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Microscopical investigation of three *Datura* species

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Abstract

Background/Objectives: Most of the species of *Datura* imposes a wide lacuna in our knowledge especially for proper identification and pharmacognostical studies. Anatomical structure is most likely to provide evidence concerning the interrelationships of families or in establishing the real affinities of species of uncertain taxonomic status.
Methods/Statistical Analysis: The wood anatomical characters and certain 'anomalous' or uncommon structures of the axis have been extensively employed by the systematic anatomists for drawing conclusion with reference to systematic position and phylogeny of plant taxa. Apart from anatomical and trichome studies, palynological investigation is also to be attempted. This will give additional information both for diagnosis of the plants and for conformation their taxonomic position.
Findings: In *Datura* species epidermal trichomes have been cited as one of the criteria for distinguishing the species. The cross sectional outline of young stem differs significantly in the species studied. The cross section shape is circular and densely pubescent in *Datura innoxia*, angular in *Datura fastuosa*. The secondary xylems of roots are uniformly dense with wide cylinder of libriform fibres and vessels. The vessels of the root are wider than those of the stem. The roots of *Datura* species deviate in their secondary xylem features from the rest of the taxa. These cross sectional configurations are easy access for identification of fragmentary stem materials.
Application/Improvements: An attempt will be made to prepare an artificial dichotomous taxonomic key involving anatomical and palynological data obtained during the present investigations.
Keywords: *Datura fastuosa* L., *Datura innoxia* Miller., *Datura metal* L., Trichomes, Palynology.

1. Introduction

Solanaceae Juss. is a taxon which includes about 90 Genera and 2000 species, most of which are tropical and temperate in distribution [1]. The Solanaceae members merit our attention as well as their economic applications [2]. Oblique zygomorphy of the flower, adnation of the petiole and peduncle, and intra xylary phloem are some of the unique features which readily distinguish Solanaceae from its related taxa. Several members of Solanaceae are known as food plants (*Solanum tuberosum*, *S. melangena*, *Lycopersicon*, *Physalis* and *Capsicum* etc.); some are ornamentals (*Petunia*, *Cestrum*, *Brunfelsia*, *Datura* etc.), several members contain alkaloids that produce disorders of the brain and marked excitation when smoked or eaten. Several species of *Datura* are used for their narcotic hypnotic properties. There are several other weed taxa of this order, which are equally important, especially in the Indian System of Medicine. There seems to be a wide lacuna in our knowledge of pharmacognosy and phytochemistry of these and many other drug yielding plants of Solanaceae. There has been a perennial controversy with regard to certain species of *Datura*. The floristic botanists, pharmacognostic scientists and taxonomists have contributed to their mite towards creating more controversies coupled with confusion while circumscribing the taxonomic status of *D. fastuosa* (Figure 1), *D. innoxia* and *D. Metal*. Most of the descriptions of these taxa have been at 'alpha level' which may not obviously help to resolve the controversy. It is believed that most reliable taxonomic conclusions result from the synthesis of pertinent information drawn from many related disciplines such as cytology, anatomy and embryology etc. Anatomical structure is most likely to provide evidence concerning the interrelationships of families, or in establishing the real affinities of genera of uncertain taxonomic status. Literature pertaining to the taxonomic aspect of Solanaceae seems to be voluminous. This is partly because of the economic importance of many taxa included in this family. However, anatomy of various vegetative organs of the Solanaceae members has not been studied

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in detail barring certain genera of medicinal value, such as *Atropa*, *Withania*, *Nicotiana* and *Datura*. These plants have been described by the pharmacognosists [3] and by economic botanists [4]. The majority of the review of literature gives the most important clues relevant to the present study [5,6]. It may be realised that only those taxa which were very popular in pharmaceutical field have been given due attention with the respect to botanical and phytochemical analyses. Yet there are several Solanaceae plants which are of equal values in medicinal properties. Such plants have not been studied adequately both by herbal scientists as well as phytochemists. This fact prompted us to take taxonomical and anatomical studies of some common weeds of Solanaceae which are of high medicinal values. The present study combines both systematic anatomy and pharmacognosy of a few selected taxa. Apart from anatomical studies palynological investigation is also to be attempted. This will give additional information both for diagnosis of the plants and for conformation their taxonomic position.

