**RESEARCH ARTICLE**

# Pharmacognostical Studies on the Leaves of *Caesalpinia sappan* Linn (Fabaceae)

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**ABSTRACT**

Botanical medicine, herbalism, and herbal medicine are all terms that describe the same practice. There are many different chemical compounds found in and produced by herb plants that have physiological effects. Traditional herbal medicine is an integral part of the medical systems of all indigenous peoples, including Ayurvedic, naturopathic, homeopathic, traditionally oriental, and native American Indian medicines. The amount and quality of medicinal components determine the medicinal plant's therapeutic effectiveness. Misidentification is the first step in the unlawful use of plant-based materials or herbal remedies. Pharmacognostical studies of medicinal plants by proper standardization parameters ensures plant identification and authentication. Brazil, or Sappan wood, is a popular name for *Caesalpinia sappan* Linn., which belongs to the Fabaceae family. In Hindi, it is referred to as Bakam/Patang. The Malay Peninsula, Sri Lanka, India, Myanmar, and Vietnam all have a long history of using the medicinal herb *C. sappan* Linn. heartwood is beneficial for Pitta, burning feelings, injuries, ulcers, leprosy, dermatological ailments, dysentery, diabetes, loose stool, and more, according to Ayurveda. The tree's huge, decorative panicles of yellow blossoms are the reason it's grown in gardens. The plant grows rapidly after being produced from seed. Cultivated throughout every region of tropical Asia, this spreading shrub or tree may reach a height of 10 m. It is native to and exists in the wild in places like the southern part of India, the West Bengal region, Orissa, Madhya Pradesh, Malaya, as well as Sri Lanka. The wood has an especially smooth, uniform appearance, is somewhat massive, and has a strong, orange-red color. Its grain is straight. Branchlets, pubescent and rufous, equipped with tiny prickles. Pinnae are 9 to 14 pairs in number. Leaves are big, hairy to glabrous, and have little prickles at the base and leaflets are subsessile, oblong, membranous, and obliquely truncate, with 10 to 20 pairs per pinna. Primarily, it promotes blood flow and acts as an analgesic, emmenagogue, hemostatic, and anti-inflammatory in traditional Chinese medicine for traumatic diseases. One of the ingredients of the world-famous Indian toothpaste and powder Vicco-Vajradanti is the wood of the *C. sappan Linn* Tree. Some research suggests that *C. sappan* Linn. may have antidiabetic effects. The flavonoid of *C. sappan* Linn. wood extract is protosappanin, Brazilin, and gallic acid compounds. Brazilin has a mechanism of action to increase the production of fructose 2,6 bisphosphate and hexose six phosphate. Fructose 2,6 bisphosphate in the metabolic process has a role in regulating glycolysis and gluconeogenesis in the liver. Increased fructose 2,6 bisphosphate in a state of hyperglycemia will stimulate the process of glucose breakdown (glycolysis) by activating phosphofructokinase 1 intended for the purpose of can be a reduction in blood glucose levels in body. This paper deals with the morphological studies and microscopical studies carried out on the leaves of *C. sappan* Linn according to the World Health Organization (WHO) accepted parameter for the identification of medicinal plants. For a better understanding of structure, zooming in using a Zeiss Axio Cam Erc5s digital camera and a Nikon ECLIPSE-E200 trinocular microscope under strong field lighting also employed.

**Keywords**: *Caesalpinia sappan* Linn., Brazilin, Antidiabetic, Morphological, Microscopical, Trinocular microscope attached with digital camera.

