ANATOMICAL STUDIES ON THE LEAF OF *LUDWIGIA PERENNIS* L.

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**ABSTRACT**

The present investigation is to provide information on the anatomical studies on the leaf of *Ludwigia perennis*. The fresh leaf was undertaken by rotary microtome and examined on photomicrographs, to find out identical characteristics. The leaf consists of a thin lamina and thick midrib. The midrib has adaxial part, abaxial part, epidermis, parenchyma cells, ground tissues, deeply boat shaped vascular strand, xylem, phloem elements are present. The lateral veins are small, prominent, contains compact parenchyma cells, spindle shaped epidermal cells with cuticle; small, circular collateral vascular strand present in the upper part of the vein. Lamina is distinctly dorsiventral and heteromorphic, thick, mesophyll tissue contains long cylindrical palisade cells, small loosely arranged spongy cells, and wide air chambers are present. Calcium oxalate crystals are abundant in mesophyll and it consists of two types. They are druses and raphides. The stomata are diffusely distributed, actinocytic and cyclocytic; some are diacytic. The guard cells are broadly elliptical.

**KEYWORDS**: *Ludwigia*, leaf, anatomy, lamina, midrib, veins.

**INTRODUCTION**

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. The use of medicinal plants in traditional medicine is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005; Gupta *et al.*, 2005). According to World Health Organisation (WHO) more than 80% of the World’s Population rely on traditional medicine for their primary health care...
needs and have found a place in day-to-day life (Ammara et al., 2009). Medicines obtained from plants are relatively safer than synthetic alternative (Idu et al., 2007 and Iwu et al., 1999). According to the world Health Organization the macroscopic and microscopic description of medicinal plants is the first step towards the establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken (WHO, 1998). Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of year as a result of man’s inquisitive nature. So that today we possess many effective means of health care. In the past almost all the medicines used were from the plants being, man’s only chemist for ages. Today a vast store of knowledge concerning therapeutic properties of different plants has accumulated (Anandanayaki and Uma 2014). Plants are major sources of herbal medicines and the presence of secondary metabolites many therapeutic activities (Ogunleye and Ibitoye 2003). The medicinal plants with time tested healing properties are now in vogue. An urgent need is therefore being felt for their proper identification and utility.

Plant anatomy is the general term for the study of the internal structure of plants. It was originally included in plant morphology, and plant morphology describes the physical form and external structure of plants. Plant anatomy is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy. Anatomical studies have much significance in different sectors of investigation. Studies on anatomy of plants can explain where, what, when and how chemical components are produced. The anatomical studied can be clarified the qualities of the wood properties. Anatomic studies have shown to be an important tool associated with taxonomic studies; mainly when there is no reproductive organ in the investigated sample (Solereder, 1908; Metcalfe and Chalk, 1950). The present study deals with the study of anatomical studies of the leaf of *Ludwigia perennis* L.

**MATERIALS AND METHODS**

**Collection of plant materials**

The fresh plant *Ludwigia perennis* L. (Onagraceae) were collected in Erode district, India and were authenticated at Botanical Survey of India (BSI), Coimbatore, India.

**Microscopic Analysis**

**Preparation of specimens**

Care was taken to select healthy leaves of *Ludwigia perennis* The fresh sample of leaves were cut into small pieces and fixed in FAA solution (Formalin-5 ml + Glacial acetic acid-5 ml +
70 % Ethyl Alcohol-90 ml) as per the schedule given by Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60ºC) Tertiary Butyl Alcohol (TBA) 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. De-waxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per method published by O’Brian *et al.* (1964).

**Staining**

For anatomical studies the following staining schedules were followed by toluidine blue stain was prepared by dissolving 0.25gm of the stain in the mixture of benzoic acid 0.25gm, sodium benzoate 0.29gm and distilled water 200ml with pH of 4.2 - 4.4. Since toluidine 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. wherever, necessary sections were also stained with safranin and Fast-green and IKI (for starch). (IKI- lugol’s iodine is a brown solution that turns black in the presence of starch). For studying stomata morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of the leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid (Sass,1940) were employed. Glycerin mounted temporary preparations were made for macerated materials.

