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Histological study and thin layer chromatographic profile of stem-bark and leaf of *Holoptelea integrifolia* (Roxb.) Planch

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ABSTRACT

Holoptelea integrifolia (Roxb.) Planch., a multifaceted drug, extensively used in Ayurveda, Siddha and other various systems of medicine. Leaves and bark are the officinal parts of the plant and used for various ailments. In the present investigation, histological and thin layer chromatographic identification of stem-bark and leaf has been studied in detail and provided diagnostic key to identify the original drug from the adulterant(s).

Keywords: *Holoptelea integrifolia*, Bark, Leaf, Histology, Thin layer chromatography, Ayurveda.

INTRODUCTION

Holoptelea integrifolia (Roxb.) Planch. belongs to the family Ulmaceae, is a versatile herbal drug in Ayurveda, popularly known as *Cirabilvaḥ*, *Pūtīkarañjaḥ*. Bark and leaves are the officinal parts. The drug is reported to be bitter, astringent, hot, digestive, laxative and anthelmintic. It purifies blood and overcomes dyspepsia, vomiting, intestinal disorders, piles, flatulence, sprue, diabetes, leprosy and other skin diseases and used to prepare *Cirivilvādi kaṣāyaṃ, Ayaskṛti, Indukāntaghṛtaṃ* and *Valiya Pañcagavyaghṛtaṃ* [1]. Plant used in the treatment of edema, bronchitis and obesity. Juice and mucilage taken from the bark used for rheumatism and intestinal tumour. Bark paste externally applied for inflammation of lymph gland, scabies, common fever, ring worm, swellings and herpes simplex infection [2, 3]. Bark extracted in coconut oil and mixed with garlic applied to treat eczema. Bark cut into small circle or a coin shape and fixed on left arm for the treatment of malaria and also used to treat intestinal cancer [4-6]. Leaves decoction used to treat ringworm, eczema, regulate fat metabolism and cutaneous diseases. Leaf bud mixed with lemon juice externally applied for the treatment of hair loss. Leaf past is externally applied for treatment of leucoderma [7, 8]. The drug also possesses antibacterial, anti-inflammatory, adaptogenic, antioxidant, antitumour, anticancer, wound healing, antiulcer, analgesic, hepatoprotective activities [9].

Many workers have been studied the pharmacognosy of leaf and root-bark of *Holoptelea integrifolia* [2, 10, 11]. However, there is lack in comprehensive anatomical characters. Hence, the present study was carried out to provide the complete anatomical diagnostic characters of the leaf and stem-bark of *Holoptelea integrifolia*.

MATERIALS AND METHODS

Anatomical studies

Fresh stem-bark and leaves were collected from mature tree growing near the place Uthukkottai, Thiruvallur district, Tamilnadu and authenticated using regional flora. The samples were cut in to small pieces and fixed immediately in FAA for 24 hrs and embedded in paraffin wax after dehydration and infiltration. Sections were taken using rotary microtome to the thickness of 8-12 μm, stained with toluidine blue and photographed. For leaf clearing, small pieces were treated in 5% NaOH at 60 °C for 3 hrs, washed with distilled water thoroughly and stained with safranin [12].

Thin Layer Chromatographic (TLC) Analysis

For the TLC analysis, stem-bark and leaves samples were shade dried for a week and powdered. 2 g of each powdered samples was extracted under reflux with methanol in a water-bath 3 times, then concentrated and dried. The residue was re-dissolved in methanol and used for the TLC spotting. For stationary phase precoated Silica Gel F^{254} (Merck) plate and for mobile phase Toluene : ethyl acetate (3:1) was used. After the plate development, dried, observed under UV-254 nm and recorded the spots. Then the plate was dipped in 1 % Vanillin H_2SO_4 and heated at 105 °C for colour development and the spots are

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recorded.

RESULTS

Macroscopic characters of stem-bark (Figure – 1: 1-2)

The bark is even or somewhat curved, from thickness of 8 to 15 mm, external surface is grey with thin flakes, small irregular longitudinal fissures and uneven due to exfoliation of cork. Internal surface is smooth, pale white to light brown, fibrous with characteristic odour.

Microscopic characters of stem-bark (Figure – 1: 3-5)

Bark is separated into periderm (outer) and secondary phloem (inner). Periderm consists of phellem, phellogen and phelloderm regions. Phellem cells are structured into thin tangential layers and the older layers exfoliates to thin membranes with distinct fissures. The phelloderm region is wide and distinctly thin walled cells and fibres. Secondary phloem distinguished into narrow non-collapsed zone inner and broad collapsed zone outer. The non-collapsed region consist radialy sieve tube members, axial parenchyma and group of fibres. The external collapsed phloem has dilated rays, crushed sieve tube members, thick walled lignified fibres (Figure – 1: 3). Phloem rays are uniseriate, multiseriate and homocellular (Figure – 1: 4). Prismatic type of calcium oxalate crystals found abundantly (Figure – 1: 5).

