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Anatomy of two species of *Plumbago* : a traditional medicinal plant and its relevance for taxonomy

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ABSTRACT

Plumbago is a traditional medicinal plant in Ayurveda. The paper presents anatomical study of leaf, petiole, stem and root of two species of *Plumbago* namely *P. zeylanica, P. auriculata* and, its relevance in discrimination of these two species.Anatomical features of leaf which are of diagnostic value in delimitation of both taxa are outline of T. S.,shape and size of epidermal cells, presence of sclerenchyma surrounding the vascular bundles, number of tannins cells. Characters of taxonomic significance in petiole anatomy are outline of T. S, presence of trichomes, shape and size of epidermal cells, abundance of collenchyma, arrangement and number of vascular bundles, presence of sclerenchyma surrounding vascular bundles, number of tannin cells. The diagnostically useful anatomical features of stem to discriminate both taxa of *Plumbago* are degree of elevation of stem ridges, occurrence of double layered epidermis, size of epidermal cells, distinctness of endodermis, abundance and distribution of pericyclicsclerenchyama, number of vascular bundles. Anatomical features of taxonomic significance in root are width of cortex and abundance of starch grains in cortex cells, abundance and distribution of pericyclics clerenchyama, amount of vascularization, distribution, diameter and density of vessels, width of medullary ray.

Figures : 04 References : 15 Tables : 04
KEY WORDS : Anatomy,*P. auriculata, P. zeylanica,* Taxonomy

Introduction

अमंत्रमक्षरं नास्ति नास्तिमूलमनौषधीः।
अयोग्यपुरुषोनास्ति योजकस्तत्र दुर्लभः।।

"Every letter has the potential to become Mantra. Every plant (root) has the potential to become a medicine. Every person is competent but '*Yojaka*' (*Dristaa*), a visionary,a seer, a planner is very rare[12].

The above mentioned Sanskrit verse is cited in our ancient Indian spiritual literature. This verse depicts the significant role of plants in maintaining human health since antiquity. Since time immemorial, medicinal plants are well known to our country through Ayurveda. Ayurveda is the ancient Indian system of healthcare and longevity[7].

Plumbago is important medicinal plant in Ayurveda. It belongs to family Plumbaginaceae. It is called as'leadwort' and in Sanskrit it is called 'Chitrak'.The word *Plumbago* is derived from Latin word *Plumbum*(=lead) referring to its use as remedy for lead palsy or the power of plant sap to make lead coloured stains on skin. So the plant is also called'leadwort'[4].

There are variations in opinions about types of chitrak in Ayurveda. Three types of chitrak : black, white and red are mentioned in the following verse of Yogaratna samuccayam[4].

त्रिविधः सतु विज्ञेयः कृष्णः श्वेतोऽथ रक्तकः।

Following verse of *Vagbhata's Astangahrdayam* cited three types of chitrak *viz.* yellow flowered, white flowered and black flowered –more effective in successive order.They act as rejuvenat or when used in proper procedure[4].

यथास्वं चित्रकः पुष्पैर्ज्ञेयः पीतसितासितैः।।
यथोत्तरं स गुणवान् विधिना च रसायनम्।।62।।

Thus based on colour of flowers, four types of chitrak are mentioned in Ayurveda-White (*Sveta)*, Yellow (*Pita)*, Red (Rakta) and Black (*Krishna*). But actually yellow and red types are synonymous and blue chitrak and black chitrak are same.Thus three types of chitrak in ayurveda are- White (*Plumbagozeylanica* Linn), Red

ACKNOWLEDGEMENT : The author is grateful to Dr. G. S. Chuadhari (Retd.), Reader, P. G. Department of Botany, M. J. College, Jalgaon for his guidance during my research work.

TABLE-1: Comparative account of anatomical features of mid rib region of leaf lamina of two species of *Plumbago*.

