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Histochemical studies on *Delonix elata* (L.) Gamble. (*Cesalpiniaceae*) Leaves

M. SENTHILKUMAR and *N. SAMI VEERAPPA.
* Department of Botany, Periyar EVR Arts College, Tiruchirappalii, Tamil Nadu, India.

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Author(s):
M. SENTHILKUMAR and N. SAMI VEERAPPA*

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Corresponding Author:
N. SAMI VEERAPPA.
Department of Botany, Periyar EVR Arts College, Tiruchirappalii, Tamil Nadu, India.

E-mail: enesveerappa@gmail.com

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Abstract

The histochemical studies of the *Delonix elata* of the leaflet were investigated to certain the relevance of these characters in establishment of interspecific similarities and differences in these taxa. The results showed that leaflet is thin with fairly prominent lateral vein and midrib. The lamina is 110 mm thick, the lateral vein is 170mm thick and the midrib is 180 mm thick. Adaxial epidermis 10-20 mm thick. Adaxial groove, it is 1 mm thick. The outer ground tissue is narrow while the central ground tissue is wide and parenchymatous.

INTRODUCTION

The Casealpinoidae includes 171 genera and about 2250 species of tropical and sub tropical trees and shrubs (Lewis et al., 2005) listed 5 tribes, namely Cercideae, Ceasalpinieae, Cassieae, Amheristeae and Detarieae as the of phylogenetic analysis (Polhill and Raven, 1981), (Bruneau, et al., 1980). Listed only 4 tribes, Cassieae, Detarieae, Caesalpinieae and Cercideae as the components of the Casealpinoidea (Herendeen, et al., 1980). Herbal medicine has been practiced worldwide and is now recognized by World Health Organization (WHO) as an essential building block for primary healthcare (Stuessy, 1990). Though the traditional Indian system of medicine has a long history if use, they lacked adequate scientific documentation, particularly in the light of modern scientific knowledge. *Delonix elata* (L.) Gamble. (Casealpiniaceae).

A genus of tree with large showy flowers, distributed in tropical Asia and Africa. Two species are grown in India, mostly for ornamental purposes. *Delonix elata* Gamble syn. *Poinciana elata* Linn. An erect tree, 20-30ft high reported to occur wild in some parts of Kathiawar and South India and frequently planted as an avenue tree. It bears feathery foliage and handsome pale yellow flowers with reddish flowers filaments (Stuessy, 1990), Gamble J. S and Fisher C.E.C. (1915-1936), (Ramjani and Krishnamurthy, 1988).

Pharmacognostical Studies

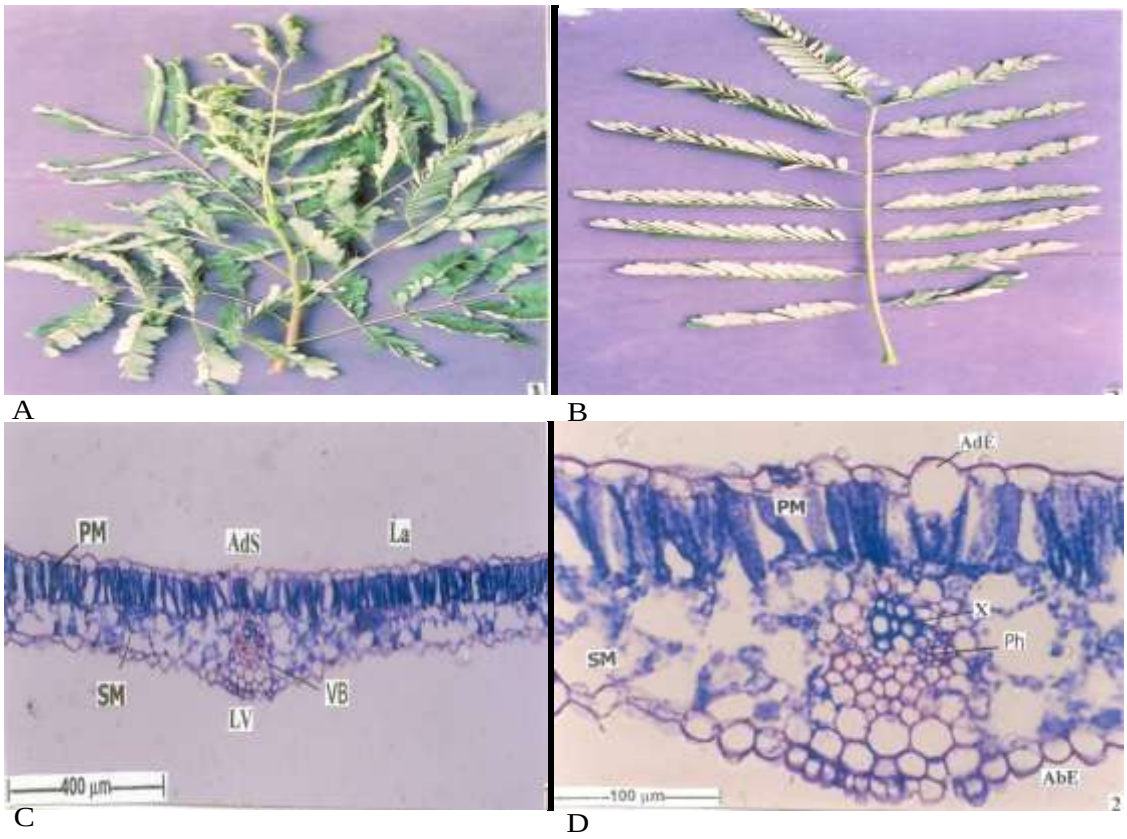
Macrosocpial studies and morphological characters of the medicinally useful parts of *Delonix elata* (L.) were studied. Microscopical Studies of leaves were collected from the plants and fixed in FAA (Formalin 5 ml +Acetic acid 5ml +70% Ethyl alcohol 90 ml). After 24 hrs of fixation the specimens were dehydrated with a graded series of Tertiary Butyl Alcohol (TBA), as per the schedule given by Sass (1940). Infiltration of the specimen was carried by the gradual addition of paraffin wax (melting point 58^{o}C -60^{o}C) until the TBA solution attained super saturation. The specimens were case into paraffin blocks.