2. Materials and Methods

Three species belonging to one genera of Solanaceae were selected for the present study. Table 1 provides the details of taxa studied and other aspects of the species. To begin with, taxonomic studies of all three taxa were undertaken. Plant specimens were collected from different localities within Chennai and its neighbourhood. Data pertaining to both vegetative and floral characters were obtained from fresh specimens. The specimens were also compared with the herbarium materials preserved in the Presidency College, Chennai. Taxonomic descriptions were followed as per the method of standard books [7,8]. The materials were collected from the natural habitats wherever possible. Small portions of properly trimmed leaf, petiole, internode (mostly thin) and root (taproot or lateral root) were cut with the help of sharp blade directly in the field and fixed in FAA (Formalin, Acetic Acid and Alcohol). For epidermal peeling of upper and lower sides of the leaf, pulling with forceps was found to be satisfactory. The epidermal peelings so removed from the leaf were immediately soaked in a stain on the slide, rinsed after sometimes and mounted in a drop of glycerin for microscopic observations. Partial maceration by employing Jeffrey's fluid was also followed wherever hand-pulling did not work out. For leaf, petiole and stem paraffin embedded rotary microtome method of sectioning was followed. The materials fixed in FAA were washed in water and dehydrated, cleared wax infiltrated by passing through graded series of ethyl alcohol and xylene [9]. Leaf and petiole sections were cut at 8-10 µm thickness, stem sections cut at 10-12 µm thickness [10-12]. Microtome sections were stained with tannic acid ferric chloride and safranin [13] or Toluidine blue [14]. Epidermal peelings were stained with either Toluidine blue or safranin. Pollen grains were collected from unopened flowers and observed directly after staining with crystal violet. The pollen grains were also acetalysed and studied. Measurements of stomata were based on 25 readings and range with mean values was given. Other readings such as stomatal frequency pollen diameter etc were also computed on 25 readings. Photomicrographs were taken with NIKON-Lab Phot – II Microscope. Identification of plants was done with the help of different Floras guides [5, 7, 15].

Table 1. List of plants studied

S. No.	Name of thep	Month & Year of collection	Place of collection	Period of flowering
1.	*Datura fastuosa* L.	October 2017	Pattabiram	August to November
2.	*Datura innoxia* Miller.	August 2017	HVF Estate, Avadi	August to November
3.	*Datura metal L.*	September 2017	West Tambaram	August to November

3. Observations

(i) *Datura fastuosa* L. (Figure 1) - A large annual plant often with zigzag stem to 1 m. height; Leaves: Ovate, acute, margin frequently lobed, base unequally truncate, 15 x 11 cm, petiole 9.5 cm; Inflorescence: Axillary cyme; Flowers: Bisexual, complete, pentamerous, actinomorphic, hypogynous; Calyx : Sepals 5, gamosepalous, valvate, 7 cm long; Corolla: Petals 5, gamopetalous, valvate, 5 toothed, often double or tirple, purple outside and white within, 13 cm long; Androecium: Stamens 5, epipetalous, alternipetalous, filament flat, adherent with the petal to the total length; filament 10 cm long, anthers 1.5 cm long; Gynoecium: Bicarpellary, syncarpous, bilocular, superior ovary, ovules many attached to the axile placentation; Fruit: Capsule, small, 2 cm across, short stalked, strait, spines short, stout, 0.2 cm, blunt; Seeds: Many, circular.

ISSN (online): 2320-9836
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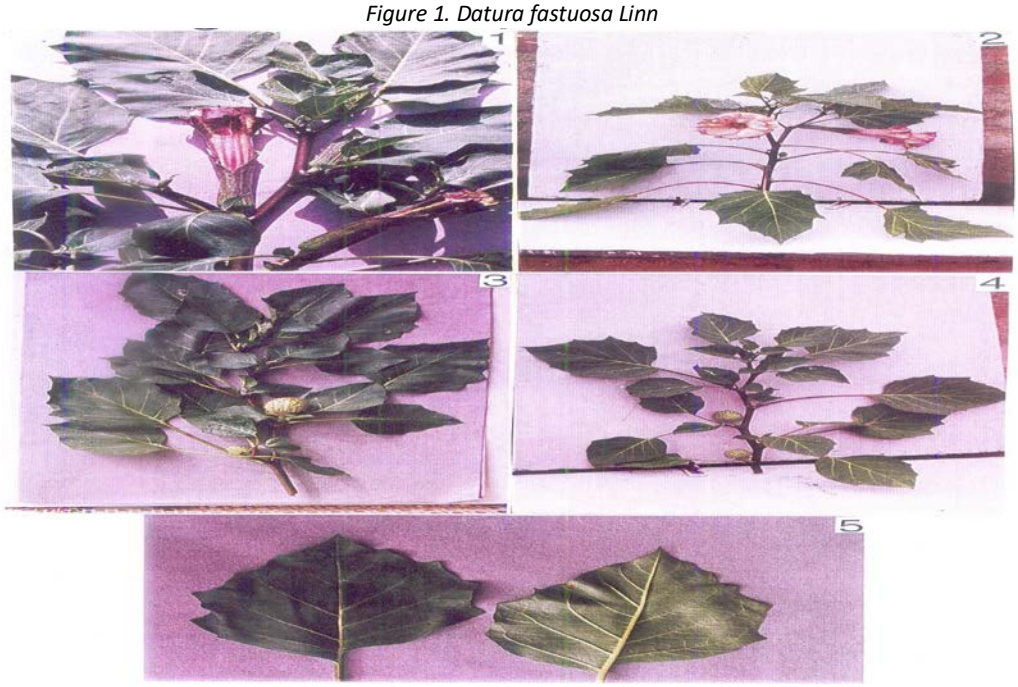
Figure 1. Datura fastuosa Linn