**Photomicrographs**

All permanent slides, after staining were dehydrated by using graded series of Ethanol + Xylol and mounted in DPX. Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo-2microscopic using Konica colour film (100 ASA). For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used since these structures have bi refringent property, appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features were taken from the standard anatomy books (Esau, 1964).
RESULT AND DISCUSSION

Anatomical studies of the leaf of *Ludwigia perennis* – T.S of Leaf

The leaf consists of a thin lamina and thick midrib (Fig: 1.1).

**Midrib**

The midrib consists of a thick dome shaped adaxial part and wide boat shaped abaxial part. The adaxial hump is 300 μm wide and the abaxial part is 700 μm thick in vertical axis. The adaxial midrib has fairy, large, square shaped, thin walled epidermal cells. The abaxial part consists of small, less prominent, rectangular or circular epidermal cells. There are three sub epidermal layers, angular, thin walled parenchyma cells in the adaxial hump. The abaxial part includes six or seven layers of polyhedral compact thin walled parenchymatous ground tissues (Fig: 1.2).

**Vascular Strand**

Vascular strand is deeply boat shaped and it consists of xylem and phloem elements.

Xylem elements comprise an arch of several rows of xylem elements. There are about three xylem in each rows. The xylem elements are 15 μm in diameter, circular / elliptical, thick walled and wide (Fig: 1.2, 1.3).

Phloem elements are located along lower part of the xylem arc. They are in small discrete groups. These are also scattered, discrete phloem units in the adaxial concavity of the xylem (Fig: 1.3).

**Lateral Veins**

The lateral veins are smaller, leaf prominent, having small adaxial cone and swollen hemispherical abaxial part (Fig: 2.1). The adaxial cone of the lateral vein is made up of five dilated compact parenchyma cells. The abaxial part consists of spindle shaped, fairy large epidermal cells with thin cuticle. The abaxial ground parenchyma cells are highly dilated angular, compact and thin walled (Fig: 2.1). There is a small circular collateral vascular strand located in upper part of the vein.

**Lamina**

The lamina is distinctly dorsiventral and heteromorphic. It is 60μm thick. The adaxial epidermal cells are circular to rectangular and thin walled. The abaxial epidermal cells are small, squarish and thin walled. The mesophyll tissue consist of single adaxial compact rows...
of long cylindrical palisade cells. The lower zone includes about four layers of small, lobed, loosely arranged spongy mesophyll cells and wide air chambers. (Fig: 2.2)

**Leaf -Margin**

The marginal part of the lamina becomes slightly thicker and conical. The epidermal cells assume larger size with conical outer tangential walls. A small vascular strand is found in the marginal region. The mesophyll tissues do not alter much in the marginal region. The leaf margin is 80µm thick. (Fig: 2.3)

**Crystal distribution**

Calcium oxalate crystals are abundant in the leafy mesophyll. Two types of crystals are seen. They are druses and raphides. Druses are spherical bodies with spiny surface, located in the wide mesophyll cells (Fig: 3.1). Raphides are thin, long pointed needles aggregated into thick Spindle shaped bundles (Fig:3.2) located inside. A wide, modified parenchyma cells called idioblasts (Fig: 3.3) are seen. The raphides are seen up to 130µm long and 25µm thick.

**Epidermal cells and Stomatal morphology**

Paradermal sections of the lamina were studied in surface view of the epidermis. In the surface view, the epidermal cells are wide with wavy anticlinal walls. The stomata are diffusely distributed. The stomata are actinocytic type (Fig: 4.2) and cyclocytic type (Fig: 3.3). Some stomata are diacytic type (Fig: 4.1). The guard cells are broadly elliptical, 15×25 µm in size.

**Venation pattern**

The veins of the lamina are less conspicuous. The major lateral veins are fairly thick and thin lateral veins form wide polygonal vein islets and simple (un branched) or branched dendroid vein terminations (Fig: 5.1, 5.2, and 5.3). Thick, cylindrical dark coloured raphide bundles are seen sparsely distributed in the lamina.