Materials and Methods

Collection of specimens

The *Delonix elata* leaves specimens were collected from Narthamalai, Pudukottai, Tamil Nadu (Figure 1a, 1b). This investigation was conducted at the Plant Anatomy Research Centre, Pharmacognosy Institute, West Tambaram, Chennai 2009. Care was taken to select healthy plant and normal organs. The required samples

Figure 1: PM: Palisade Mesophyll; SM: Secondary Metaxylum; AdS: Adaxial Side; LV: Lateral Vein; La: Lamina;VB: Vascular Bundle; X:Xylem; Ph:Phloem: AbE: Abaxial Epidermis; AdE: Adaxial epidermis



(a) A twig showing compound leaf (b) Leaf Enlarged (c)T.S of leaf-let through midrib with lamina (d) Lamina Enlarged

of different organs were cut and removed from the plant fixed in FAA (Farmalin-5ml+Acetic acid-5ml +70% Ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary - Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{o}C) until 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 by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toludine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Where ever necessary sections were also stained with safranin and Fast-green and IKI (for Starch).

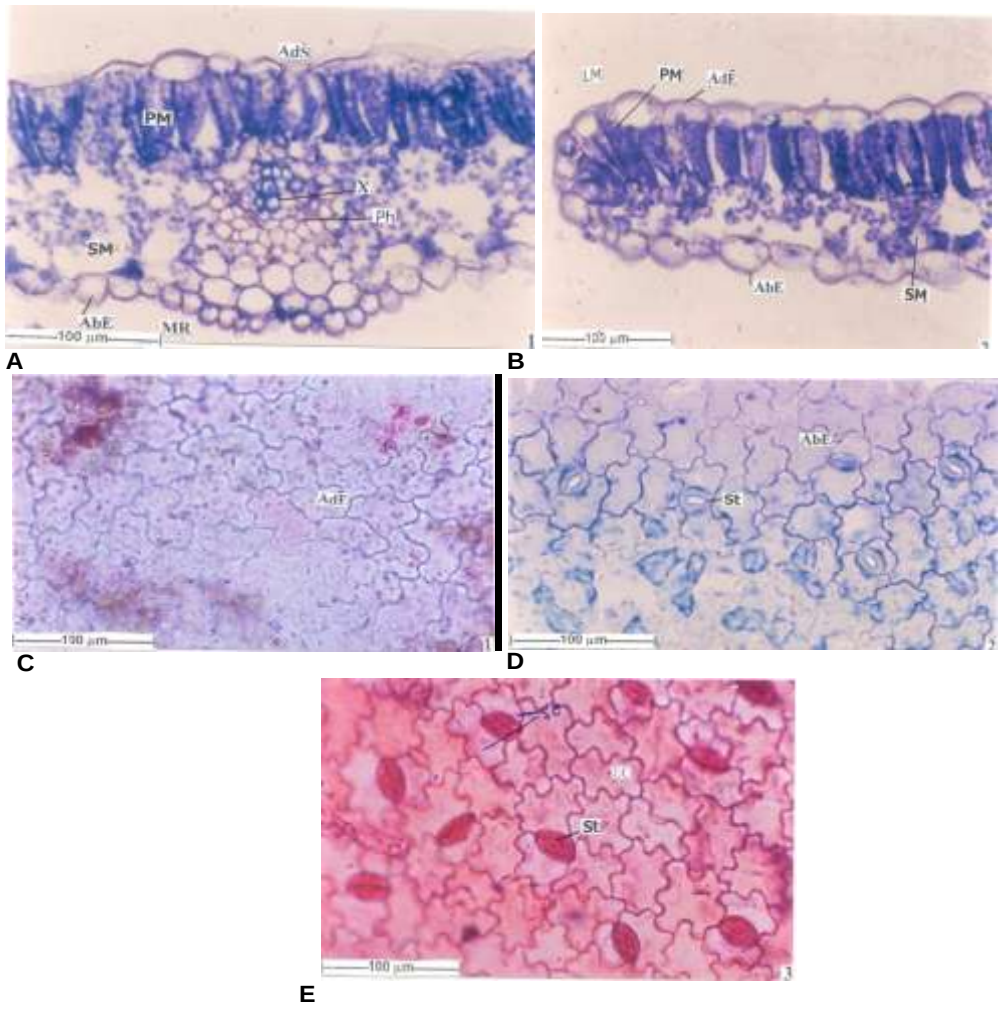
Stomatal morphology