1-4: Flowering and Fruiting shoots; 5-Leaf abaxial and adaxial sides

Anatomy (Figure 2) Leaf: Lower epidermis stomatiferous, stomatal type anomocytic; stomatal frequency 50/mm^2; stomatal size 24-36 (30) x 24-28 (26) µm. Epidermal cells lobed and irregular in outline; Radial walls wavy and thin. Epidermal trichomes frequent, Trichomes 2-4 celled, uniseriate, unbranched, straight, pointed; surface geniculate, trichome 160-260 (174) µm long. Upper side: Stomatiferous; stomata shrunken with closed poses and seem to be non functional. Stomatal type mostly anisocytic with 3 unequal subsidiary cells. Frequency 30-40 (35) / mm^2. Stomatal size 32-36 (35) µm x 14-26 (18) µm. Epidermal cells slightly lobed with undulate radial walls. Trichomes less in abundance, 2-3 celled, straight, unbranched, non-glandular pointed, surface crenellate; length 130-200 (140) µm. Petiole: Diameter of the specimen studied; 2.6 mm; cross-sectional shape-circular; surface even; adaxial depression not evident; glabrous all around except a narrow zone of the adaxial surface, where short stalked, multicellular glandular trichomes are seen; Epidermis: Unistratose, epidermal cells cubical, outer tangential walls broadly papillate; cuticle thin. A layer of larger hypodermal cells filled with purple pigments is present; outer ground tissue differentiated into 5 or 6 layers of collenchyma cells, and rest of the ground tissue parenchymatous, thin walled, circular and compact. Vascular system consists of a C-shaped strand with adaxial opening; xylem elements angular, thick walled, and occur in radial rows; the vascular strands in the adaxial side are smaller than the rest of the strands. Phloem occurs on both outer and inner sides of the xylem masses. Stem: Diameter of the specimen 8 mm; cross sectional outline somewhat angular, even and glabrous. Epidermis unistratose, narrow and tabular in shape. Cortex wide, heterogeneous outer zone collenchymatous, 350 µm wide; inner zone, large, circular less compact parenchymatous, 350 µm wide, sand crystals frequent in the parenchyma zone. Secondary phloem 100 µm wide, cells randomly oriented; phloem sclerenchyma elements absent. Secondary xylem thin hollow cylinder of about 600 µm thickness including primary xylem; vessel elements wide, thin walled, angular, up to 100 µm in diameter; ground tissue of the xylem thin walled sclerenchyma cells. Medullary phloem larger masses phloem elements occur around the periphery of the pith. Small nests of pith-fibres are found amidst the medullary phloem strands. Root: Specimen examined is tap root of about 1 cm thick. Epidermis is collapsed, no periderm is evident; Cortex is homogeneous, 100 µm wide, cells tangentially oblong, contain sand crystals. Secondary phloem zone narrow and cells random in distribution. Secondary xylem wide and prominently occupying the entire volume of the root. The xylem is unusual in that there are isolated clusters of vessels ensheathed by xylem fibres which are embedded in parenchymatous ground tissue. Vessel elements angular, thin walled, up to 100 µm in diameter.