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## INTRODUCTION

The practice of employing herbs for their medical benefits or therapeutic properties is known as herbal medicine, herbalism. Herbs are plants or parts of plants that are prized for their culinary, medicinal, or fragrant properties. Herb plants possess and release a wide range of physiologically active compounds. Four billion individuals, or 80% of the global population, utilize medicines made from herbs in some way for basic medical treatment, according to the World Health Organisation (WHO). Integral to the medicinal practices of Ayurveda, homeopathy, naturopathy, and traditional eastern medicine, including native American Indian medicines, is the consumption of herbs.[1] Herbal medications have little to no negative effects, are inexpensive and are widely available; nonetheless, they are susceptible to contamination. The greater the efficacy of an herbal medicine, the higher the demand and the greater the likelihood that it may be unavailable. The amount and quality of chemical components determine the medicinal plant's therapeutic effectiveness. Misidentification is the first step in the abuse of natural goods or herbal remedies. The most prevalent mistake is the incorrect assignment of a usual vernacular term to many species.[2] Investigating medicinal plants *via* a pharmacognostic perspective may resolve all these issues. Establishing pharmacognostic criteria for medicinal plants employed in a variety of medications is crucial. In order to avoid adulterations, pharmacognostical investigations verify plant authenticity and provide helpful standardization standards. Natural product safety and effectiveness may be assured by these investigations, which also aid in plant authentication and guarantee the repeatable quality of herbal goods.[3]

A member of the Fabaceae family, *Caesalpinia sappan Linn*. goes by many names: Sappan in English, Pattanga in Ayurveda, Bakam in Unani, and Patangam in Siddha.[4] The plant's native homeland was India, but it has since expanded over Thailand, Burma, Indo-China, Malaysia, the Philippines, Sri Lanka, Hong Kong, including Hawaii. Additionally, this species of plant variety prospered and expanded throughout Asia, Europe, and the Americas. The scientific name of Secang (Indonesia) is *C. sappan Linn*, and its synonym Biancaea is 'sibukao' (Filipino), 'sbaeng' (Cambodia), 'teingnyet' (Burma), 'fangdeeng' (Laos), then 'faang' (Thailand).[5] There are around 500 species in this genus, and they're all throughout the subtropical as well as tropical regions.[6]

The Malay Peninsula, Sri Lanka, India, Myanmar, and Vietnam all have an extensive record of cultivating the medicinal herb *C. sappan Linn*. Heartwood is beneficial for Pitta, burning feelings, injuries, ulcers, leprosy, skin illnesses, dysentery, diabetes, diarrhea, and more, as stated by Ayurveda.[7] It is usual practice to extract red dye from the plant's heartwood. Traditional Thai medicine has anecdotally treated TB, diarrhea, dysentery, skin diseases, and anemia using the heartwood of the *C. sappan Linn*. tree.[7] The tree's huge, decorative panicles of yellow blossoms are the reason it's grown in gardens. When its branches are interwoven, they form an immense barrier. It grows rapidly after being produced from seed. Cultivated all throughout tropical Asia, this spreading shrub or tree may reach a height of 10 m. It is native to and found in the wild in places like the southern part of India, West Bengal, Orissa, Madhya Pradesh, Malaya, as well as Sri Lanka.[8] The wood has a fine, uniform appearance, is somewhat hefty, and has a strong, orange-red color. Its grain is straight. Fringed with tiny prickles, the branches are rufous and hairy. Each pinna has 9 to 14 pairs of leaves, which range in size from big and hairy to glabrous and carry little prickles at the base. The leaflets are subsessile, oblong, membranous, and obliquely truncate, with 10 to 20 pairs per pinna.[9]

The panicles of flowers are approximately 30 to 40 cm long, ending at the base of the top leaves; the pedicels measure 1.3 to 1.5 cm long; the caducous bracts are approximately 8 mm long; and the panicles hold the flowers. A long, leathery, hairless calyx about 11 mm in length and 2 cm in diameter supports a yellow corolla with orbicular subequal petals that are spotted with red at the base. The stamens are thin and fragile, white and waxy, with thickly woolly filaments at the base. Indehiscent pods measuring 7 to 10 through 3.8 to 5 cm in size, with a firm returning small beak at the upper curve of the obtuse apex, are densely woody, obliquely oblong, laterally squeezed and polished. Nuts 3/4. Sapwood, the exterior layer of wood, possesses a light buff or white tone, whereas heartwood, the interior layer, is a vibrant orange.[10,11] Many years ago, this wood was a key component in the calico printing process for silk, wool, and cotton. Synthetic dyes have mostly supplanted it, actually.[12] The species *C. sappan Linn* is known. In addition to its function in improving complexion, heartwood is known to alleviate swelling and discomfort from exterior injuries.[13]