For studying the stomatal morphology, venation pattern and trichome distribution, 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 (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Naoh and mounted in glycerine medium after staining. Different cell component were studied and measured.

Pharmacognostical Studies

Pharmacognostical Studies were carried out the following the method of Wallis (1997) to ascertain the correct identity of the drug plants.

Figure 1: AdS: Adaxial Side;PM: Palisade Mesophyll; SM: Secondary Metaxylum; AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; MR: Midrib;X: Xylem;Ph: Phloem; LM: Lamina



(a - b) T.S. of leaf let showing lateral vein with lamina (b - c) Adaxial epidermis (d) Abaxial epidermis with stomata

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo II 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 appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Anatomical descriptions were made employing descriptive terminology given in standard Anatomy books (Esau, 1965; 1979; Fahn.1982).

Preparation of Leaf powder

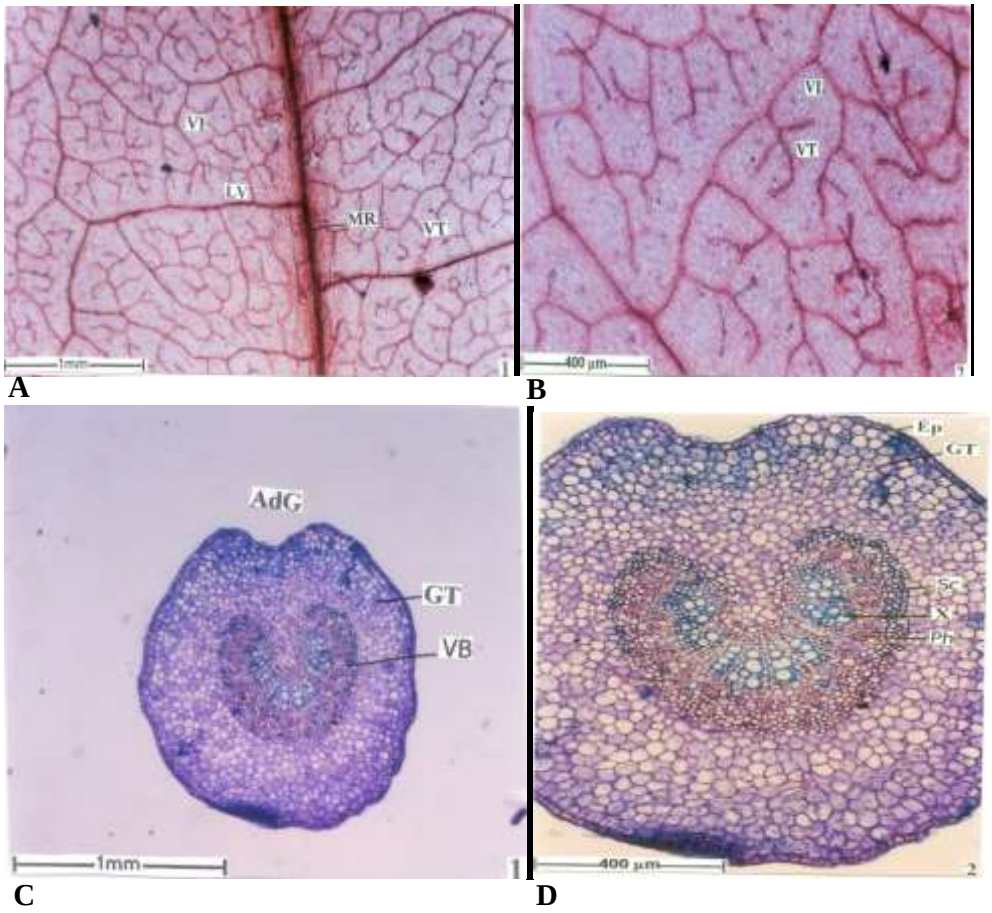
Leaves of the study plants were collected and shade dried for four days, sun dried for a day and then stored in black polythene bags. The leaves were powdered in a pulverizer as and when required, sieved, labeled and stored in Polyethylene terithalate (PET) bottle (Harbone, 1973).

