

Anatomical and Histochemical Studies On *Alysicarpus longifolius* (Spreng.) Wight & Arn

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ABSTRACT: Traditional medicine is found to be effective and lasts long in maintaining health status than modern medicine. It shows biological compatibility to our system and hence is preferred to natural medicine. The floras are found to be the source, and hence there is a need to find out the importance of certain medicinal plants unknown to the world and bring to limelight their importance. Many plants are not exposed well to human race with regard to their medicinal properties. One such plant of this category which is not yet completely experimented is *Alysicarpus longifolius*. To understand about the plant and its biological importance, anatomical and histochemical localization studies were conducted. The studies revealed the presence of alkaloids, terpenoids and flavonoids which brings forth the medicinal importance and can serve as a source for traditional medicine.

KEYWORDS: Traditional medicine, floras, alkaloids, terpenoids, flavonoids, anatomical, histochemical localization.

I. INTRODUCTION

Our universe is a rich source of different flora and fauna. Flora plays a vital role in our daily life. Plants serve not only as food sources but also as medicinal sources. Many plants are not exposed well to human race with regard to their biological importance. In order to elucidate their biological importance, the prime step is to understand the anatomical and histological features of the plants. One among the plants of this category which is not yet completely experimented is *Alysicarpus longifolius*. The studies may reveal the biologically important constituents in the plant.

II. MATERIALS AND METHODS

Collection of the plant material: The plant, *Alysicarpus longifolius* (Spreng.) Wight & Arn., selected for the present study was collected from Thirumalaisamudram area, Thanjavur, South India, identified with the help of Flora of Presidency of Madras and authenticated by Dr. R. Banumathy, Reader, Post Graduate and Research Department of Botany, Queen Mary's College, Chennai. The voucher specimen numbered PCHC/2009/56 was deposited at the Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai.

Anatomical Studies

Macroscopic Studies: The external characters were determined through simple observations.

Microscopic Studies: Healthy and disease-free plants were selected. The required quantity of whole plant was cut, removed, and then fixed in FAA (formalin 5 mL + acetic acid 5 mL + 70% ethyl alcohol 90 mL). After fixing them for 24 hours, the specimens were dehydrated with graded series of tertiary-butyl alcohol⁷. The specimens were infiltrated by gradually adding paraffin wax (m.p. 58–60°C) till super saturation of TBA solution. They were then embedded in paraffin blocks.

Sectioning work: The specimens embedded in paraffin were sectioned using a rotary microtome. The section thickness was 10–12 µm. The sections were dewaxed by a customary procedure and stained with toluidine blue⁵. The dye rendered blue color to the protein bodies, pink to the cellulose walls, violet to the mucilage, and blue to the lignified cells. The sections were also stained with safranin and Fast-green and KI (for starch). Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after suitable staining.

Photomicrographs: Photographs of different magnifications were taken with Nikon lap photo 2 microscopic units. For normal observations, bright field and for the study of crystals, starch grains and lignified cells, polarized light was employed⁵.

Histochemical study: Histochemical localization of alkaloids, terpenoids, and flavonoids in the root, leaf, and stem was carried out by using the respective staining methods³. The photographs were taken with a Nikon F301 camera or a digital Nikon coolpix 4500 camera.

Alkaloids(Dragendorff's test):The transverse sections of root, leaf, and stem were immersed for a minute in Dragendorff's reagent and then mounted in glycerine-water (10 : 90, v/v) solution. Appearance of golden yellow color in the cells showed the presence of alkaloids.

Terpenoids(2,4-Dinitro phenyl hydrazine test):The transverse sections of root, leaf, and stem were immersed for a minute in 2,4-dinitro phenyl hydrazine reagent and then mounted in glycerine-water (10 : 90, v/v) solution. Appearance of orange yellow color in the cells showed the presence of terpenoids.

Flavonoids(Neu's test):The transverse sections of root, leaf, and stem were immersed in 1% (w/v) 2-aminoethyl-diphenylborinate in absolute methanol for 2–5 minutes and then mounted in glycerine-water (10 : 90, v/v) solution. Appearance of blue fluorescence in the cells showed the presence of flavonoids.