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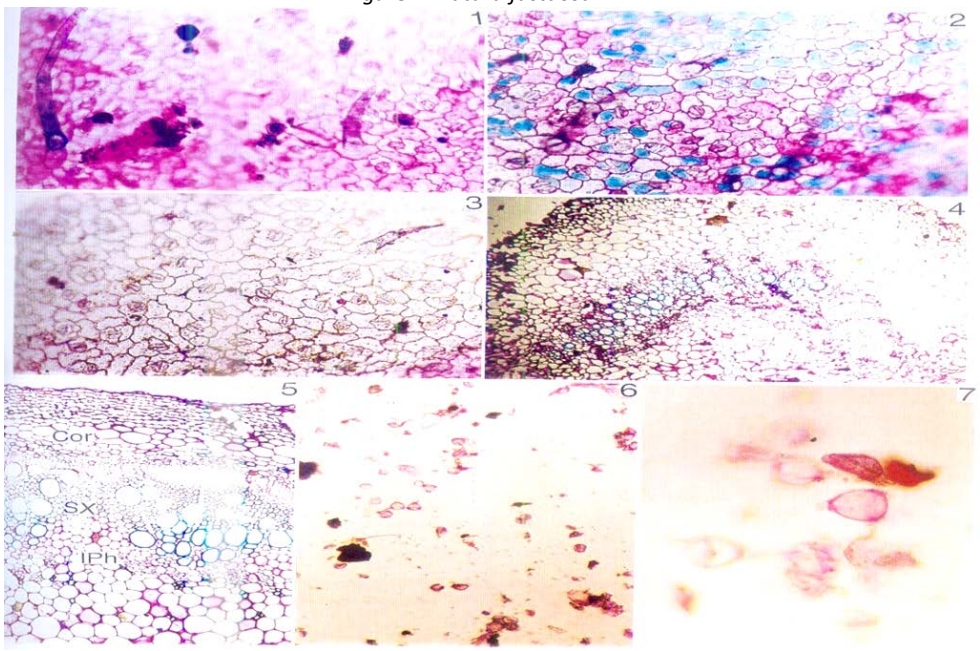
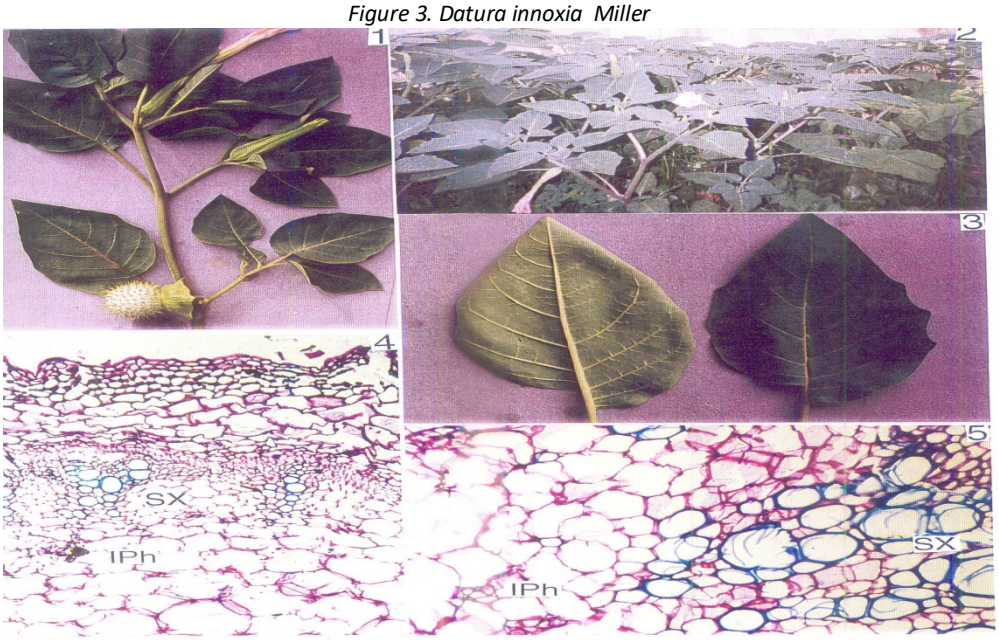
Figure 2. Datura fastuosaLinn



1, 2 : Epidermal trichomes and stomata (Adaxial sides); 3-Abaxial stomata; 4-Petiolar vasculature 5-Stem T.S.; 6, 7-Pollen grains (Cor – Cortex; IPh – Inner Pholoem; SX – Secondary Xylem)

(ii) *Datura innoxia* Miller (Figure 3) A herb to 60 cm tall, stem covered with soft hairs; Leaves: Elliptic ovate, 9-16 x 6 -12 cm, soft pubescent on either side, base usually truncate or rounded, margin almost entire, rarely shallowly lobed, petiole 10 cm long; Inflorescence: Solitary cyme; Flowers: Axillary, white, bisexual, pentamerous, hypogynous, actinomorphic, pedicel 1.5 cm long; Calyx: Sepals 5, gamosepalous tubular, herbaceous, upper part deciduous, lower part persistent; Corolla: Petals 5, gamopetalous, white funnel shaped, ten toothed, 10 -12 cm long; Androecium: Stamens 5, epipetalous, filaments filiform, 7 cm long, anthers oblong, 1 cm long; Gynoecium: Bicarpellary, syncarpous, bilocular, hypogynous ovary, 0.5 cm across, ovules many on bifurcate placentation, style elongate, 13 cm long, stigma 2 lobed; Fruit : Capsule, ovoid, 3.5 cm across, spines long, weak, sharp, to 1 cm long; Seeds : Many, circular.

Figure 3. Datura innoxia Miller



1,2 Flowering and Fruiting shoots; 3 Leaf abaxial and adaxial sides; 4, 5 Stem T.S. (IPh : Inner Phloem; SX Secondary Xylem)

