INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-2, April-June-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490
*Received: 14th Feb-2016* *Revised: 20th Mar 2016* *Accepted: 24th Mar 2016*
***Research article***

# EVALUATION OF ANATOMICAL FEATURES OF *Adenia wightiana* (Wall. ex Wight & Arn.) Engl. OF PASSIFLORACEAE FOR PROPER IDENTIFICATION OF THE SPECIES

Presena, J[a*] and Pragasam, A[b]

[a]Indira Gandhi College of Arts and Science, Puducherry, India.
[b]Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India.

**ABSTRACT:** *Adenia wightiana*, a tendril climber of the family Passifloraceae, is used as an important medicinal plant and also as a leafy vegetable. Since the genus *Adenia* comprises of a large number of species the proper identification of the particular species is very important. Cross section of leaf, petiole, stem, root, root tuber and powder microscopic studies were done to bring out the anatomical significance of the species *Adenia wightiana*. Paracytic stomata of unequal subsidiary cells; dendroid vein terminations; irregularly lobed sclereids and tannin cells in the upper epidermis of lamina; druse idioblasts in the mesophyll of leaf and cortex of root and root tuber; cuticular projections with annular striations in the epidermal cells of leaf; accessory vascular bundles in the petiole; cleaved vascular cylinder, wide vascular rays, abundant axial parenchyma, diffuse porous wood of stem; discrete clusters of vascular tissue and highly proliferated ground parenchyma in the root tuber; vessel elements with simple perforations and alternate lateral wall pitting; fibre-tracheids cylindrical, four lobed starch grains with polarimarks in the root tubers are some of the distinctive characters for identification of the taxon studied.
**Key words:** *Adenia wightiana*, Passifloraceae, Anatomy, Pharmacognosy.

*Corresponding author: Presena, J, [a]Indira Gandhi College of Arts and Science, Puducherry, India.
Email: presena@gmail.com


## INTRODUCTION

*Adenia wightiana* belongs to the family Passifloraceae which includes 27 genera and 530 species. The genus *Adenia* includes about 80-100 species which are distributed throughout the Old World tropics, and more species are likely to be discovered [1]. A*denia wightiana* is a tuberous, perennial liana with axillary, branched tendrils. It is one of the medicinal plants commonly used in the folklore medicine for curing the ailment like peptic ulcer [2]. Leaves are eaten as vegetable by the tribes of Anamalai hills, Western Ghats [3].

Since *Adenia* comprises of large number of species and *A. wightiana* is medicinally important its proper identification is very important. In addition to macroscopic examinations and use of various chemical tests, the microscopic evaluation is essential for the correct identification of the species. The anatomy is of primary importance for all aspects of research in plant sciences. When a plant part is powdered some of the morphological characters such as tracheary elements, fibres, sclereids, leaf epidermal cells, stomata, trichomes, crystals of silica bodies, starch grains etc., remain unaffected. Thus microscopic studies of sections and powder of plant parts are essential for plant identification [4]. The anatomy of 58 species of *Adenia* were described to bring out the anatomical diversifications [5]. Ayensu and Stern [6] provided the first and only comprehensive anatomical survey in Passifloraceae.

Although they investigated 44 species in Passifloraceae, they characterized the anatomy of *Adenia lobata* only [5]. The complete anatomy of the whole plant of *A. wightiana* is not reported so far. So a detailed anatomical study of the complete plant of *Adenia wightiana* is carried out in the present work for its proper identification which will be useful in Taxonomy as well as Pharmacognosy. The study is also aimed to provide valuable and reliable illustrated anatomical descriptions of the plant.

## MATERIALS AND METHODS

### Specimen collection

The plant specimens of *Adenia wightiana* were collected from the Tropical Dry Evergreen Forests (TDEF) of Puthupet which is about 20 kilometers on the North of Pondicherry, South India. The plant was identified with the help of the Flora of the Presidency of Madras [7] and the Flora of Tamil Nadu Carnatic [8]. Care was taken to select healthy plants and normal organs (Fig. 1A). It was photographed and herbarium specimens were prepared. The required samples of different organs were cut from the plant and fixed in FAA (5ml Formalin, 5ml Glacial Acetic acid, 90 ml of 70% Ethyl alcohol). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA) as per the schedule [9]. Infiltration of the specimens was done by gradual addition of paraffin wax (melting point 58-60˚C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

### Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure [10]. The sections were stained with Toluidine blue [11]. Since Toluidine blue is polychromatic stain, it rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and fast-green and iodine potassium iodide for starch. For studying the epidermal cells, stomatal morphology and venation pattern paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [9] were done. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered material of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and the dimensions were measured.