<table>
<tr><th>Sr. No.</th><th>Anatomical Feature</th><th>Plumbago zeylanica</th><th>Plumbago auriculata</th></tr>
<tr><td>1</td><td>Outline of mid rib region</td><td>Conical on both sides but upper (adaxial) surface is more bluntly conical as compared to lower (abaxial) surface</td><td>Bluntly conical on upper (adaxial) surface and convex on lower (abaxial) surface</td></tr>
<tr><td>2</td><td>Upper Epidermis</td><td>1) Single layered
2) Covered by thick cuticle
3) Cells of Upper epidermis are -Oval
- Not alike but vary considerably in size
4) Dimensions of cells of upper epidermis
Height(µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>14.867</td><td>30.330</td><td>21.295 ± 4.742</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>15.587</td><td>42.797</td><td>27.379 ± 8.818</td></tr></table></td><td>1) Single layered
2) Covered by thick cuticle
3) Cells of Upper epidermis are -Rectangular
- Not alike but vary considerably in size
4) Dimensions of cells upper epidermis
Height(µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>4.460</td><td>22.148</td><td>14.516 ± 3.731</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>3.154</td><td>34.982</td><td>14.291 ± 7.607</td></tr></table></td></tr>
<tr><td>3</td><td>Hypodermis</td><td>1) Collenchymatous
2) Present below upper epidermis and above lower epidemis
3) Multilayered (6-12 layered),adaxial (upper)hypodermis is more layered as compared to abaxial (lower) hypodermis</td><td>1) Collenchymatous
2) Present below upper epidermis and above lower epidermis
3) Multilayered (4-12 layered) , adaxial (upper) hypodermis is more layered as compared to abaxial (lower) hypodermis</td></tr>
<tr><td>4</td><td>Vascular Bundles</td><td>1) Vary in their number , size , arrangement throughout the length of leaf
2) Number varies from 2 to 7
3) Sclerenchyma found on both the sides of vascular bundles as bundle caps</td><td>1) Vary in their number , size , arrangement throughout the length of leaf
2) Number varies from 2 to 8
3) No Sclerenchyma found</td></tr>
<tr><td>5</td><td>Lower Epidermis</td><td>1) Single layered, covered by thick cuticle
2) Cells of lower epidermis are
- Oval
- Smaller than cells of upper epidermis
- Not alike but vary
3) Dimensions of cells of lower epidermis</td><td>1) Single layered, covered by thick cuticle
2) Cells of lower epidermis are -Rectangular
-Smaller than cells of upper epidermis
-Not alike but vary
3) Dimensions of cells of lower epidermis</td></tr>
</table>

<table>
<tr><td></td><td></td><td>Height (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean ± S.D.</th></tr><tr><td>8.619</td><td>21.448</td><td>14.557 ± 4.490</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>7.702</td><td>28.748</td><td>17.176 ± 5.538</td></tr></table></td><td>Height (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean ± S.D.</th></tr><tr><td>7.372</td><td>16.669</td><td>11.317 ± 2.667</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>5.292</td><td>15.560</td><td>11.045 ± 3.341</td></tr></table></td></tr>
<tr><td>6</td><td>Tannin cells</td><td>Comparatively less in number</td><td>Comparatively more in number</td></tr>
</table>

(Note- Min. means Minimum, Max. means maximum, S. D. Means Standard Deviation)

(*Plumbagorosea*), Blue (*Plumbagocapensis*). These three types were recorded from India[4].

P. zeylanica is wild multivalent medicinal plant while *P. auriculata* is ornamental cultivated plant with least medicinal potential. The present investigation was undertaken to discriminate both species correctly on the basis of anatomy

Materials and Methods

Transverse sections of leaf, petiole stem and root were taken manually. Then sections were double stained with safranin and light green[2,8].Histochemical tests were conducted for identification of starch and plumbagin in sections. Starch gives blue colour with iodine[15]. Plumbagin gives pink colour with 5% potassium hydroxide[10].

Photo micrographs and measurements were taken using LM 52-1712 Digiscope (LCD Digital Microscope) of Lawrence and Mayo.Measurements of cells were taken at the widest points. Mean values of 20 observations with standard deviation were taken for consideration. To explain mid rib region of leaf terminology[5,14] is used. Terminology used to explain petiole anatomy follows[1]. Terminology used to explain stem is in accordance[6]. To explain root, terminology[3,9,13] are used.

Results and Discussion

T.S of Mid rib region of leaf

Structural organization of lamina of leaf of both the species of *Plumbago* is similar to that of typical dicotyledons. Lamina is bifacial / dorsiventral. Mid rib region of leaf lamina shows upper and lower epidermis, collenchymatous hypodermis, central parenchyma and vascular bundles. Collateral vascular bundles are surrounded by bundle sheath. Xylem faces upper epidermis and phloem faces lower epidermis. Tannin cells are also found (Fig. 1). The comparative anatomy of mid rib region of leaf of both taxa is explained in detail in Table-1.