ISSN (online): 2320-9836
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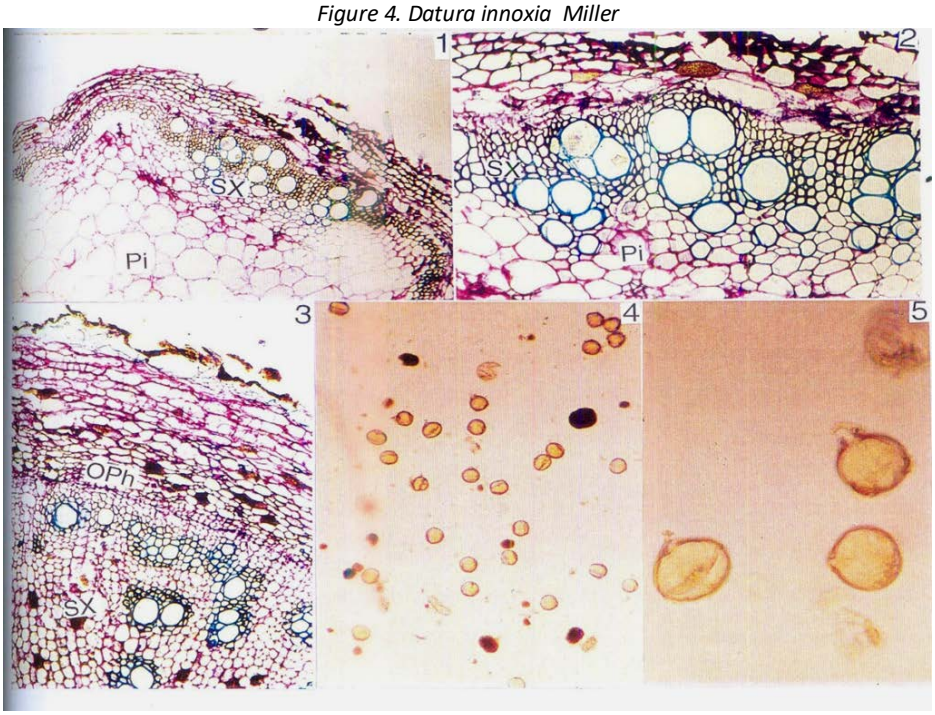
Anatomy (Figure 3 & 4) Leaf: Lower Epidermis stomatiferous, stomatal type anisocytic with three unequal subsidiaries; stomatal frequency 12 -17 / mm^2, size of the ground cells 30-45 x 30 µm; Epidermal cells irregular and lobed, tangential walls wavy and thin; Epidermal trichomes abundant, especially along the costae; the trichomes are attached to the one-four epidermals which form a circle around the basal cell of the trichome. Upper epidermis stomatiferous, stomatal type anisocytic with three unequal subsidiaries encircling a stoma; stomatal frequency 24-28 / mm^2, size of the guard cells 37.5-40 x 17.5-22.5 µm; Epidermal cells polygonal; tangential walls of the epidermal cells straight or slightly wavy trichomes present, but less frequent. Trichomes: Glandular, 4-6 celled, uniseriate, unbranched, cells narrowly oblong, terminal cells spherical and densely cytoplasmic; long 260-400 µm; pointed cells less frequent. Petiole: Circular in cross sectional outline with a shallow depression on the adaxial side; surface even; densely pubescent, trichomes multicellular, uniseriate, unbranched, straight, terminating its spherical glandular cell or nonglandular pointed tip. Epidermis: Unistratose, cubical in CS view; cuticle fairly thick; cortical ground tissue differentiated into outer zone of 5-7 layers of angular collenchymas and inner zone of larger, circular, thin walled, 6-7 layers of parenchyma; Vascular Tissues occurs in the form a ring, open by a narrow gap below the adaxial notch; xylem elements are in discrete and widely separated radial files with the phloem elements disposed past of each xylem strand. Xylem elements, central part wide, parenchymatous, sometimes a few central cells lysed to form a cavity. Stem: Circular and even in CS view. Surface densely pubescent clothed with multicellular, uniseriate, unbranched hairs. Hairs 2-6 celled, thin walled with spinulose surface. Epidermis:unistratose, cubical to barrel shaped; cuticle thin. Cortex: heterogeneous with outer zone of angular type of collenchymatous cells of 5-7 layers and inner zone of 3-8 layers of circular, thin walled, less compact parenchyma cells. Pith: wide, thin walled, compact, parenchymatous. Vascular System: A continuous cylinder vascular cambium has been formed functionally in usual bidirectional plane. There are isolated portions xylem elements arranged in radial files, the intervening spaces being occupied by immature secondary xylem tissue. The outer secondary (normal) phloem occurs in continuous cylinder; inner medullary phloem occurs in small isolated nests. Sclerenchyma elements are totally absent in the cortex or pith. Sand crystals are very frequent in the cortex and pith. Root: Maturetap root, measuring 0.5 mm was examined. The epidermis is replaced by a thin, less conspicuous periderm.