### Photomicrographs

Microscopic description of tissues was supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appeared bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book [12, 13].

## OBSERVATIONS

### Leaf- midrib

The leaf in cross section is thin and bifacial with plano-convex midrib. The midrib is 230 µm thick, flat on the adaxial side and convex and semi-circular on the abaxial side (Fig. 1B). The adaxial epidermis of the midrib is thin with short cylindrical thick walled cells. The abaxial epidermis is thick with squarish thick walled cells and prominent conical, cuticular projections. The ground tissue of the midrib is parenchymatous, the cells are wide, angular, compact and thick walled. The vascular strand is single and collateral. The xylem strand includes about four short vertical lines of xylem elements which are angular and thick walled. Phloem occurs in thin arc at the lower end of the xylem strand.

### Lamina

The lamina is 110 µm thick, dorsiventral and hypostomatic. The adaxial epidermis consists of thick and wide cylindrical cells. The abaxial epidermis is thin and made of rectangular thin walled cells with thick conical cuticular projections. The mesophyll consists of single row of vertically elongated conical and compact palisade cells on the adaxial side and 3 or 4 layers of small, lobed spongy parenchyma with large intercellular spaces on the abaxial side (Fig. 1C). The spherical idioblasts containing prismatic calcium oxalate crystals are sparsely distributed in the palisade tissue of the lamina each measures 70 to 100 µm in diameter and may have 1-3 druses (Fig. 2A).

**Leaf epidermis in surface view**

Surface view of the epidermal cells was studied in paradermal sections. The adaxial epidermis consists of polyhedral thick walled cells with straight anticlinal walls. Tannin cells and large, irregularly lobed, deeply staining sclereids are found distributed here and there (Figs. 1D & H). The epidermal cells have several successive annular cuticular striations and form small conical mounds (Fig. 1E). The abaxial epidermis is stomatiferous, the epidermal cells are larger in size and have fairly thick wavy anticlinal walls. The annular cuticular striations are more prominent on the cells of the abaxial epidermis. The stomata are of paracytic type and 15x10 µm in size. Of the two subsidiary cells one is much smaller and the other is larger (Fig. 1F).

**Venation**

The venation is densely reticulate, veins are thin and wavy, vein islets are wide and the vein boundaries are not well defined (Fig. 1G). The vein terminations occur in all vein-islets. The termination is much branched repeatedly forming large, dendroid outline.

**Petiole**

The petiole is roughly circular in cross section. It is 1.5 mm in diameter and exhibits dorsiventral polarity. The epidermis has barrel shaped, fairly thick epidermal cells with prominent cuticle. Internal to the epidermis there are 3 or 4 layers of small collenchyma cells. The remaining ground tissue is parenchymatous and made of thin walled angular and compact cells. The vascular system consists of a pair of adaxial small accessory vascular bundles. The main vascular strand includes wide and deep bowl shaped vascular bundle and fairly thick wedge shaped medullary vascular bundle which is situated above the concavity of the main bundle (Fig. 2B). All the bundles are collateral with well-developed vertical line of xylem elements which are circular or elliptical, wide and thick walled. Phloem occurs in the form of thin band on the outer end of each vascular bundle.

**Stem**

The stem is circular in transverse section and measures 6.5 mm in thickness. The epidermal layer is thin with cylindrical cells and thick cuticle. The cortical zone is thick and parenchymatous with discontinuous, discrete masses of cortical fibres (Fig. 2C). The vascular tissues occur in wide six segments with narrow parenchymatous gaps in between the segments. The vascular segments are collateral with outer phloem and inner mass of xylem. Phloem consists of fairly wide thick walled, angular compact cells which are diffuse in distribution. Xylem strands include secondary xylem as well as primary xylem (Fig. 2D). The secondary xylem consists of fairly large number of wide circular, thin walled solitary vessels and xylem fibres. The secondary xylem vessels are 90-150 µm wide. The primary xylem vessels are comparatively narrow, fairly thick walled and occur in tangential row in the inner border. Pith is wide, parenchymatous and homogenous.

