc) in 2008 with specialization Molecular Biology and completed Masters in Philosophy in 2010. Recently I submitted my Phd Thesis namely "Study of Phytochemicals and Anatomical Biomarkers of some Medicinal Plants Mentioned in Atharvaveda" under the guidance of Dr. Shubhangi N. Ingole in August 2014 and waited for the degree to be awarded. I worked as CHB (Clock Hour Basis) Lecturer and have 3 years of teaching experience. I also attended National and some International conferences and present a paper in the same.

Dr. Shubhangi N. Ingole is working as Head, Department of Botany at Bar. R.D.I.K. and N.K.D. College, Badnera-Amravati (M.S.); Specialized area of research Angiosperm Taxonomy, Anatomy, Phytochemistry, Pharmacognosy and Floristic Studies, Grasses having experience of 25 years; She is recognized Ph.D. guide; She completed 2 Research Projects funded by U.G.C.; Published more than 25 Research Papers in reputed journals and 2 books; Presented about 50 papers in National and International Conferences of which some awarded as Best Papers; She was Member of Board of Studies in Botany of S.G.B. Amravati University since 2007-2012; She is working on several University Committees; She is a life member of more than 10 reputed National and International Associations; Successfully organized National Conference "Status of Biodiversity in India" in 2011; She established Botanical Society, Nature Club and Botany Research Cell; Conducts several innovative Co-curricular and Extension Activities for promotion of love for nature, environment conservation and promote botanical interest; She is active social worker; was NSS Programme Officer; won prizes in flower shows, essay competitions, etc; She is active writer and authored several diverse articles for Newspapers and Magazines.