Macroscopic characters of the leaf (Figure – 2: 1)

Leaves simple, alternative, dark green above, light green below, penninerved, 8 – 13 × 4 – 6 cm., elliptic, acuminate, glabrous, entire, base rounded or cordate, leathery; petiole 6 – 13 × 0.1 – 0.15 cm.

Microscopic characters of the leaf (Figure – 2: 2-7)

Petiole (Figure – 2: 2-3)**:**

The transverse section of petiole shows bowl shape in outline, slightly concave in adaxial surface. The epidermis is single layer, thin, cubical shaped cells with unicellular filiform trichomes. Four to five layers of collenchymatous cells are present followed by epidermis. In center a large bowl shaped closed type of vascular bundle is present. Xylem region is surrounded by the phloem. Three to four layers of tannin containing parenchyma cells are distinctly surrounded the vascular bundle. The center region of vascular bundle also occupied by the tannin cells (Figure – 2: 2). Druse types of calcium oxalate crystals are present in the phloem region (Figure – 2: 3).

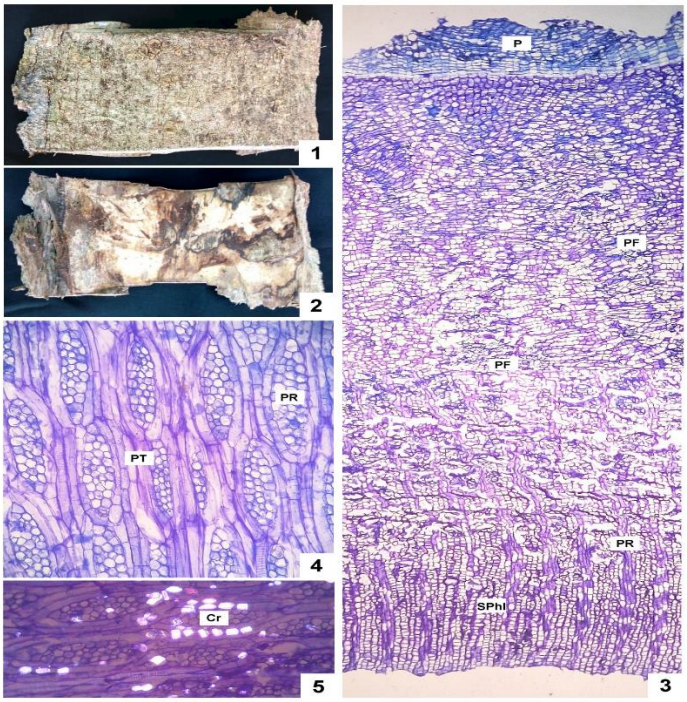
Lamina (Figure – 2: 4-7)**:**

The midrib has convex in adaxial surface, a single layer epidermis having cuboid shape cells with thick cuticle. Two to three layers of collenchyma cells followed by epidermis. A bowl shaped large vascular bundle in dorsal side and small circular shaped vascular bundle in ventral side consisting xylem towards inner side and phloem towards outer side. A distinct layer of tannin containing cells are surrounded the vascular bundles. Unicellular filiform trichomes are present in the dorsal epidermis of the midrib (Figure – 2: 4). Druse type crystals are present in the phloem region of the vascular bundles (Figure – 2: 5). In lamina, the upper epidermis has large, cuboid or rectangle shaped cells and the lower epidermis has comparatively small, cuboid shaped cells. Both epidermis has thick cuticle. Followed by the upper epidermis, narrowly cylindrical shaped palisade parenchyma cells are present followed by spherical shaped spongy parenchyma cells forming a network. Vascular bundles are distinct, circular, containing tannin cells. Large size of air chambers present in the spongy parenchyma region (Figure – 2: 6). The stomata anomocytic type and hypostomatic. Glandular types of trichomes are present in lower epidermis (Figure – 2: 7). Calcium oxalate crystals are no evident in the lamina region.

Thin Layer Chromatographic (TLC) analysis (Table -1; Figure – 3)

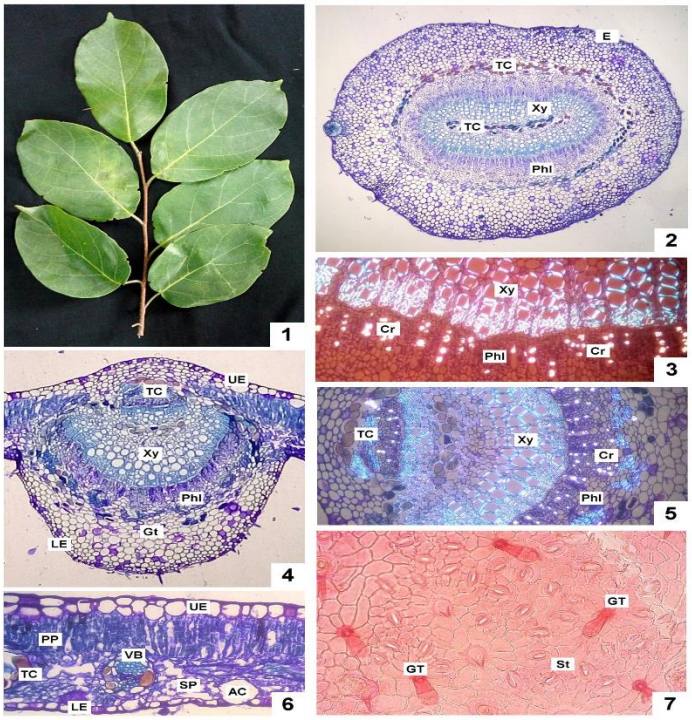
Table 1: The TLC profile and Rf – values of leaf, stem-bark as follows