Its primary functions in traditional Chinese medicine are those of an emmenagogue, hemostatic, anti-inflammatory, analgesic, and agent that promotes blood flow in cases of trauma.[14] Vicco Vajradanti, a well-known Indian dental paste and powder, contains wood from the *C. sappan* Linn. tree. Movable tooth structure, aphthous ulcerations, stomatitis, as well as gum erosions may all benefit from the wood's strong astringent, haemostatics, and healing characteristics, which aid in halting bleeding throughout the gums as well as provide them rigidity and strength. Asavams and Arishtams, including Chandranasavam, Useerasavam, Dasamoolarishtam, Mritasanjeevani Sura, and Saribadyasavam, are just a few of the many ayurvedic formulations that often include *C. sappan* Linn. Some examples of ghritams are Mahakalyana ghritam, Kalyana ghritam, Sarvamayanthaka ghritam, Dasaswarasa ghritam, and Brihachagaladi ghritam. Some examples of Thailams such as Triphaladi, Irimedadi, and Bala, and Kashayams such as Drakshadi, Mahatiktakam, and Kashayam.[15] The species of plant is utilized to make the traditional medicine "Lukol," which has shown promising benefits in treating non-specific leucorrhoea (post IUD) and bleeding after the installation of an intrauterine device (IUD).[16] "Yellowwood" refers to the species of *C. sappan* Linn. tree, the outer layer of bark of which has been utilized to create a yellow dye. There have been reports that the dye is a harmless natural colorant with therapeutic properties and is also utilized in medicines.[17]

In contrast to other metabolic disorders, diabetes mellitus does not cause death directly. Amputations caused by gangrene, failure of the kidneys, cardiovascular disease, and visual impairment are all outcomes of diabetes mellitus. Chronically elevated blood glucose levels harm blood vessels, which in turn impacts the functioning of the kidneys, nerve cells, and heart, and individuals having diabetes mellitus are 2 to 3 times more likely to suffer from cardiovascular disease.[18] There were 14.9% of 187 diabetes mellitus patients experiencing cardiovascular complications, and as many as 57.7% had died due to cardiovascular complications in diabetes mellitus patients.[19] The cardiovascular complication in diabetics is associated with hyperlipidemia, obesity, and high blood pressure.[20] Plants of many different kinds contain phenolic substances called flavonoids. The antidiabetic effect of this compound is based on its ability to regenerate injured pancreatic beta cells, which in turn boosts glucose tolerance, minimises glucose absorption, and regulates the expression of enzymes are substances that involved in carbohydrate synthesis.[21,22] Flavonoids found in the wood extract of *C. sappan* Linn. include gallic acid compounds, protosappanin, Brazilin, and Brazilin.[23,24]

It has been shown that *C. sappan* Linn may have anti-diabetic qualities.[25,26] The main chemical constituent of *C. sappan* Linn is flavonoid Brazilin. Moreover, the molecule enhances glucose transport by engaging GLUT4. Brazilin has an additional effect on the pancreas that causes blood glucose levels to drop. Brazilin enhances the peripheral utilization and activity of enzymes that break down glucitiose. An acting mechanism of Brazilin is to enhance the synthesis of hexose-6-phosphate (H-6-P) and fructose-2,6-bisphosphate (F-2,6-BP).[27] Fructose-2,6-bisphosphate in the metabolic process has a role in regulating glycolysis and gluconeogenesis in the liver. Increased fructose 2,6 bisphosphate in a state of hyperglycemia will stimulate the process of glucose breakdown (glycolysis) by activating phosphofructokinase-1 in order for the body's blood glucose levels to drop.[28,29] In this present study medicinally important drug of *C. sappan* Linn. is studied from microscopy and morphological point of view.