The present observations of internal structure of leaf conform totally to report[11]. The general structural plan of mid rib region of leaf in both species is same with some diagnostic differences. In *P. zeylanica* outline of mid rib region is conical on both sides but upper surface more bluntly conical; epidermal cells oval and larger (21.295 ± 4.742 × 27.379 ± 8.818 µm in upper epidermis and 14.557 ± 4.490 µm × 17.176 ± 5.638 µm in lower epidermis); vascular bundles with sclerenchyma on both sides; less number of tannin cells while in *P. auriculata* outline of mid rib region is bluntly conical on upper surface and convex on lower surface; epidermal cells rectangular and smaller (14.516 ± 3.731 µm × 14.291 ± 7.607 µm in upper epidermis and 11.317 ± 2.667 × 11.045 ± 3.341 µm in lower epidermis); vascular bundles without surrounding sclerenchyma, more number of tannin cells.

T.S of petiole

In T.S. petiole appears sulcate and differentiated into epidermis, hypodermis, ground tissue, vascular bundles (Fig. 2). The comparative anatomy of petiole of both taxa is explained in detail in Table-2.

The general structure of petiole is same in both taxa. Some differences of diagnostic importance are observed in petiolar anatomy of both taxa. In *P. zeylanica* petiole the outline more broadly sulcate with acute margin and distinct ridges; trichomes absent; epidermal cells oval, larger (22.698 ± 11.956 µm x 27.861 ±11.908 µm); collenchymatous hypodermis comparatively more massive; vascular bundles arranged in 2 semicircles, more in number (9 to 14), sclerenchyma patches surrounding vascular bundles may occur; tannin cells comparatively less in number while in *P. auriculata* petiole the outline broadly sulcate with erect acute margin and indistinct ridges; trichomes present on the middle portion of epidermis on the upper side; epidermal cells rectangular or some time oval, smaller (12.272 ± 4.397

TABLE-2 : Comparative account of anatomical features of petiole of two species of *Plumbago*

<table>
<tr><th>Sr. No</th><th>Anatomical Feature</th><th>Plumbago zeylanica</th><th>Plumbago auriculata</th></tr>
<tr><td>1</td><td>Outline</td><td>1) More broadly sulcate with acute margin
2) Crescent shaped with distinct ridges
3) Presence of one ridge in the center of adaxial side and many ridges on the abaxial side (Ridges are more distinct in mature leaf petiole as compared to young leaf petiole)</td><td>1) Broadly sulcate with erect acute margin
2) Roughly pentagonal with two erect horn like margin on the adaxial side
3) Presence of ridges (but these are not distinct as compared to that of P. zeylanica leaf petiole), presence of one indistinct ridge in the center of adaxial side three ridges on the abaxial side (which are prominent in mature leaf petiole)</td></tr>
<tr><td>2</td><td>Epidermis</td><td>1) Single layered
2) Presence of thick cuticle
3) Absence of trichomes
4) Cells of upper epidermis are
-oval
-vary considerably in their size
-smaller in ridge region than other epidermal cells
5) Dimensions of epidermal cell
Height (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>7.232</td><td>40.020</td><td>22.698 ± 11.956</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>11.423</td><td>50.165</td><td>27.861 ± 11.908</td></tr></table></td><td>1) Single layered
2) Presence of thick cuticle
3) Presence of many multicellular trichomes onepidermis in the middle portion of petiole on the upper side
4) Cells of upper epidermis are
-rectangular or some may be oval
-vary considerably in their size
-smaller in ridge region than other epidermal cells
5) Dimensions of epidermal cell
Height (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>4..078</td><td>20.086</td><td>12.272 ± 4.397</td></tr></table>Breadth (µm)<table><tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr><tr><td>2.342</td><td>32.080</td><td>16.934 ± 7.928</td></tr></table></td></tr>
<tr><td>3</td><td>Hypodermis</td><td>1) Collenchymatous
2) Represented by patches present in ridge regions below epidermis and at the corner of crescent
3) 1 to 8 layered in ridge region and 1to 6 layered at the corners
4) More massive as compared to Plumbagoauriculata</td><td>1)Collenchymatous
2) Represented by patches present in ridge regions below epidermis and at both the corners
3)1 to 7 layered in ridge region and 1to 4 layered at the corners
4)Less massive as compared to Plumbagozeylanica</td></tr>
<tr><td>4</td><td>Ground Tissue</td><td>1) Parenchymatous , thin walled
2) Outer 1-4 layers of ground tissue below epidermis contain chloroplast (i.e.chlorenchymatous)
3) Consist of polygonal / oval cells of various sizes</td><td>1) Parenchymatous , thin walled
2)Outer 1-3 layers of ground tissue below epidermis contain chloroplast (i.e.chlorenchymatous)
3) Consist of polygonal / oval cells of various sizes</td></tr>
<tr><td>5</td><td>Vascular bundles</td><td>1) Vary in their number , size
2) Arranged in two semicircles in ground tissue (one semicircle below epidermis on adaxial side and other above epidermis on abaxial side) and one or two vascular bundles in center of petiole
3) Number varies from 9 to 14
4) Vascular bundle surrounded by parenchymatous bundle sheath. Sclerenchyma patches may present on both sided or one side of vascular bundles
5) Conjoint, collateral with xylem towards adaxial side and phloem towards abaxial side</td><td>1) Vary in their number , size
2) Remain scattered in ground tissue
3) Number varies from 5 to 8
4) Vascular bundle surrounded by parenchymatous bundle sheath. No sclerenchyma patches surrounding the vascular bundles are found
5) Conjoint, collateral with xylem towards adaxial side and phloem towards abaxial side</td></tr>
<tr><td>6</td><td>Tannin Cells</td><td>Comparatively less in number</td><td>Enormous number of tannin cells</td></tr>
</table>