Figure 4. Datura innoxia Miller



1, 2 Stem T.S.; 3 Root T.S. (Anomalous Feature); 4, 5 Pollen grains (Pi-Pith; Oph-Outer Phloem; SX Secondary Xylem)

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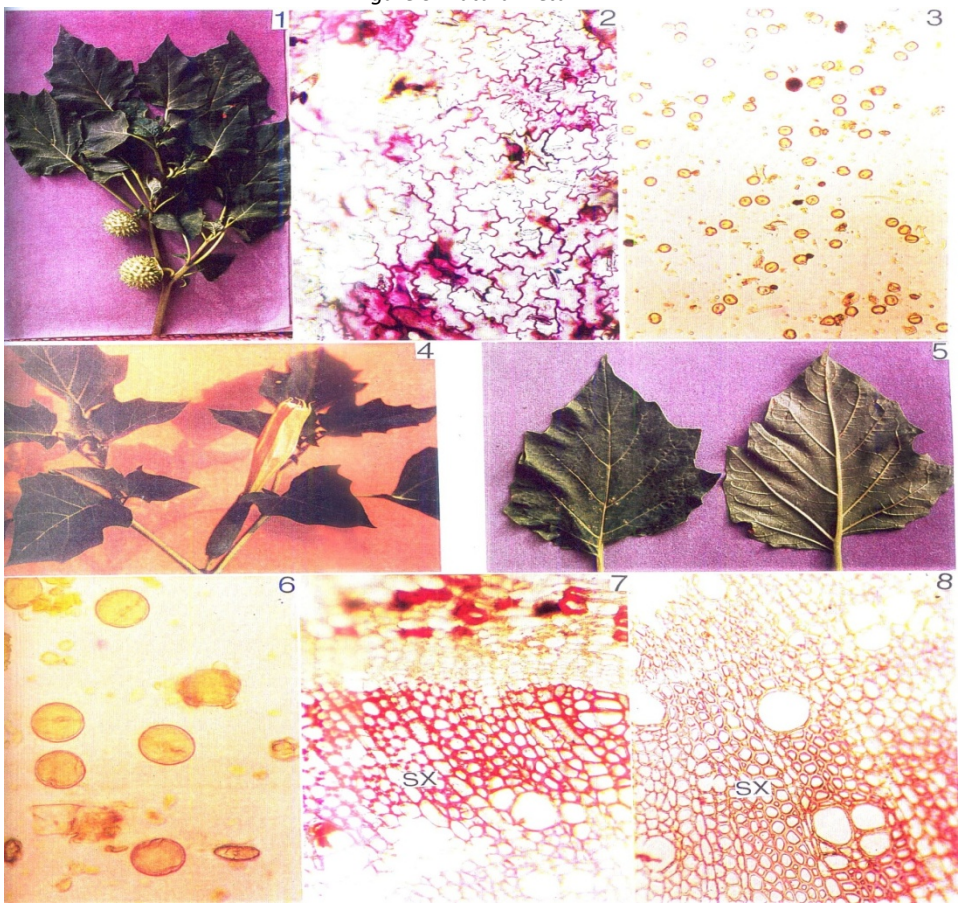
A narrow zone of cortex, made up of tangentially of long parenchyma cells. The central core of the rootis occupied by a dense mass of wide vessels and sclerenchyma elements. Outer to the central part and major part of root is formed by thin walled, undignified parenchymatous ground tissue within which are embedded patches of vessel groups ensheathed by fibres. Because of the predominance of parenchyma and reduced vessel clusters, the secondary xylem appears to be storage in function. Secondary phloem occurs as continuous sheath around the xylem cylinder sand-clusters are very frequent in the cortex starch grains are common in the xylem parenchyma.

(iii) *Datura metal* L. (Figure 5 & 6) Erect, rounded, soft-stemmed herb to 60 cm tall, branches somewhat zigzag, almost glabrous; Leaves: Elliptic to broadly ovate, 14-18 x 9-15 cm, base unequally truncate, margin frequently lobed, petiole 10 cm long; Inflorescence: Solitary cyme; Flowers: Axillary, bisexual, pentamerous, hypogynous, actinomorphic, pedicel 1.5 cm long; Calyx: Sepals 5, gamosepalous, tubular, herbaceous, usually divided at apex; Corolla: Petals 5, gamopetalous, white or creamy white, funnel shaped, 5 toothed, 15 cm long; Androecium: Stamens 5, epipetalous, filaments filiform, 11 cm long, anthers oblong, 1 cm long; Gynoecium: Bicarpellary, syncarpous, bilocular, hypogynous ovary, 0.5 cm across, ovules many on bifurcate placentation, style elongate. 11 cm long, stigma 2 lobed; Fruit: Capsule, ovoid, 3 cm across, spines stout and sharp at tip, to 0.5 cm long; Seeds: Many, circular, brown, 5 mm diameter.

Figure 5. Datura metal L.