## MATERIALS AND METHODS

### Collection and Identification

The preset substantial for investigation was collected from Tirunelveli, Tamil Nadu, India. For identification of *C. sappan* Linn. leaf sample submitted to Sidha Central Research Institute, Chennai and it was examined and verified by Dr. K.N. Sunil Kumar Research Officer and Head of Department of Pharmacognosy department, Sidha Central Research Institute, Arumbakkam, Chennai 600106, Tamil Nadu. The plant was identified then given voucher specimen (PCOGOO2-ACF) Certificate no .331.18072201.

### Organoleptic Characters

The easiest and fastest way to determine the identification and purity of a medicine is by organoleptic characteristics, such as sensory organs. Characteristics of organoleptic interest include the size, shape, color, odor, taste, and cracks in the bark of stems and leaves, as well as venation, inflorescence, and the leaf's edge, apex, and base surface.[30]

### Morphological Studies

The macroscopical investigation is the morphological description of the components of plants that can be observed with a microscope or the human eye. External feature of test sample was documented using Nikon D-5600 Digital camera.

*Microscopical studies*

Taking the right sections of the plant components under research allows for the microscopical investigation, which is anatomical examination. It is possible to record each unique attribute, and the powder study even preserves some of them. Clear sections may be obtained using a variety of compounds, including safranine, phloroglucinol, methyl orange, chloral hydrate, and others. *C. sappan* Linn. samples were fixed in FAA for over 48 hours so that microscopic examination could proceed. Safranin was applied to thin transverse portions cut from the conserved specimens utilizing a sharp blade.[31-33] Under nearly strong field lighting, images of the transverse sections were captured utilizing a digital camera equipped with a Zeiss-Axio Cam Erc5s and a Nikon-ECLIPSE E200 trinocular microscope. The scale bar was utilized to denote the magnifications.

*Quantitative microscopy*

To identify, determine purity, and evaluate crude leafy drugs, it is necessary to boil fresh leaf samples with a 0.1% chloral hydrate solution and prepare slides for quantitative microscopy of specific pharmacognostic parameters, such as vein-islet numbers, vein-termination numbers, stomatal index, stomatal numbers and palisade ratio. The following parameters were measured: palisade ratio, stomatal index, epidermal number, vein termination, stomatal number, and vein islets.

*Powder microscopy*

The only difference between microscopic research and powder microscopy is that powder microscopy uses dried powder rather than plant sections. Some of the compounds utilized for powder microscopy include phloroglucinol, methyl orange, safranine, chloral hydrate, and others. Before using chloral hydrate to clean the sample, followed by potassium iodide solution to analyze the starch grains, a little pinch of the powdered *C. sappan* Linn was positioned on a microscope slide with a small amount of 50% glycerol.[34-36] A Nikon ECLIPSE E200 trinocular microscope coupled with a Zeiss ERc5s digital camera was utilized to interpret the characters in strong field light. Observations of diagnostic characteristics were reported using photomicrographs.

## RESULT

### Macroscopy

Leaves compound bipinnate, upto 20 to 45 cm long and 10 to 20 cm wide, individually contained of 8 to16 (pairs) sets of as much as 20 cm lengthy pinnae. Individual pinnae are

contained of 10 to 20 (pairs) leaflet sets. Figure 1 shows the sub-sessile, oblong leaflets, which are 10 to 20 mm in length and 6 to 10 mm in width, with an oblique base and a rounded or emarginated tip, dried leaf grayish in color; odor characteristic with slightly sour in taste.

**Microscopy**

*Rachilla*

Transverse section (TS) of the rachilla is nearly oval in shape; a thick cuticle covers the single-layered epidermis and some covering trichomes; the multilayered cortex, surrounded by two layers of collenchyma cells, is formed of three to four layers of parenchyma cells; ground tissue possesses continuous vascular bundles which are closed, collateral and covered with four to five layers of thick pericyclic fibers; prismatic crystals are seen distributed throughout the pericyclic fibers; phloem consists of usual elements; xylem comprises of centrifugally organized vessels encircled by tracheid's and fibers; central parenchymatous pith is present (Figure 2a, 2b, 2c).