(Note- Min. means Minimum, Max. means maximum, S. D. Means Standard Deviation)

TABLE-3 : Comparative account of anatomical features of thin stem of two species of *Plumbago.*

Sr. No.	Anatomical Feature	*Plumbago zeylanica*	*Plumbago auriculata*
1	Diameter of stem studied	2.506 ± 0.115mm	1.571 ± 0.104mm
2	Outline	Roughly circular, showing distinct ridges and furrows (Ridges and furrows more prominent in mature stem)	Roughly circular, wavy showing indistinct ridges and furrows (not as prominent as in *P. zeylanica*) (Ridges and furrows distinct in mature stem)
3	Epidermis	1) Single layered but not uniformly single layered. At some places it is double layered. However it is less frequent as compared that of *P. auriculata* 2) Presence of thick cuticle 3) Cell of upper epidermis are -Rectangular -Vary in their size - Smaller in ridge region with their corners somewhat rounded -Larger in furrow region and show more elongation parallel to surface of stem 4) Dimensions of epidermal cells Height (µm) Min. | Max. | Mean± S.D. 5.885 | 24.859 | 17.521 ± 4.879 Breadth (µm) Min. | Max. | Mean± S.D. 6.156 | 42.969 | 21.034 ± 9.761	1) Single layered but not uniformly single layered. At some places it is double layered 2) Presence of thick cuticle 3) Cells of upper epidermis are -Rectangular -Vary in their size -Smaller in ridge region with their corners somewhat rounded -Larger in furrow region and show more elongation parallel to surface of stem 4) Dimensions of epidermal cells Height (µm) Min. | Max. | Mean ± S.D. 4.721 | 24.658 | 10.512 ± 4.819 Breadth (µm) Min. | Max. | Mean± S.D. 5.122 | 42.073 | 15.683 ± 8.886
4	Hyperdermis	1) Collenchymatous 2) 2-8 layered 3) Present below ridge region 4) Not continuous but interrupted by chlorenchyma of cortex region below furrow	1) Collenchymatous 2) 2-8layered 3) Present below ridge region 4) Not continuous but interrupted by chlorenchyma of cortex region below furrow
5	Cortex	1) Parenchymatous and interrupted by hyperdermis 2) Cells oval 3) Cortex has outer 2-4 layered chlorenchyma 4) Cells of chlorenchyma smaller than remaining cells.	1) Parenchymatous and interrupted by hypodermis 2) Cells Oval 3) Cortex has outer 2-4 layered chlorenchyma 4) Cells of chlorenchyma smaller than remaining cells.
6	Endodermis	1) Single layered 2) Cells of endodermis contain starch grain	1) Not Distinct
7	Pericycle	1) Pericycle has outer sclerenchyma and inner parenchyma 2) Outer sclerenchyma is in the form of ring which is uneven in thickness. It is 1-7 layered 3) Inner parenchyma 1-2 layered	1) Pericycle has outer sclerenchyma and inner parenchyma 2) Outer sclerenchyma is in the formof ring of uneven thickness which is interrupted at many places. It is 1-9 layered 3) Inner parenchyma 1-2 layered