III. RESULTS

Anatomical Studies:

Macroscopic observations: *Alysicarpus longifolius* comes under the family Fabaceae¹. It is an erect herb. The stem is around 1.2–1.5 m tall, slightly striate, and glabrous. The leaves are found to be unifoliate and stalks are 3–10 mm long. The leaflets are 5–15 × 1–2 cm, oblong or lanceolate, and the base is heart shaped. The inflorescence is a dense raceme, 15–30 cm long. The bracts are often longer than 1.3 cm, ovate, and pointed.

Flowers are around 1 cm in pairs, blooming from the base of the spike upward. The standard petal is yellow flushed with red. The wing and keel are dark pink. The pods are 1 cm and 4–6 jointed. The flowering period is the month of September. It is distributed in Saurashtra, Madhya Pradesh, Bombay, Andhra Pradesh, and various regions of Tamilnadu, particularly Coimbatore, Thanjavur, and Tiruchirappalli.

Fig 1.Experimental Plant-*Alysicarpus longifolius* (Spreng.) Wight & Arn

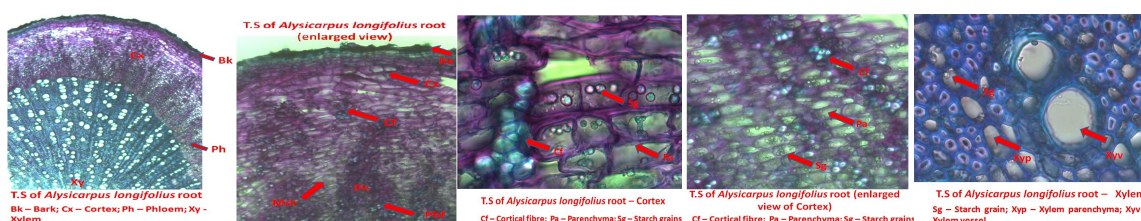


Microscopic observations:

Transverse Section of Root: The transverse section of the root is shown in Figure 2a. It contains root cork, cortex, and vascular bundles. The cork regions consist of the epidermis and the hypodermis. The epidermis is made up of two to three layers of dead cells and is followed by the hypodermis, which consists of three to four layers of horizontally compressed and elongated cells.

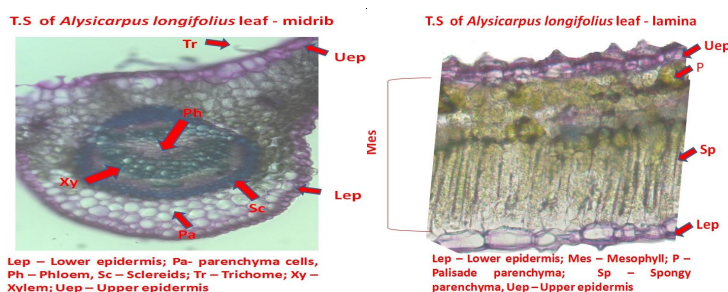
The cortex regions consist of several layers of parenchymatous cells; some of the parenchymatous cells are filled with compound and simple starch grains and prism-type calcium oxalate crystals. Cortical fibers are present as groups and scattered throughout the cortex; one to five fiber cells are present in each group.

The vascular bundles consist of phloem, cambium, and cortex. Phloem regions are made up of two to six layers of irregularly arranged cells; phloem fibers are also present in this region. Some of the phloem cells are filled with starch grains. The cambium is made up of two to four layers of small cells. Xylem occupies the major portion of the root; secondary xylem vessels are larger than primary xylem vessels. Xylem parenchymatous cells are filled with simple and compound starch grains.



Transverse Section of Leaf: The transverse section of the leaf is shown in Figure 2b. Dorsiventral leaves with upper and lower epidermis are present. The epidermal cells are covered with cuticles. Some of the epidermal cells contain unicellular trichomes. Mesophyll tissue consists of palisade cells and spongy cells. Palisade cells are four to six-layered globular, ovoid, elongated, and irregularly arranged cells with chloroplasts. Spongy parenchymatous cells are one to two-layered loosely arranged cells with intercellular spaces.