**Root**

The root is 8.5 mm thick. It consists of outer thick, highly fissured periderm followed by cortex. The vascular cylinder includes secondary phloem and secondary xylem enclosing narrow pith. The periderm is irregularly fissured at several places, thin at certain places and thick in other places. It includes thin walled, tubular, suberized phellem cells. The secondary phloem is very thick measuring one mm in radial plane. It consists of outer portion of collapsed phloem and inner intact phloem. The collapsed phloem includes crushed and obliterated sieve elements which are seen as dark thin tangential or irregular markings. In the intact phloem, the sieve elements are intact and they occur in wide circular masses (Fig. 2E). The secondary phloem is followed by thick wavy cylinder of secondary xylem which is cleaved into many thick radial segments by the wide, dilated xylem rays. In the segmented xylem the segments include numerous solitary or paired vessels. The vessels are wide, fairly thick walled, circular or angular and are 100-170 µm in diameter. The ground tissue of the secondary xylem includes xylem fibres and thin tangential segments of parenchyma. Druse idioblasts are distributed in the parenchyma cells (Fig. 2A).

**Root tuber**

Because of the storage function of the root tuber, the structure is unusual in general. The tuber contains more quarters of storage parenchyma than vascular tissues. The tuber consists of a thick dark superficial layer and less distinct periderm layers. The cortical zone is wide and parenchymatous. The cortical tissue includes cells of various shape and size and highly distorted in orientation. The central part of the tuber consists of large number of small clusters of vessels and fibres. The vessel clusters are distorted and occur in different orientation. The cluster includes two to four vessels which are ensheathed by thick walled lignified fibres. The vessels are wide, circular and fairly thick walled. They are 120-170 µm wide. Towards the periphery of the tuber, the vessel clusters become reduced in number and are radially stretched. Each vessel cluster is associated with a narrow, radially oriented phloem strand. The phloem strand consists of small, angular thick walled sieve elements. The ground tissue in between the vascular strands is composed of proliferated cells of the cambial derivatives. The cells possess dense accumulation of the starch grains and large druse idioblasts (Fig. 2F & G).

**Distribution of crystals and starch grains**

The druse idioblasts of spherical calcium oxalate crystals are fairly abundant in the mesophyll of leaf and different tissues of the root and root tuber. The druse idioblasts in the leaf are large and spherical with 1-3 druses. In root they are mainly distributed in the parenchyma of phloem and ray parenchyma of xylem. In the root tuber they are distributed in the ground parenchyma. The cells bearing the druses in root and root tuber are similar to the ordinary cells in size and shape. They are irregularly distributed and range from 20-25 µm in diameter. The parenchyma cells of the root tuber contain dense accumulation of the starch grains (Fig. 2G). They are cylindrical and longitudinally four lobed with dark cross marks on the polar ends.

**Powder microscopic observations**

The powder preparation of the plant material exhibited the following inclusions when examined under the microscope. Xylem fibres are of libriform type, long, uniformly thick with lignified walls and narrow lumen. They are about 500 µm long and less than 5 µm wide. Fibre-tracheids are 550 µm long and 20 µm wide, similar to fibres but with wide lumen and dense slit like lateral wall pittings. The pits occur in two or three vertical rows. The tracheids are about 450 µm long and 30 µm wide with thick lateral walls and wide cell lumen and blunt ends. They have bordered pits densely distributed on the lateral walls. Vessel elements are 220-270 µm long and common in the powder (Fig. 2H). They are short, cylindrical and wide with multiseriate, elliptical pits on the lateral walls. The end wall may be horizontal or slightly oblique with wide circular simple perforation plate.

## RESULTS AND DISCUSSION

Anatomy is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution and genetics [14]. Though the anatomy of 58 species of *Adenia* was described [5], the complete anatomy of vegetative parts of the species *Adenia wightiana i*s not reported so far. The present study brings out the microscopic observation of anatomy of leaf, petiole, stem, root, root tuber and crude powder for proper identification of the plant which will be useful in systematic botany and pharmacognosy.