8	Vascular bundle	1) 25-47 Vascular bundles arranged in ring 2) Conjoint , Collateral open 3) Endarch xylem	1) 18-21 Vascular bundles arranged in ring 2) Conjoint , Collateral open 3) Endarch xylem
9	Pith	1) Large ,well developed 2) Parenchymatous 3) Pith cells polygonal, thin walled and lager in the centre whereas smaller towards the periphery	1) Large , well developed 2) Parenchymatous 3) Pith cells polygonal, thin walled and lager in the centre whereas smaller towards the periphery

(Note- Min. means Minimum, Max. means maximum, S. D. Means Standard Deviation)

µm × 16.934 ± 7.928 µm); collenchymatous hypodermis less massive, vascular bundles scattered, less in number (5 to 8); no sclerenchyma patches surrounding vascular bundles; tannin cells enormous in number.

T.S. of thin stem

In T. S. outline of stem is roughly circular with ridges and furrows which become prominent at maturity. It is differentiated into epidermis, hypodermis, cortex, endodermis and stele (pericycle, vascular bundles and pith) (Fig. 3). The comparative anatomy of stem of both taxa is described in detail in Table-3.

The present observations of internal structure of thin stem of *Plumbago* are in conformity with observations[11].The general structure of thin stem of *Plumbago* is same in both taxa with some differences. In *P. zeylanica* stem outline is showing distinct ridges and furrows; epidermis rarely double layered, epidermal cells larger (17.521 ± 4.879 µm × 21.034 ± 9.761 µm); single layered endodermis; pericyclic sclerenchyma in the form of continuous ring; vascular bundles more in number (25-47) while in *P. auriculata* stem outline is wavy showing ridges and furrows which are not prominent as in *P. zeylanica* stem; epidermis frequently double layered, epidermal cells smaller (10.512 ± 4.819 µm × 15.863 ± 8.886 µm); endodermis not distinct; pericyclic sclerenchyma ring interrupted at many places; vascular bundles less in number (18-21)

T.S. of thin root

Outline of root in T.S. is almost circular. Secondary growth is observed in the thin root of present investigation. Anatomically root is differentiated into periderm, cortex, pericycle, vascular tissue (Fig. 4). The comparative anatomy of root of both taxa is described in detail in the following Table-4.

Though the general plan of anatomical structure of root in both the plants is same, differences of taxonomic importance are observed. Presence of comparatively broad cortex with abundant starch grains in its cells; pericyclic sclerenchyma in the form of patches; narrower vascular tissue (about 55.723% of root diameter) ; occurrence of clusters of vessels, comparatively larger vessels with thicker wall which may be uniform or uneven, higher vessel density; narrow medullary rays are features of root of *P. zeylanica* which differentiate it from root of *P.auriculata* which has comparatively narrow cortex with less starch grains in its cells; percyclic sclerenchyma well developed and form almost ring of uneven width; wider vascular tissue (about 79.076% of root diameter); absence of clusters of vessels, comparatively smaller vessels with thinner walls of uniform thickness, lower vessel density; broad medullary rays. Our observations of almost a ring of pericyclic sclerenchyma, wider vascular tissue in root of *P.auriculata* and scattered pericyclic sclerenchyma patches, narrower vascular tissue in root *P.zeylanica* is in accordance[11] but our observation of larger vessels in *P.zeylanica* and smaller vessels in *P.auriculata* is in contrary[11] which reported larger vessels in *P.auriculata* and smaller vessels in *P.zeylanica*. In root T. S. distinct endodermis is not observed. It was also stated that endodermis in many angiospermic root remains only in primary form and shed together with cortex with the development of secondary growth[9].