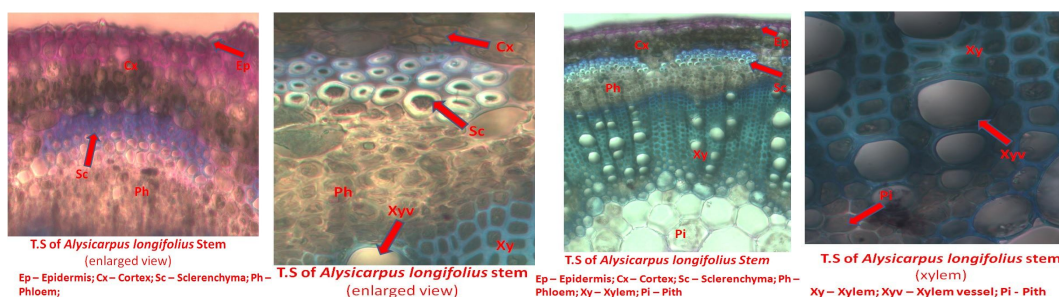
The vascular bundle is bicollateral, and a distinct parenchymatous bundle sheath is found on both sides of the midrib, but the upper side of the parenchymatous cells contains chloroplasts and some of the cells contain prismatic calcium oxalate crystals. Sclerenchyma cells form a sheath on both sides of the vascular bundles. Large xylem and phloem cells are present toward the lower side of the midrib than on the upper side. Protoxylem vessels exist toward the upper side of the leaves. The leaves contain paracytic stomata.



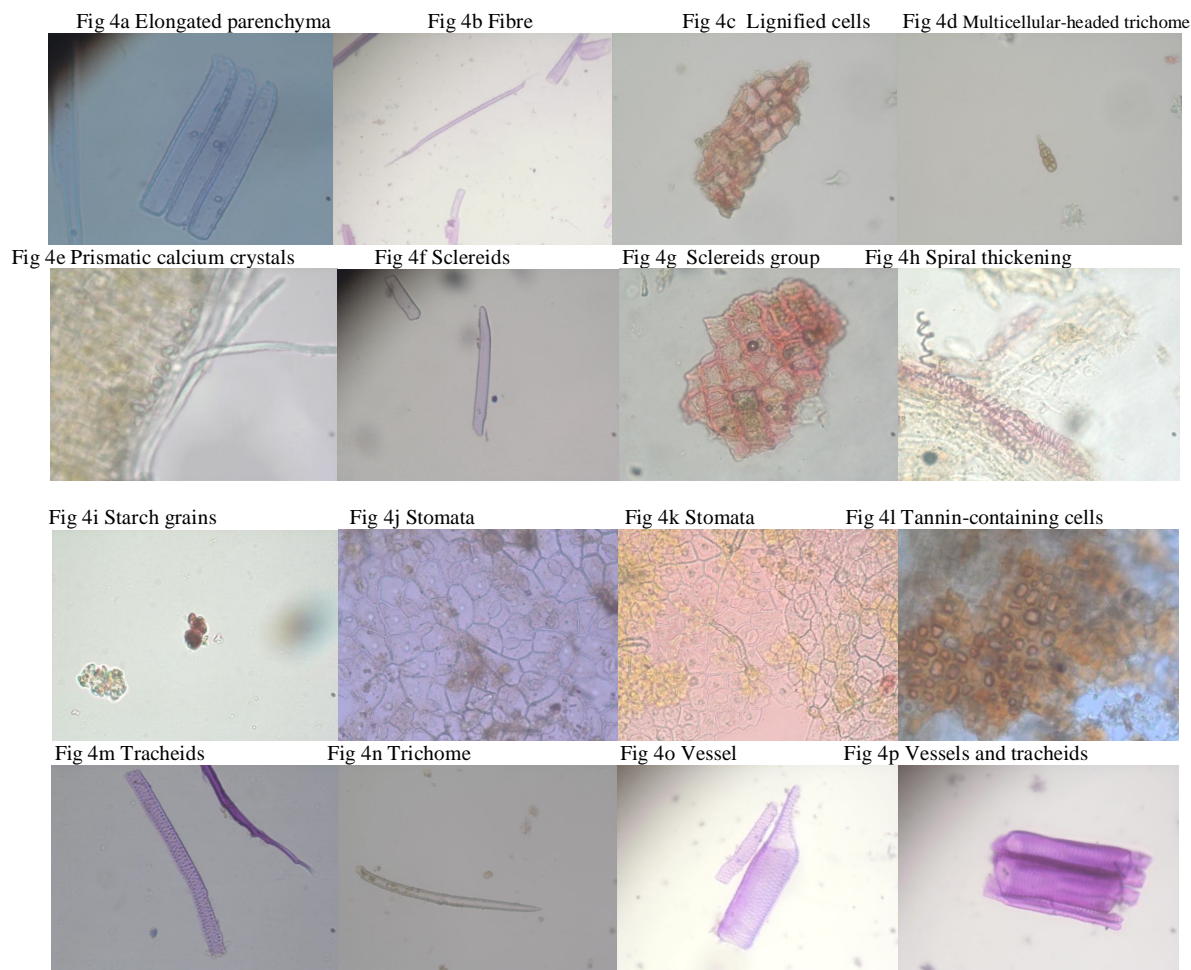
Transverse Section of Stem: The transverse section of the stem is shown in Figure 2c. The stem is circular. The transverse section consists of the epidermis, cortex, pericycle, vascular bundles, and pith. The epidermis contains two to three layers of ovoid parenchymatous cells. The outer epidermal cells have a thick cuticle and unicellular warty trichomes.