1-Fruiting branch; 2. Abaxial stomata; 3, 6-Pollen grains; 4-Flowering branch; 5-Leaf Adaxial and abaxial view; 7, 8 Root Secondary Xylem(SX-Secondary Xylem)

Anatomy (Figure 5 & 6) Leaf: *Lower epidermis:* stomatiferous, stomal type anomocytic, stomatal frequency 40-60 (49) / mm^2. Stomatal size 28-34 (31) µm x 24-30 (26) µm. Epidermal cells polygonal, radial walls less situate, thin stomatal pores open; stomata functional. *Upper epidermis:* stomatiferous, stomata with shrunken guard cells and closed pores, non-functional. Stomatal type anomocytic. Stomatal frequency 20-30 (26) / mm^2. Stomatal size 30-34 (31) µm x 12 x 18 (14) µm. Epidermal cells polygonal to oblong; radial walls straight and thick. Petiole: Diameter 3.6 mm; circular, even, glabrous, adaxial groove not pronounced. Epidermis: Unistratose, epidermal cells radially oblong, cuticle fairly thick. Epidermis in followed by a sub-epidermal layer

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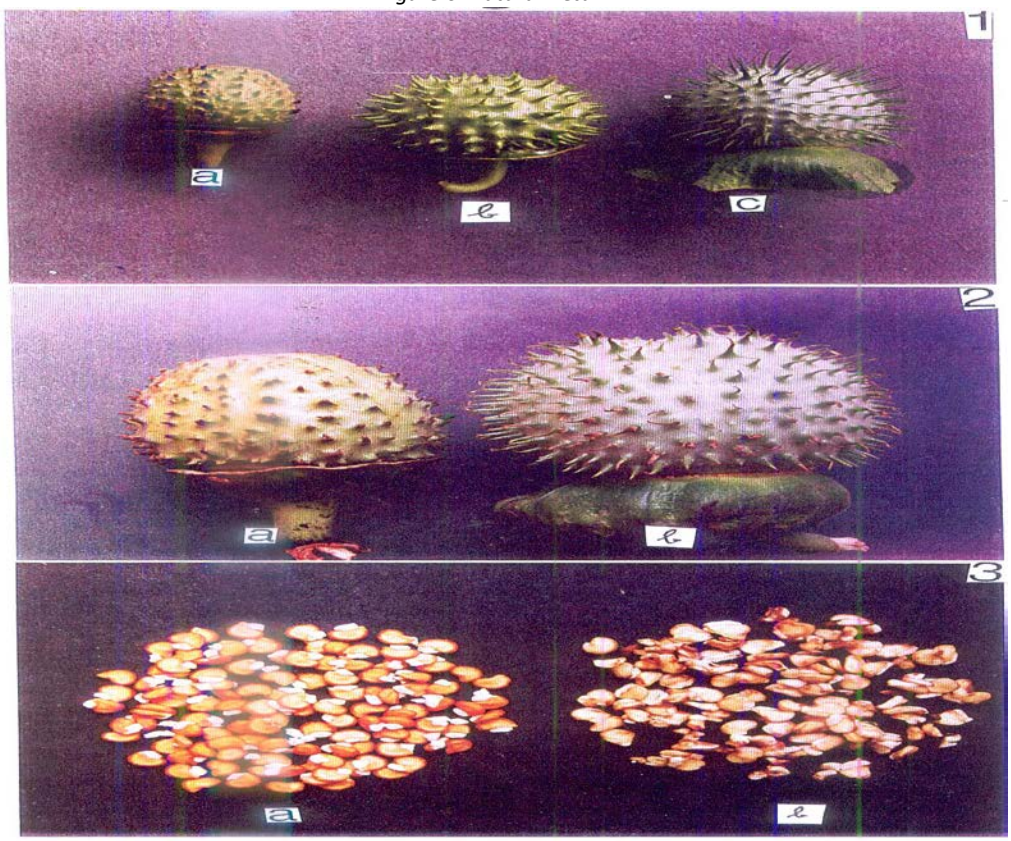
of fairly large, cubical and circular thin walled parenchyma cells; Ground tissue: differentiated into outer zone of 6 or 7 layers of angular collenchyma cells followed by inner zone of 6-10 layers of thin walled, compact, polygonal parenchyma cells. Central part is also parenchymatous with a narrow lysigenous canal. Vascular system consists of discrete radial rows of xylem elements with small nests of inner and outer phloem elements; towards the adaxial side are about 6 small, independent accessory strands an either side of a narrow median gap. Xylem elements are thick walled. Stem: Diameter of the specimen examined 8 mm cross sectional view circular, even, sparsely hairy or glabrous. Epidermis unistratose, epidermal cells oblong or squarish, cuticle fairly thick. Cortex heterogeneous, outer broad zone of collenchyma cells, inner zone of narrow of 2-4 layers of large circular parenchyma cells, delimited by widely separated small groups fibres.

Secondary phloem district and broad, cells occur in regular radial rows. Secondary xylem forms a hollow cylinder of uniform radial thickness. Thick walled angular vessel elements are organized in radial multiples; xylem fibres are thick walled and occur in radial rows; Rays are thin and narrow and run parallel to the xylem elements. Medullary phloem forms large circular strands just inner to the xylem grinder. Inner to the medullary phloem strands are seen prominent patches of thick walled pith-fibres all around the pith. Pith portion is wide, parenchymatous, circular and contain sand-crystals. Root: Both lateral and tap roots exhibit well developed secondary growth. There is a broad soft periderm followed by a narrow zone of cortex. Secondary phloem is broad and possesses phloem elements and thick walled sclereids. The sclereids occur in tangential lines in regular succession. The secondary xylem has dense thick walled libriform fibres and circular wide vessels. During later of secondary growth small islands of parenchyma are seen embedded in the xylem so that the xylem becomes less dense. The fibres are also thin walled in the periphery of the xylem cylinder.