Taxonomic significance of anatomy

Anatomical characters are helpful to delineate two species of *Plumbago*

Keys to *Plumbago* species

1. Key based on mid rib region of leaf anatomy

Outline conical on both sides (upper surface more bluntly conical), epidermal cells oval and larger (21.295 ± 4.742 µm × 27.379 ± 8.818 µm in upper epidermis and 14.557 ± 4.490 µm × 17.176 ± 5.538 µm in lower epidermis), sclerenchyma found on both sides of vascular bundles, tannin cells comparatively less in number

-Plumbago zeylanica

Outline bluntly conical on upper surface and convex

TABLE-4: Comparative account of anatomical features of thin root of two species of *Plumbago*

Sr. No.	Anatomical feature	*Plumbago zeylanica*	*Plumbago auriculata*
1	Diameter of root studied	3.442mm	2.772mm
2	Outline	Almost circular	Almost circular
3	Periderm	1) Epidermis disintegrated 2) Width - 0.046 to 0.102mm 3) Outer cells not distinct and appear yellowish brown and inner periderm 5 to 8 layered 4) Cells tangentially elongated, rectangular (rarely polygonal) and arranged in radial files	1) Epidermis disintegrated 2) Width - 0.067 to 0.116 mm 3) Outer cells not distinct and appear yellowish brown and inner periderm 5 to 7 layered 4) Cells tangentially elongated, rectangular (rarely polygonal) and arranged in radial files
4	Cortex	1) Width - 0.660 mm 2) Made up of polygonal cells 3) Cells are heavily loaded with starch grains	1) Width - 0.085 to 0.117 mm 2) Made up of polygonal cells 3) Cells shows presence of starch grains which are less as compared to *P.zeylanica*
5	Pericylic sclerenchyma	Few small groups of thick walled sclerenchyma cells between the inner boundary of cortex and outer part of phloem.	Patches of thick walled sclerenchyma cells between inner boundary of cortex and outer part of phloem. These patches form interrupted ring of uneven width (0.027 to 0.064mm)
6	Vascular tissue	1) 0.959 mm wide 2) Vascular tissue constitute about 55.723 % of the diameter of the root 3) Consists of outer zone of phloem, cambium and inner thick cylinder of xylem 4) In phloem zone sieve elements occur in radial lines 5) Xylem consists of vessels, parenchyma and fibers 6) Xylem fibers thick walled 7) Xylem parenchyma show presence of starch grains 8) Vessels - Elliptical (oval) or almost circular outline - Arrangement a. In radial chain b. Occur singly or in multiples (of 2 vessels in oblique row or 2 to 6 vessels in radial row) or in cluster of 6 vessels - variable in diameter Radial lumen diameter of oval vessel (µm)	1) 1.096 mm wide 2) Vascular tissue constitute about 79.076 % of the diameter of the root 3) Consists of outer zone of phloem, cambium and inner thick cylinder of xylem 4) In phloem zone sieve elements occur in radial lines 5) Xylem consists of vessels, parenchyma and fibers 6) Xylem fibers thick walled 7) Xylem parenchyma show presence of starch grains 8) Vessels - Elliptical (oval) or almost circular outline - Arrangement a. In radial chain b. Occur singly or in multiples (of 2 or 7 vessels in radial row or rarely 2 vessels in oblique row. Cluster not observed - variable in diameter

