Macro and Microscopical evaluation of Trunk bark of *Ailanthus excelsa* Roxb.

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**INTRODUCTION**

There has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance \(^1\). The use of plant drugs is subject to their correct identification. In general potent drugs are either adulterated or substituted depending upon morphological characters or biological activity. Despite the modern techniques of investigation, identification of plant drugs by pharmacognostical studies is more reliable. *Ailanthus excelsa* Roxb. (Simaroubiaceae) is commonly known as Mahanimb. *Ailanthus excelsa* is a large tree originally from China, is known as the 'tree of heaven'. Different parts of this plant are used widely in traditional medicine for a variety of diseases \(^2\). However there is none or very minute pharmacognostical report on the Macro and Microscopical standards which is required for the quality control of the Trunk bark of crude drug.

**MATERIAL AND METHODS**

**Plant material**

Trunk barks of *Ailanthus excelsa* Roxb. were collected in Aug. 2008 from local area of Pimpri, Pune (INDIA) and identified by the Regional Research Institute of Ayurveda Kothrude, Pune (INDIA). A voucher specimen - 899 was authenticated and provided.

**Chemical and instruments**

The different materials used for the study include basic microscopical instruments like compound microscope, trianocular microscope, glass slides, cover slips, watch glass, and other common glasswares. Microphotographs were taken using Lecia DMLS microscope attached with Letiz MPS 32 camera. Common solvents like ethanol (95%), and reagent like glycerine, Toluidine blue, iodine solution, Phloroglucinol, hydrochloric acid, chloral hydrate, and sodium hydroxide were procured from Ranbaxy fine Chemical ltd, Mumbai (India).

**Macroscopical analysis**

The Macroscopy and morphology of the plant were studied according to the method of Brain and Turner \(^3\).

**Microscopic study of Trunk bark**

The paraffin embedded specimens was sectioned with the help of Rotary Microtome \(^4, 5, 6, 7\). The thickness of the sections was 10 – 12 μm. Dewaxing of the sections...
was done. The sections were stained with Toluidine blue. 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 iodine (for starch). The micro powder analysis was done according to the method of Brain and Turner [8, 9].

EXPERIMENTAL

Macroscopic Evaluation

Barks examined were light greyish-brown colour on outer side and yellow or yellowish brown in inner side. It has rough with irregular fissures. Size: 1.2-2.2cm in length, width 2-3 cm and it varies according to size. Bark is approximately 1 cm thick. Taste: aromatic, slightly bitter. Texture: fibrous and rough. Odour: earthy characteristics. Lenticels are vertically elongated with pointed tips, centrally blistered and whitish brown in colour.

Microscopic characteristics of Ailanthus excelsa Roxb. Trunk bark

Microscopic features

The Trunk bark of Ailanthus excelsa Roxb. can be differentiated into distinct region i.e. the outer bark and inner bark. The entire bark is 2.8 mm wide.

Outer bark

The outer barks measures about 1.5mm in width. The outer bark is thick and broad, differentiated into several successive bands of periderm and non-peridermous tissue of secondary phloem. This compound structured is termed as rhytidome. The phellem has tangential width of 100 μm and phelloderm is 150 μm wide. (Fig. 2.1) Phellem cells are thick walled, suberised, tabular and

Structure of the bark:

![Figure 2.1. T.S of phellem oragnified.](image_url)
occur in radial series. (Fig. 2.1) Phelloderm cells are thin walled and 5 to 8 layers in thickness cubical or rectangular, arranged in compact radial files (Fig. 2.2). The outer surface Trunk bark consists of many irregular fissures of various shapes. The successive periderm originates from the secondary phloem region, hence the sclerides of the secondary phloem held captive in between the periderm zones (Fig. 2.2).

**Inner bark:**

The inner bark is broader than the outer bark and it includes all the secondary phloem tissue. Microscopically the inner bark can be distinguished into two distinct regions i.e. (a) Collapsed secondary phloem region and (b) non Collapsed secondary phloem region.