The salient features observed in this study which will be useful for its specific identification are: Dorsiventral, hypostomatic leaf with paracytic stomata measuring 15x10 µm with distinctly unequal subsidiary cells. The clear vein islets and dendroid vein terminations. Irregularly lobed, deeply staining scelereids and tannin cells in the upper epidermis of the lamina. Large spherical crystalliferous idioblasts with 1-3 druses in the mesophyll of leaf. The idioblasts in the root and root tuber are similar to the cortical cells in size and shape. Successive concentric cuticular striations in the epidermal cells of leaf. A pair of adaxial small accessory vascular bundles in the petiole. Vascular cylinder of the stem cleaved into about six wide fan shaped segments separated by wide vascular rays. Root tubers exhibiting anomalous structures with division of the vascular tissue into discrete clusters and highly proliferated ground parenchyma. Wide, circular vessel elements with transverse or slightly inclined end walls with simple perforations and alternate lateral wall pitting. Fibre-tracheids with elliptical lateral wall pitting. Abundant axial parenchyma. Diffuse porous wood frequent with solitary vessels, sometimes in pairs. Large number of cylindrical and longitudinally four lobed starch grains with dark cross shaped polarimarks in the root tubers.

According to Selvam [15] the type and distribution of starch grains are characteristic and species specific. The location of the crystals within a taxon is also often very specific and may be represented as a taxonomic character [16]. Thus the present study reveals the complete anatomical descriptions supplemented with photomicrographs for the proper identification of *Adenia wightiana* for the first time.