<table>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D</th></tr>
<tr><td>8.891</td><td>62.460</td><td>29.762 ± 19.681</td></tr>
</table>
Tangential lumen diameter of oval vessel (µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D</th></tr>
<tr><td>5.929</td><td>61.670</td><td>28.588 ± 21.165</td></tr>
</table>
Thus vessels are larger
- Wall thickness
1) Comparatively thicker.
Wall thickness (µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean ± S.D</th></tr>
<tr><td>2.159</td><td>12.178</td><td>7.395 ± 2.789</td></tr>
</table>
2) Some vessels with uniform wall thickness but some are with uneven wall thickness they have more thick radial wall as compared to tangential wall
Radial thickness(µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D.</th></tr>
<tr><td>5.797</td><td>12.178</td><td>8.995 ± 2.638</td></tr>
</table>
Tangential thickness(µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D</th></tr>
<tr><td>2.159</td><td>7.232</td><td>4.538 ± 2.067</td></tr>
</table>
- Vessel density (mean number of vessels per mm^2) is 198 ± 24.891. It is relatively high
</td>
<td>
Radial lumen diameter of oval vessel (µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean ± S.D</th></tr>
<tr><td>7.857</td><td>44.310</td><td>26.830 ± 15.667</td></tr>
</table>
Tangential lumen diameter of oval vessel (µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D</th></tr>
<tr><td>7.052</td><td>31.388</td><td>18.150 ± 9.477</td></tr>
</table>
Thus vessels are smaller
- Wall thickness
1) Comparatively thinner.
Wall thickness (µm)
<table>
<tr><th>Min.</th><th>Max.</th><th>Mean± S.D</th></tr>
<tr><td>2.110</td><td>10.426</td><td>3.787 ± 2.379</td></tr>
</table>
2) Vessels with uniform wall thickness
- Vessel density (mean number of vessels per mm^2) is 123.6 ± 33.860. It is relatively low.
</td>
</tr>
<tr><td>7</td><td>Medullary ray</td><td>Narrow</td><td>Broad</td></tr>
<tr><td>8</td><td>Pith</td><td>Absent, occupied by xylem</td><td>Absent, occupied by xylem</td></tr>
<tr><td>9</td><td>Plumbagin</td><td>1) Present abundantly in periderm, cortex and xylem phloem
2) It is dark yellow in colour</td><td>1) Present in periderm, cortex and xylem, phloem
2) It is dark yellow in colour</td></tr>
</table>

(Note- Min. means Minimum, Max. means maximum, S. D. Means Standard Deviation)

A **B**

Fig.1: (A-B) Leaf anatomy

A. T.S of mid rib region of leafof *P. zeylanica* (100x).

B. T.S of mid rib region of leaf of *P. auriculata* (100x).

on lower surface, epidermal cells rectangular and smaller (14.516 ± 3.731 µm × 14.291 ±7.607µm in upper epidermis and 11.317 ± 2.667 × 11.045 ± 3.341 µm in lower epidermis), no sclerenchyma around vascular bundles observed, tannin cells comparatively more in number

– *Plumbago auriculata*

2. Key based on petiole anatomy

Petiole outline more broadly sulcate with acute margin and distinct ridges, trichomes absent, epidermal cell oval and larger (22.698 ± 11.956 µm × 27.861 ± 11.908µm), collenchmatous hypodermis comparatively more massive, vascular bundles arranged in two semicircles, more in number (9 to 14) and may show

A **B**

Fig. 2: (A-B) Petiole anatomy

A. T. S. of petiole of *P. zeylanica* (100x)

B. T. S. of petiole of *P. auriculata* (100x)

A B

Fig. 3: (A-B) Stem anatomy

A. T. S. of stem of *P. zeylanica* (100x)

B. T. S. of stem of *P. auriculata* (100x)

presence of surrounding sclerenchyma patches, tannin cells comparatively less in number

- Plumbago zeylanica

Petiole outline broadly sulcate with erect acute margin and indistinct ridges, trichomes present on the middle portion of epidermis on upper side, epidermal cells rectangular or sometime oval, smaller (12.272 ± 4.397 × 16.934 ± 7.928 µm), collenchymatous hypodermis less massive, vascular bundles scattered, less in number (5 to 8) and show absence of surrounding sclerenchyma patches, tannin cells enormous in number

-Plumbago auriculata

3. Key based on stem anatomy

Stem outline show distinct ridges and- furrows, epidermis rarely double layered, epidermal cell larger (17.521 ± 4.879 × 21.034 ± 9.761 µm), endodermis single layered, pericyclic sclerenchyma form continuous ring, vascular bundles more in number (25 - 47)

- Plumbago zeylanica

Stem outline wavy showing ridges and furrows which are not prominent, epidermis frequently double layered, epidermal cells smaller (10.512 ±4.819 µm × 15.583 ± 8.886 µm), endodermis not distinct, pericyclic sclerenchyma ring interrupted at many places, vascular bundles less in number (18-21)

- Plumbago auriculata

4. Key based on root anatomy

Presence of comparatively broad cortex with abundant starch grains in its cells, pericyclic sclerenchyma in the form of patches, narrower vascular tissue, occurrence of clusters of vessels, comparatively larger vessels with thicker walls which may be uniform or uneven, higher vessel density, narrow medullary rays