Similarly Florence and Domettila (2016) investigated the anatomical features of leaves and stems of the plant *Gmelina asiatica*. Leaf and stem of this plant was undertaken by rotary microtome and examined on photomicrographs. Anatomical characters such as echinate epidermal cells, glandular trichomes, anomocytic stomata, calcium oxalate crystals, periderm cylinder, phloem cells and vascular bundle of leaf and stem explains typical features of Verbinaceae. The cross section of leaf *Myrciaria glomerata* shows simple, opposite,
lanceolate, pinnate, hairy, involute margin, hypostomatic, dorsiventral; stomata anomocytic. Epidermal cells show uneven thickening of periclinal outer walls on the adaxial side of the leaf. The bundles are collateral, crystals of calcium oxalate spread throughout the mesophyll (Nemes Veiga, Ana Maria, 2016). The anatomical and histochemical characteristics of the leaf, stem and root of *Cordia obliqua* has been studied by Uthiraselvam *et al.*, (2016). The result showed that there is concentration of vascular bundles at the central portion of root, cortex, calcium oxalate present in the root powder and non glandular trichome were present in the leaves.

Rubaiyat Sharmin (2017) reported that cross section of the stem *Euphorbia hirta* has a circular shape, epidermis was uniseriate and isodiametric. Cortex are distinctly formed, 5-6 rows composed of chlorenchyma and found laticifers. Trachieds, vessels are seen. Pith were filled with laticifers at young stage, it has distinct gap at maturation stage. In leaf, the epidermis was uniseriate, regular, thin walled, usually similar in diameters and covered with thin cuticle layer. Multicellular uniseriate or gland-like trichomes occur in rugose hairs at epidermis. Mesophyll was differentiated into palisade and spongy layers, was composed of parenchyma cells. The palisade layer assembled with 2 rows of cells. The spongy layer thickness was different around the midrib region, compared with other parts, has 2-6 rows of cells. Laticifers were present at the middle part of the mesophyll. Xylem elements in midrib initiated perfectly and composed of many straight rows of mainly vessels where the phloem elements were abundant and occupied a good part of the vascular bundle as a semicircle shape. The stem and leaf anatomy of this species studied here showed laticifers in cortex zone and pith cells, and middle part of the mesophyll, respectively, was a taxonomic trait for this species.

**Anatomical studies of the leaf of *Ludwigia perennis* L.**

![Fig: 1.1 - T.S of leaf through midrib](image1)

**Fig: 1.1 - T.S of leaf through midrib**
AdH - Adaxial Hump; La – Lamina; MR – Midrib; VS – Vascular Strand

![Fig: 1.2 - T.S of midrib](image2)

**Fig: 1.2 - T.S of midrib**
Ep – Epidermis; GP – Ground Parenchyma; La – Lamina; Ph – Phloem; PM – Palisade Mesophyll; X - Xylem
Fig: 1.3 - Vascular bundle of midrib
AbPh – Abaxial Phloem; AdPh – Adaxial Phloem;
X – Xylem

Fig: 2.1 - T.S of leaf through lateral vein
AbP – Abaxial Parenchyma; AdC – Adaxial Cone;
Ep – Epidermis; GP – Ground Parenchyma;
Ph – Phloem; PM – Palisade Mesophyll;
SM – Spongy Mesophyll; X – Xylem

Fig: 2.2 - T.S of Lamina
AbE – Abaxial Epidermis; AdE – Adaxial Epidermis;
PM – Palisade Mesophyll; SM – Spongy Mesophyll

Fig: 2.3 - T.S of Leaf Margin
AbS – Abaxial Side; AdS – Adaxial Side; Ep – Epidermis
LM – Leaf Margin; MT – Mesophyll Tissue;
VS – Vascular Strand

Fig: 3.1 - T.S of Lamina with Druse
AdS – Adaxial Side; Dr – Druse;  
PM – Palisade Mesophyll;  
SM – Spongy Mesophyll

Fig: 3.2, 3.3 - T.S of Lamina with Raphides  
Dr – Druse; PM – Palisade Mesophyll; Ra - Raphides

Fig: 4.1, 4.2, 4.3 - Paradermal section of the Lamina showing Stomatal morphology  
AW – Anticlinal Wall; EC – Epidermal Cells; St – Stomata
CONCLUSION

Plant anatomy has been found to be very essential in plant taxonomy. Hence, the purpose is to develop a system of classifying plants in a way that all the differences and similarities are set out in ordered manner at a glance. Therefore, the leaf sectional anatomy provides extensive taxonomic data and the literature on the subject is now vast. Thus, from the leaf anatomical characters observed in the plant *Ludwigia perennis* L.

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REFERENCES