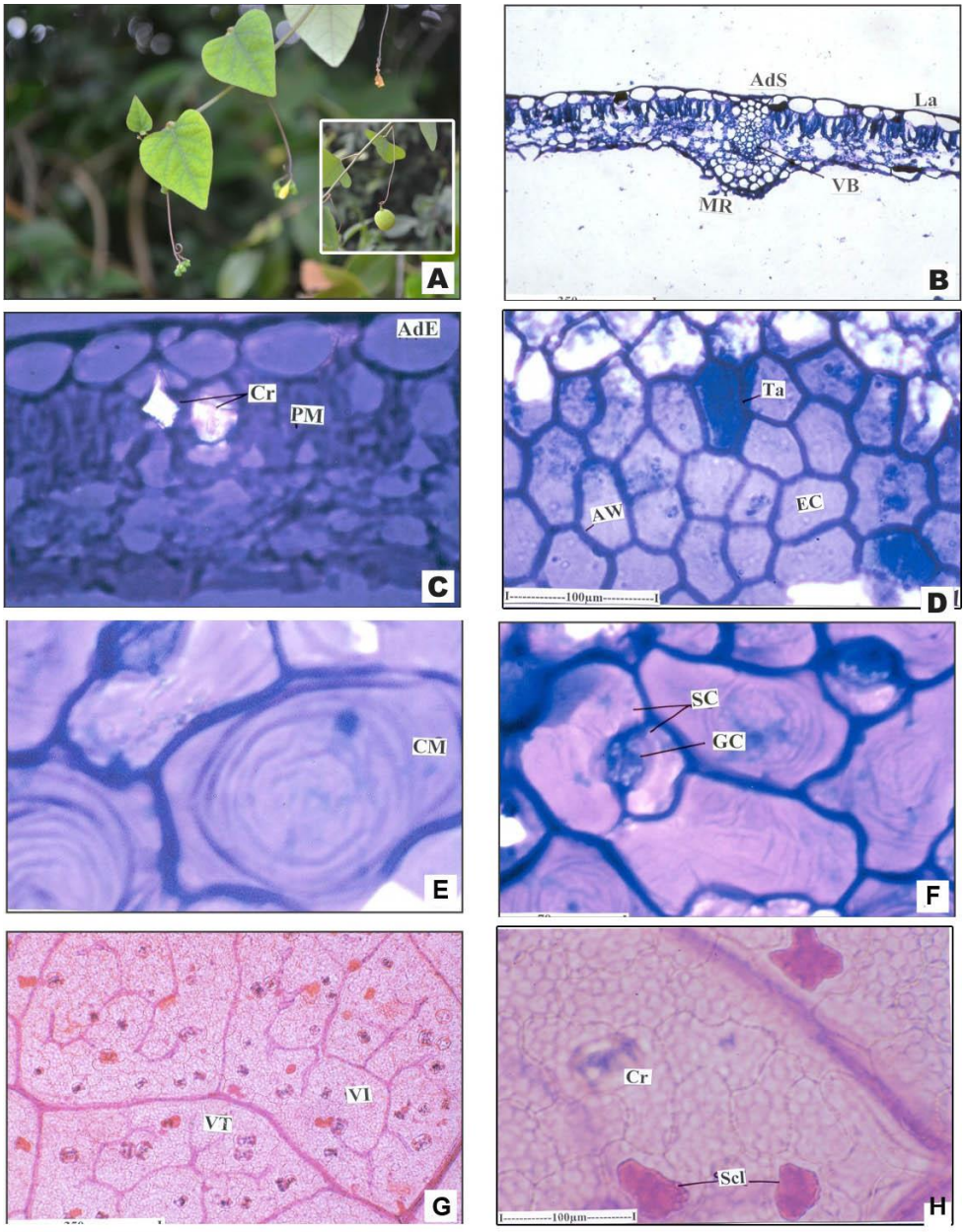


**Fig.1.** ***Adenia wightiana*****: (A) A twig (B) T.S. of leaf (C) Crystals in Lamina (D) Tannin cells (E) Cuticular striations of epidermal cells (F) Paracytic stomata (G) Vein islets with terminations (H) Sclereids**

**Key: AdE-Adaxial Epidermis, AdS-Adaxial side, AW-Anticlinal Wall, CM-Cuticular Mound, Cr-Crystal, EC-Epidermal Cell, GC-Guard Cell, La-Lamina, MR-Midrib, PM-Palisade Mesophyll, SC-Subsidiary Cell, Scl-Sclereid, Ta-Tannin cell, VB-Vascular Bundle, VI-Vein Islet, VT –Vein Termination.**