(a) Collapsed secondary phloem region: (Fig. 2.2; 2.3)

Collapsed phloem region is the outer to inner part of the bark and middle in position. It is the broadest zone it consists of thick block of phloem sclereids forming tangential cylinders; the cylinders are broken by radially intruding phloem rays. Crushed phloem is seen as dark streaks or lines. The collapsed phloem region measures about 1.8mm in width and extends upto the periderm zone. The dilation of phloem rays towards the periphery gets disturbed and occurs in random due to thick blocks of phloem fibers present in this region.

(b) Non collapsed secondary phloem region: (Fig. 1.3; 2.3)

The non collapsed secondary phloem region is innermost region of the bark and it is the narrow zone lying next to the cambial zone. The region is 600 μm in width. In the non- collapsed phloem regions, the cells are intact and occur in radial files. (Fig. 2.3). Sieve tube members, companion cells, phloem parenchyma are intact (Fig. 3.1; 2.3). The phloem rays are narrow and undilated. In transverse section the sieve tube members are tangentially rectangular or polygonal, the walls are thin. The sieve tube members are 40 μm to 50 μm in tangential plane. Phloem parenchyma cells are prominent and occur along the narrow lateral corners of the sieve element. Sieve plate is simple or compound and prominent. (Fig. 3.2)

In tangential longitudinal section, (TLS) the phloem rays appear non storied and the sieve tube members and axial parenchyma are also non storied (Fig. 4.1). (Fig. 4.1, 2; 5.1, 2) The phloem rays are broad, multiseriate, low in height and heterocellular (Fig. 4.2; 51). The cells are angular and thick walled. The rays are 200-900 μm in height and 40-90 μm in breadth. Occasionally uniseriate rays are also evident. The ray cells are angular and compact without intercellular spaces. In TLS view the sieve tube members appear slightly curved; the sieve plates are compound and oblique (Fig. 5.2). The axial parenchyma cells occur in vertical files of rectangular cells along the sieve tube members (Fig. 5.2).

**Cell inclusions:**

(Fig. 6.1, 2; 7.1, 2). Calcium oxalate crystals and starch grains are abundant in the outer bark region. They appear bright against dark back ground when seen through the polarized light. The starch grains are small and loosely arranged. Calcium oxalate crystals are also present in the
Anatomy of the bark: -

Figure 1.1. T.S of bark- outer periderm region. [Pe - Periderm; DR - Dilated Rays; Sc - sclereids; Pd - Phelloderm].

Figure 1.2. T.S bark- middle collapsed phloem. [Cph - collapsed phloem; DR - Dilated rays; Ncph - Non-collapsed phloem; Pd - Phelloderm; Pe - Periderm; Phr - phloem ray; Sc = Scl- sclereids].

Figure 1.3. T.S of bark- collapsed and non-collapsed phloem. [Cph - collapsed phloem; DR - Dilated rays; Ncph - Non-collapsed phloem; Pd - Phelloderm; Pe - Periderm; Phr - phloem ray; Sc = Scl- sclereids].

Figure 2.3. Non collapsed phloem cambial zone. [CZ - Cambial zone; Ncph - Noncollapsed phloem; Pd - Phelloderm; PhR - Phloem ray; Pm - Phellem; Sc - Sclereids].

Structure of the non-collapsed phloem:-

Figure 3.1. T.S of non-collapsed phloem showing companion cells, phloem ray, phloem parenchyma and sieve tube member. [Php - Phloem parenchyma; PhR - Phloem ray; SPI - sieve plate; CC - companion cells; ST - sieve tube member].

Figure 3.2. Sieve plate with sieve tube members enlarged. [Php - Phloem parenchyma; PhR - Phloem ray; SPI - sieve plate; CC - companion cells; ST - sieve tube member].
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**Tangential longitudinal section (TLS) of the phloem:**

![Image 1](image1.png)

**Figure 4.1.** TLS of the phloem under low magnification. [CC - companion cells; CSP - compound sieve plate; Php - Phloem parenchyma; PhR - Phloem ray; ST - sieve tube.]