Leaf		Stem-bark	
Rf - Values UV-254 nm (Before spray)	**Rf – Values Visible light (After spray)**	**Rf - Values UV-254 nm (Before spray)**	**Rf – Values Visible light (After spray)**
0.15 (Black)	0.13 (Green)	0.28 (Black)	0.26 (Violet)
0.25 (Black)	0.20 (Green)	0.36 (Black)	0.38 (Blue)
0.39 (Black)	0.33 (Blue)	0.65 (Black)	0.50 (Purple)
0.52 (Black)	0.45 (Green)	0.78 (Black)	0.60 (Purple)
0.65 (Black)	0.50 (Purple)		0.67 (Violet)
0.76 (Black)	0.58 (sky blue)		0.84 (Purple)
	0.60 (Purple)		
	0.79 (Green)		
	0.83 (Violet)		



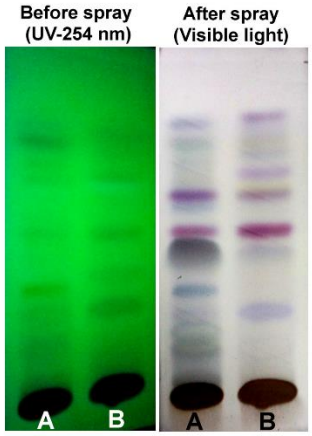
1. External feature
2. Internal feature
3. Cross section of bark
4. TLS of bark
5. Prismatic crystals in bark TLS

Figure 1: Morphology and anatomy of stem-bark of *Holoptelea integrifolia*



1 External feature 2. Cross section of petiole
3. Petiole portion enlarged (under polarized light) 4. Cross section of leaf midrib
5. Midrib portion enlarged (under polarized light) 6. Cross section of lamina
7. Leaf lower epidermis – Stomata & gland

Figure 2: Morphology and anatomy of leaf of *Holoptelea integrifolia*



A – Leaf B – Stem-bark

Figure 3: TLC fingerprint of leaf and stem bark

AC – Air chamber; E – Epidermis; Cr – Crystals; Gt – Ground tissue; GT – Glandular trichome; LE – Lower epidermis; P – Periderm; Phl – Phloem; PF – Phloem fibre; PP – Palisade parenchyma; PR – Phloem rays; SP – Spongy parenchyma; SPhl – Secondary phloem; PT – Phloem tube; St – Stomata; TC – Tannin cell; UE – Upper epidermis; Xy – Xylem;

DISCUSSION

Plant origin drugs are widely used in traditional systems of medicine such as Ayurveda, Siddha, Unani and Homeopathy. Standardization of these herbal drugs is an essential tool in order to establish their identity, purity, safety and quality. To standardize or authenticate a drug, various parameters such as macroscopic, microscopic and phytochemical analysis are done. Microscopic evaluation and thin layer chromatographic fingerprint is one of the simplest and inexpensive methods to establishing the correct identification of the source material and useful for setting standards for crude drugs [11].

Holoptelea integrifolia (Roxb.) Planch. is a multifaceted drug, extensively used in traditional medicines for various ailments. In the present investigation, we have studied the detailed anatomy characters and thin layer chromatographic fingerprint of stem-bark and leaf of *H. integrifolia* and provided the following key diagnostic features.

Stem-bark

Distinct periderm with fissures, broad zone of secondary phloem, abundant patches of phloem fibres, phloem rays uniseriate or multiseriate, homocellular, presence of prismatic type of calcium oxalate crystals.

Leaf

Petiole

Bowl shape outline, slightly concave in adaxial surface, unicellular filiform trichomes in epidermis, large bowl shaped closed type vascular bundle, layers of tannin cells distinctly surrounded the vascular bundle and center region and druse types of calcium oxalate crystals in phloem region.

Lamina

Convex adaxial surface, bowl shaped large vascular bundle in dorsal side and small circular shaped vascular bundle in ventral side, distinct layer of tannin cells surround the vascular bundles, druse types of calcium oxalate crystals are present in the phloem region, presence unicellular filiform trichomes in dorsal epidermis of the midrib, distinct circular vascular bundles containing tannin cells, presence of air chambers present in spongy parenchyma, hypostomatic and anomocytic type of stomata, presence of glandular trichomes lower epidermis and no evident of calcium oxalate crystals in lamina.

TLC fingerprint: The Rf- values and colour of the spot shown in Table – 1 and Figure - 3 can largely distinguish the leaf and stem-bark.

CONCLUSION

The present study provides key histological diagnostic characters and TLC fingerprint which can be considered as distinctive enough to identify the adulterants from commercial drug.

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