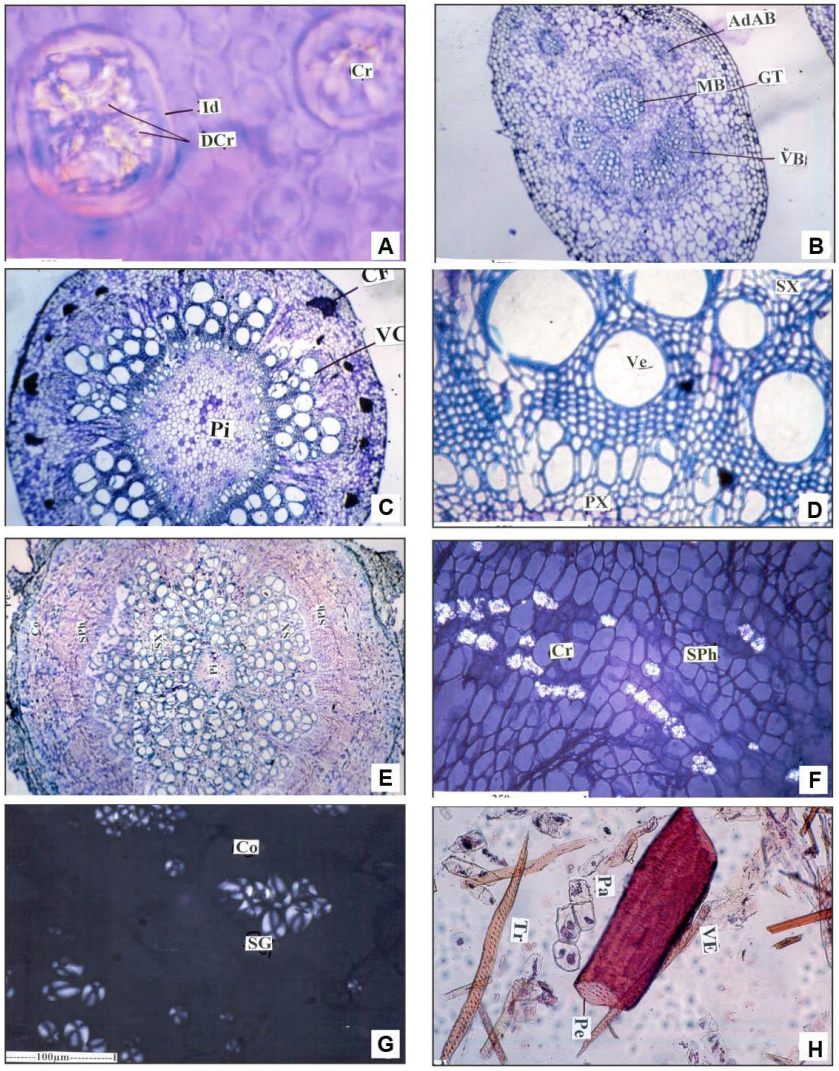


**Fig.2. *Adenia wightiana*: (A) Druse idioblast (B) T.S. of petiole (C) T.S. of stem (D) Secondary xylem & phloem (E) T.S. or root (F) Crystals in secondary xylem (G) Starch grains of the root tuber (H) Vessel elements.**

**Key: AdAB – Adaxial Accessory Bundle, CF-Cortical Fibre, Co-Cortex, DCr-Druce Crystal, ID-Idioblast, GT-Ground Tissue, MB-Median Bundle, Pa-Parenchyma, Pe-Perforation, Pi-Pith, PX-Primary Xylem, SPh-Secondary Phloem, SG-Starch Grains, SX-Secondary Xylem, Tr-Tracheid, VC-Vascular cylinder, VE-Vessel Element.**

## REFERENCES

[1] Hearn D J. 2007. Novelties in *Adenia* (Passifloraceae): Four new species, a new combination, a vegetative key and diagnostic character for known Madagascan species. Brittonia. 59: 308-327.

[2] Kottaimuthu R. 2008. Ethnobotany of the Valaiyans of Karandamalai, Dindigul District, Tamil Nadu, India. Ethnobotanical leaflets. 12: 195-203.

[3] Ramachandran V S. 2007. Wild edible plants of the Anamalais, Coimbatore district, Western Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge. 6(1):173-176.

[4] William C Dickison. 1999. Integrative plant anatomy. Academic Press, Cambridge.

[5] Hearn D J. 2009. Descriptive anatomy and evolutionary patterns of anatomical diversification in *Adenia*. A Journal of systematic and Evolutionary Botany. 27(10):5-16.

[6] Ayensu A S and Stern W L. 1964. Systematic anatomy and ontogeny of the stem in Passifloraceae. Contr. U.S. Natl. Herb. 34:45-73.
[7] Gamble J S. 1935. Flora of the Presidency of Madras. Vol. I, II & III. Botanical Survey of India, Calcutta, India.
[8] Mathew, K M. 1983. The Flora of Tamilnadu Karnatic. Vol. I. Polypetalae. Pp. 688. Rapinat Herbarium, St. John's College, Tiruchirappalli. India.
[9] Sass J E. 1940. Elements of Botanical Microtechnique. McGraw Hill Book Co. New York. Pp. 222.
[10] Johansen D A. 1940. Plant Microtechnique,. McGraw Hill Book Co. New York. Pp. 523.
[11] O'Brien T P, Feder N and Mc Cull M E. 1964. Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma. 59:364-373.
[12] Esau K. 1964. Plant anatomy. John Wiley and Sons. New York. Pp. 767.
[13] Esau K. 1979. Anatomy of seed plants. John Wiley and Sons. New York. Pp. 550.
[14] Tamilselvi N, Dhamotharan R, Krishnamoorthy P and Shivakumar. 2011. Anatomical studies of *Indigofera aspalathoides* Vahl. (Fabaceae). Journal of Chemical and Pharmaceutical Research. 3(2):738-746.
[15] Selvam A B D. 2013. Standardization of descriptive terminology of starch granules with reference to identification of raw drugs. International Journal of Pharmaceutical Research and Development. 5(7):1-6.
[16] Lersten N R and Horner H T. 2000. Calcium oxalate crystals types and trends in their distribution patterns in leaves of Prunus (Rosaceae: Prunoideae). Plant Syst. Evol. 224:83-96.

INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

# International Journal of Plant, Animal and Environmental Sciences