Figure 6. Datura metal L.



Fruits and Seeds of Datura 1.a Datura fastuosa; b. Datura metal; c. Datura innoxia 2.a Datura fastuosa; b. Datura innoxia, 3.a Datura innoxia; b. Datura fastuosa

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4. Discussion

Solanaceae exhibits such unique features as obliquely zygomorphic ovary, bicollateral vascular strands, swollen placental tissue etc. Albeit the uniformity of certain traits, the homogeneity of the family has often being questioned by certain taxonomists. Solanaceae is said to be nearly related to Scrophulariaceae in general. Certain genera of Solanceae seems to be related to Boraginaceae, Gesneriaceae, Nolanaceae etc [16]. Solereder records strands of fibres at the margin of the pith or in contact with the primary xylem in many families. However, Solereder's list does not include solanaceae. In all species of Solanaceae studied at present the pith fibres are seen in small nests around the pith inner to the medullary phloem. So, Solanaceae can be added to Solereder's list of families in which pith fibres are regularly present [17]. Controversies over *Datura* species of different species of *Datura*, it was pointed out elsewhere that *D. innoxia*, *D. metal* and *D. fastuosa* are highly in controversy. *D. innoxia* is named as *D. metal* by Mayuranathan 1929, while Mathew 1981 identifies it as *D. innoxia*, *D. metal* being synonymous. *D. metal* as recognized by is also accepted by Mathew 1981, who treats *D. alba* as synonymous. However, Mayuranathan 1929, treats it as a variety of *D. fastuosa*. *D. fastuosa* L. is treated as *D. metal* by while Mathew 1981, treats it as *D. metal* var. *fastuosa*; Mayuranathan 1929, identified it as *D. fastuosa* L on the basis of our present studies on both taxonomic anatomical, palynological characters, we recommend the following for solving the controversies:

1. Densely tomentose on all parts; fruit bearing long soft spines; stomatal frequency less than 15 on the lower surface; stomatal type anisocytic on both sides; petiole with adaxial groove; Root xylem with isolated nests of vessels embedded in parenchyma-*Datura innoxia*.
2. Plant parts glabrescent; fruit with short, strong spines; stomatal frequency more than 40 on the lower side; stomata anomocytic on both sides; Petiole without adaxial groove; Root xylem dense-*D. Metal*.
3. Plant parts glabrescent; fruits with tubercle; stomatal frequency 50 on the lower side; stomata anomocytic on the lower side; Petiole circular; Root xylem with isolated masses of vessels in the parenchymatous ground tissue; Flower violet-*D. fastuosa*.

4.1. Key on the basis of taxonomy

1. Fruit dehiscent; corolla trumpet shaped anthers away from the style; fruit armed, calyx base persistent
2. Branchlet glabrescent; capsule armed with tubercles or short stout spines
3. Branchlets pubescent; capsule armed with long, weak, sharp spines *Datura innoxia*
4. Capsules with tubercles; corolla purple; often double or triple; stem and petiole dark violet *Datura fastuosa*
5. Capsule with short, stoup spines; corolla white; single; stem & Petiole green *Datura metal*

4.2. Dichotomous key based on anatomy

1. Petiole circular without wings
2. Root with anomalous type of xylem *Datura fastuosa*
3. Root with normal secondary growth
4. Stem even in CS view *Datura metal*
5. Stem circular in CS view
6. Epidermal trichomes uniseriate, simple and straight
7. Root with "anomalous" xylem *Datura innoxia*

4.3. Key on the basis of palynological data

1. Pollen grains more than 50 µm in diameter
2. Pollen monocolpate, exine finely granular *Datura metal*
3. Pollen more than one colpate; exine with lamellate thickening *Datura innoxia*
4. Monocolpate, colpa triradiate
5. Pollen 30µm; elliptical in polar view *Datura fastuosa*

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In the present study certain interesting aspects have come into light which will be discussed in connection with the before said viewpoints. In *Datura* species epidermal trichomes have been cited as one of the criteria for distinguishing the species. The cross sectional outline of young stem differs significantly in the species studied. The cross section shape is circular and densely pubescent in *Datura innoxia*, angular in *Datura fastuosa*. The secondary xylems of roots are uniformly dense with wide cylinder of libriform fibres and vessels. The vessels of the root are wider than those of the stem. The roots of *Datura* species deviates in their secondary xylem features from the rest of the taxa. These cross sectional configurations are easy access for identification of fragmentary stem materials. The anatomical features used for the key are easy to obtain and will aid for identification of the species of the specimens do not have flower. The palynological investigation of the *Datura* species is more valuable clue to identify some unsolved problems in controversy.

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