Cortex regions consist of four to six layers of ovoid parenchymatous cells; the parenchymatous cells are filled with simple and compound starch grains. The pericycle consists of three to four layers of lignified sclerenchymatous cells forming sclerenchyma sheaths. Pericycle bundle gaps are filled with parenchymatous cells.

The vascular bundles are arranged in continuous rings and contain phloem, cambium, xylem, and xylem parenchyma. Phloem regions are made up of six to eight layers of irregular cells. Some of the phloem cells contain vascular bundles. The cambium contains two to three layers of cells. Endarch xylem consists of xylem vessels, tracheids, xylem fibers, and xylem parenchyma. The xylem parenchyma is filled with starch grains. Xylem vessels are reticulate with pitted thickenings. Pith regions consist of polygonal parenchymatous cells. Some of the parenchymatous cells are filled with calcium crystals and starch grains.



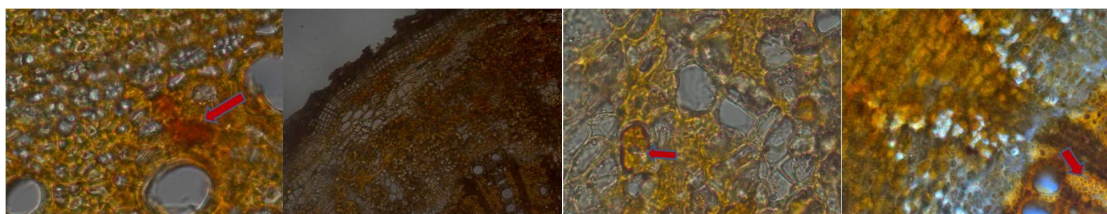
Powder microscopy (Fig 4a-4p): The powder of the whole plant contains unicellular trichomes, compound and simple starch grains, lignified fibers with blunt and tapering ends with pits. Elongated and lignified parenchymatous cells are present. Prismatic calcium oxalate crystals and short tracheids with pitted and reticulate thickenings are present. Xylem vessels with reticulate and pitted thickenings are also found in the powder of *A. longifolius*.



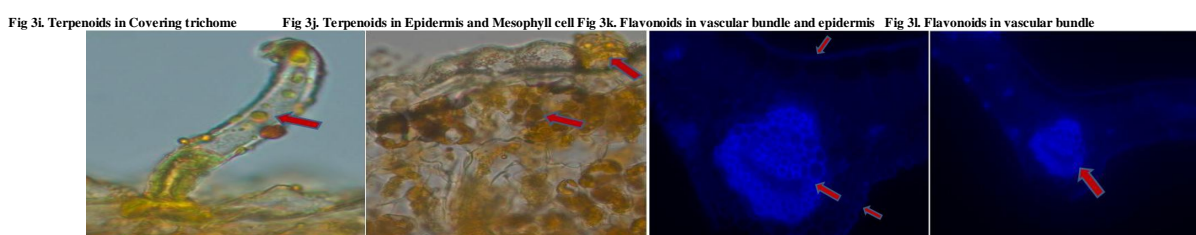
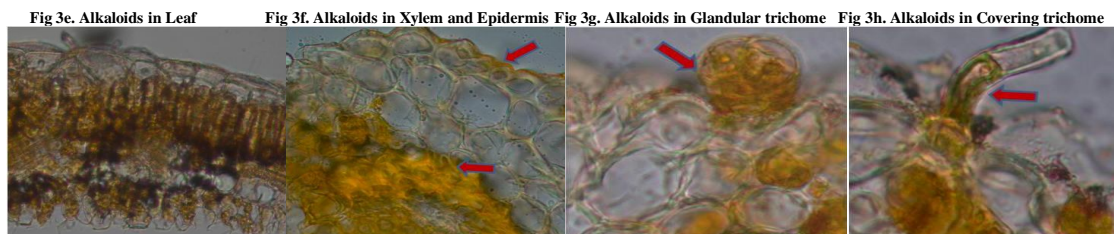
Histochemical study:

Histochemical study on the root of *Alysicarpus longifolius* (Fig 3a–3d) shows the presence of alkaloids (golden yellow color – Dragendorff’s test) in xylem and sclereids and terpenoids (orange color – 2,4-dinitro phenyl hydrazine test) in xylem.

Fig 3a. Terpenoids in Xylem Fig 3b. Alkaloids in Root Fig 3c. Alkaloids in Sclereids Fig 3d. Alkaloids in Xylem

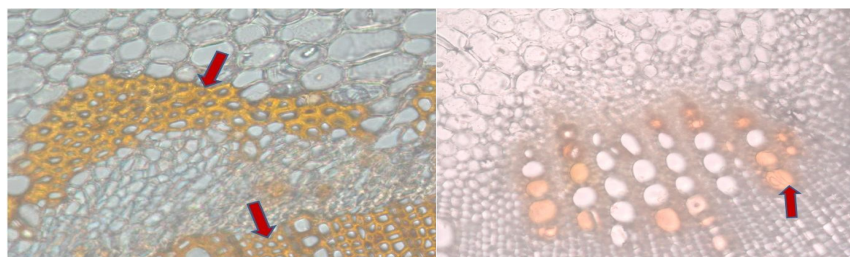


Histochemical study on the leaf of *A. longifolius* (Fig 3e–3l) shows the presence of alkaloids (golden yellow color – Dragendorff’s test) in xylem, epidermis, glandular trichome and covering trichome, terpenoids (orange color – 2,4-dinitro phenyl hydrazine test) in epidermis, mesophyll cells and covering trichome and flavonoids (blue fluorescence – Neu’s test) in the epidermis and vascular bundle of the leaf blade.



Histochemical study on the stem of *A. longifolius* (Figs 3m and 3n) shows the presence of alkaloids (golden yellow color – Dragendorff’s test) in sclereids and xylem and terpenoids (orange color – 2,4-dinitro phenyl hydrazine test) in xylem.

Fig 3m. Alkaloids in Sclereids and Xylem cells Fig 3n. Terpenoids in Xylem cells



IV. DISCUSSION AND CONCLUSION

Traditional medicine shows a positive way for a hale and healthy life style. The significance of traditional medicine as a foundation of primary health care was first authoritatively accredited by the World Health Organization (WHO). This was done in the primary health care declaration of Alma Ata (1978) and had been internationally addressed by the traditional program of WHO since 1978. To inculcate the use of traditional medicine, the biological importance of the plant and its constituents must be elucidated. These features could be revealed through anatomical and histological studies of the whole plant. Several biologically important compounds such as alkaloids, flavonoids and terpenoids have been identified with potent medicinal importance.

Alkaloids, a group of naturally occurring chemical compounds containing mostly basic nitrogen atoms, have pharmacological effects and are used as replenishing drugs⁶. Flavonoids are the ubiquitously distributed group of phenols. A range of biological activity displayed by certain individual members make the flavonoids one of the most intriguing classes of biologically active compounds, termed as bioflavonoids⁴. Terpenoids have anticancer nature². Anatomical and histological studies on plants would present the fundamental details about the plant and the family to which it belongs and provide an idea regarding the medicinal value of the plant¹. This study on the plant *A. longifolius* revealed the structural anatomy of root, leaf, and stem, histological localization of alkaloids, terpenes, and flavonoids, and powder microscopy showing the presence of fiber, calcium, and starch grains, which together contribute to a better understanding of the basic characteristic features of the plant and also its medicinal property.

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