RESULTS AND DISCUSSION

Anatomy of the leaflet

The leaflet is thin with fairly prominent lateral vein and midrib. The lamina is 110 mm thick, the lateral vein is 170mm thick and the midrib is 180 mm thick (figure 1c). The lamina has wide epidermal layers with barrel shaped thin walled cells (Figure 1d), (figure 2a 2b). The adaxial epidermis 10-20 mm thick (figure 2b, 2c). The abaxial epidermis (figure 2d) has spindle shaped dilated cells with hemispherical outer tangential walls. The cells are

Figure 3: VI: Veins; **LV:** Lateral vein; **MR:** Midrib; **VT:** Vein-terminations; **AdG:** Adaxial groove; **GT:** Ground Tissue;**VB:** Vascular Bundle; **EP:** Epidermis;**Sc:** Secretary Cavity; **X:** Xylem;**Ph:** Phloem; **AdP:** Adaxial Parenchyma



(a - b)Venation Pattern (Cleared leaf showing vein-islets and vein termination (Magnified View)
(c - d) Anatomy of the Primary rachis- T.S of rachis entire view, magnified

20 mm thick. The mesophyll tissue is differentiated into adaxial band of thick, cylindrical loosely arranged palisade cells; the cells are 40 mm in height. The abaxial portion has four to five layers lobed loosely, arranged spongy parenchyma cells and wide air- chambers.

The midrib has single collateral vascular strand which consists of conical cluster of thick walled xylem elements and two or three nests of phloem elements. The vascular strand is surrounded by thin walled parenchyma cells. The abaxial part of the vascular strand has wider, circular cells and those cells adjacent to the vascular strand are smaller and angular. The palisade zone is extended and horizontally transcurrent in between thin adaxial epidermis and the vascular bundle.

Surface view of the epidermal cells and stomata

The adaxial epidermis is apostomatic (without stomata). The cells of the epidermis have wavy anticlinal walls and amoeboid outline**.** The abaxial epidermis is stomatiferous. The stomata are paracytic type. These are two unequal subsidiaries for each stoma; these cells occur parallel and lateral to the guard cells. The epidermal cells are amoeboid in outline with thin wavy walls.

Venation pattern

The midrib is fairly prominent and straight. The lateral veins are thin and are right angles to the mid veins. The lateral veins branch profusely giving rise to tertiary veins which form distinct and indistinct vein-islets. The vein-terminations are distinct and they range from simple un branched to repeatedly branched dendroid types**.** The terminatious are curved or straight (figure 3a - 3b).

Secondary rachis

The secondary rachis is circular is sectional view with adaxial groove**.** It is 1 mm thick. It consists of a thin

epidermal layer of small squarish cell with prominent cuticle. The ground tissue is homogeneous and parenchymatous; the cells are small, angular, thin walled and compact. The ground parenchyma is 300 mm wide from the vascular cylinder to the epidermis.

The vascular strand is urn-shaped; it is wide and deep and open towards the adaxial side. It is 530 mm in horizontal plane; 200 mm thick in radial plane. The vascular strand has a thick sheath of sclerenchyma, several, circular discrete phloem nests and radial files of circular, thick walled, wide xylem elements. The meta xylem is 25 mm in diameter.

Primary rachis

The cross sectional outline of the rachis differs from base to the tip. The basal part of the rachis is wide and circular with shallow adaxial groove. It is 4 mm in diameter. The terminal part of the rachis is shield shaped with wide, fairly deep adaxial depression and lateral short ridges. The terminal rachis is 3.2 mm thick. The basal part of the rachis has wider parenchymatous ground tissue and broad, circular closed, hollow vascular cylinder. The cylinder is 2.2 mm in diameter. The cylinder has dense radial rows of fibers and circular wide vessels at frequent inter walls. The terminal part of the rachis, the vascular system consists of a wide, hollow u-shaped strand and a flat horizontal plate like strand. The outer ground tissue is narrow while the central ground tissue is wide and parenchymatous. The vascular strands have closely arranged parallel rows of xylem elements, outer zone of phloem nests and thick sclerenchyma sheath all around the vascular cylinder (**figure 3c-3d**).

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