![Image 2](image2.png)

**Figure 4.2.** Same as above enlarged. [CC - companion cells; CSP - compound sieve plate; Php - Phloem parenchyma; PhR - Phloem ray; ST - sieve tube.]

**Structure of Phloem rays:**

![Image 3](image3.png)

**Figure 5.1.** TLC of the phloem showing uniseriate biseriate and multiseriate rays. [CSP - compound sieve plate; BSR - biseriate ray; Pc - Procumbent cells; Phloem parenchyma; PhR - Phloem ray; ST - sieve tube; URc - upright cell; UR - uniseriate ray.]

![Image 4](image4.png)

**Figure 5.2.** A compound sieve plate with phloem parenchyma and phloem rays enlarged. [CSP - compound sieve plate; BSR - biseriate ray; Pc - Procumbent cells; Phloem parenchyma; PhR - Phloem ray; ST - sieve tube; URc - upright cell; UR - uniseriate ray.]

**Crystals distribution in the bark (under polarized light microscope):**

![Image 5](image5.png)

**Figure 6.1.** Non-collapsed phloem showing druses under low magnification. [Cr - Crystals; PhR - Phloem ray; ST - sieve tube.]

![Image 6](image6.png)

**Figure 6.2.** Same as above enlarged. [Cr - Crystals; PhR - Phloem ray; ST - sieve tube.]
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**Crystals distribution in the non-collapsed phloem [under polarized light microscope]:**

Figure 7.1 Prismatic crystals in the phelloderm.
[Cr - Crystals; Pd - Phelloderm; Sc - sclereids; SG - starch grains.]

Figure 7.2 Crystals and starch grains enlarged.
[Cr - Crystals; Pd - Phelloderm; Sc - sclereids; SG - starch grains.]

secondary phloem region. They are predominant in the phloem ray. The crystals are typically sphenoid or druses type (Fig. 6.1, 2). Prismatic crystals are seen in the phloem (Fig. 7.1, 2). They are rhomboidal and cuboidal type. The druses are 30μm wide. The prismatic crystals are 20×50 μm in size. The starch grains are simple type; they are ovoidal to elliptical. The hilum is excentric or centic in position (Fig. 7.1, 2).

**Powder microscopy:**

The bark powder shows druses and prismatic crystal of calcium oxalate. Starch grains are also abundant in the powder. The powder also contains Brachysclereids and fibers. Brachysclereids are isodiametric or elongated (Fig. 8.1, 2). They have thick wall and wide lumen. The fibers are long, narrow cells with pointed ends they have thick lignified walls and reduced lumen. The fibers are 600-800 μm long and 20 μm thick.

**DISCUSSION**

The quality control parameters for the crude drugs as raw materials were established with the help of several official determinations based on morphology, microscopy and physico-chemical studies. These studies were aimed at ensuring standardization of herbal drug under investigation. Morphological examination of drugs refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impression on organs of senses. All these parameters were recorded for Trunk bark of the plant *Ailanthus excelsa* Roxb. These were helpful in primary identification of *Ailanthus excelsa* Roxb. Microscopical
techniques provide detailed information about the crude drug. Microscopical inspection of crude drugs from plant origin is essential for the identification of the grounded or powdered materials. Rhytidome in outer bark, Collapsed and non Collapsed secondary phloem region in inner bark are the identification characters.

Though microscopy alone cannot provide complete evaluation profile of a herbal drug, still it can provide supporting evidence, which when combined with other analytical parameters can be used to obtain the full evidence for standardization & evaluation of herbal drugs \(^{[11]}\). The above study helps us to standardize the plant or herbal drugs which provide the healthy and maximum potent drug in the market.

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