- Plumbago zeylanica

Presence of comparatively narrow cortex with less starch grains in its cells, pericyclic sclerenchyma well developed and form almost ring of uneven width, wider vascular tissue, absence of clusters of vessels, comparatively smaller vessels with thinner walls of uniform thickness, lower vessel density, broad medullary rays

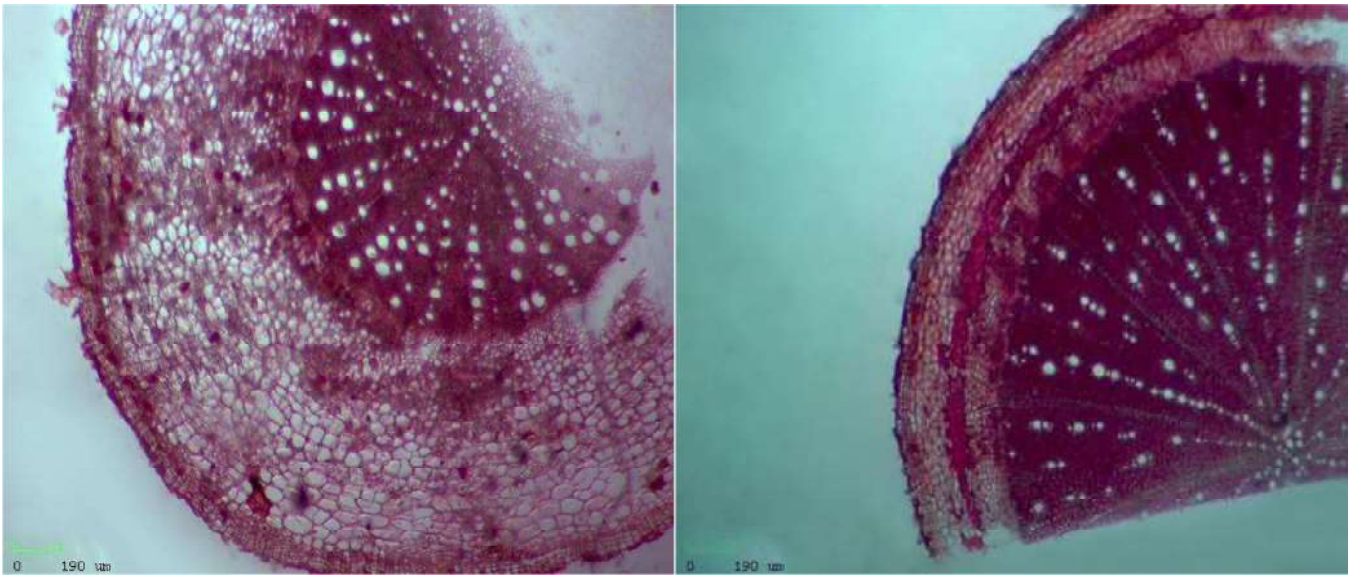
- Plumbago auriculata

Conclusions

The present work revealed distinctive differences between anatomical characteristics of leaf, petiole, stem and root which would serve as diagnostic parameters and can be used for identification and authentication of these species.

In study of midrib region of leaf, the features of diagnostic value in delimitation of both taxa are

1. Outline of T. S.
2. Shape and size of epidermal cells
3. Presence of sclerenchyma surrounding the



A **B**

Fig.4:(A-B) Root Anatomy

A. T. S. of root of *P. zeylanica* (40x)

B. T. S. of root of *P. auriculata* (40x)

vascular bundles

4. Number of tannins cells

Characters of taxonomic significance in petiole anatomy are

1. Outline of T. S
2. Presence of trichomes
3. Shape and size of epidermal cells
4. Abundance of collenchyma
5. Arrangement and number of vascular bundles
6. Presence of sclerenchyma surrounding vascular bundles

7. Number of tannin cells

The anatomical features of stem which are diagnostically useful to discriminate both taxa of *Plumbago* are

1. Degree of elevation of stem ridges
2. Occurrence of double layered epidermis
3. Size of epidermal cells
4. Distinctness of endodermis
5. Abundance and distribution of pericyclicsclerenchyama
6. Number of vascular bundles

Anatomical features of root which are diagnostically important to discriminate both taxa of *Plumbago* are

1. Width of cortex and abundance of starch grains in cortex cells
2. Abundance and distribution of pericyclicsclerenchyama
3. Amount of vascularization
4. Distribution, diameter and density of vessels
5. Width of medullary ray

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