Col refers to collenchyma, Cu refers to the cuticle, E refers to the epidermis, Pa refers to parenchyma, PCr refers to the prismatic crystal, and Per refers to pericycle. Phloem is represented by the abbreviation "Ph" while trichome is represented by the abbreviation "T". Vessel

**Leaflets**

*Midrib*

A transverse section of the leaflet, going through the midrib, reveals an epidermis that consists of a single layer and is covered by a thick cuticle; two to three layers of collenchyma cells are present upon the vascular bundle; centrally positioned vascular bundle remains closed collateral and surrounded by one to two layers of thick pericycle; phloem is present towards the lower side and xylem toward the upper side (Figure 3a, 3b).

*Lamina*

A dorsiventral, hypostomatic transverse section of the lamina reveals an epidermis with a single layer and a thick cuticle covering the bottom and top layers; few trichomes are seen in the upper epidermis; single layered palisade followed by up to 3 layers of sponge cells of parenchyma containing intercellular gaps and the mesophyll area is characterized by the presence of veins (Figure 3c, 3d).

Cu is for cuticle; LE stands for lower epidermis; Me stands for mesophyll; Pa stands for parenchyma; Pal stands for palisade; Per stands for pericycle; Ph for phloem; SP for spongy parenchyma; T for trichome; UE for upper epidermis vein, sometimes known as a vein.

*Quantitative microscopy*

Table 1 displays the quantitative characteristics derived from the microscopic examination of leaf epidermal peelings. Figure 4 demonstrates that whereas there are many paracytic stomata on the bottom epidermis, none of them are present on the top surface.

**Figure 1:** *C. sappan* Linn. pinnae

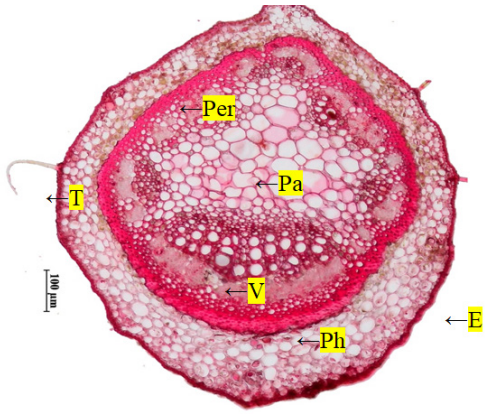



**Figure 2:** (a) TS of *C. sappan* Linn. rachilla

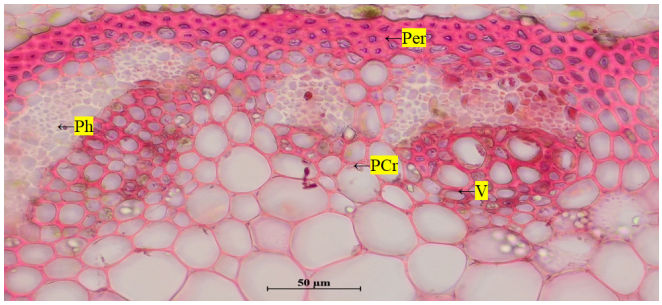



**Figure 2:** (b) Upper portion enlarged view

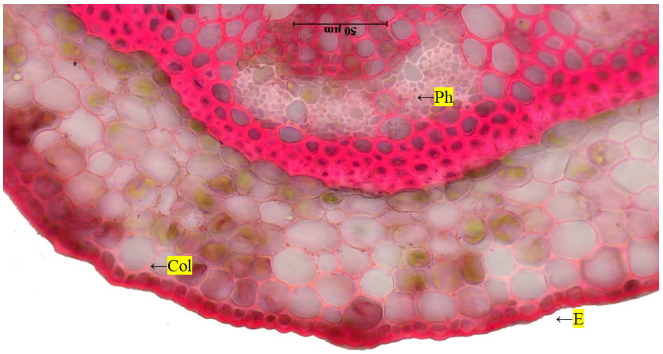



**Figure 2:** (c) Enlarged view of lower portion

TS of lamina

**Figure 3:** (a) TS of *C. sappan* Linn. leaflet

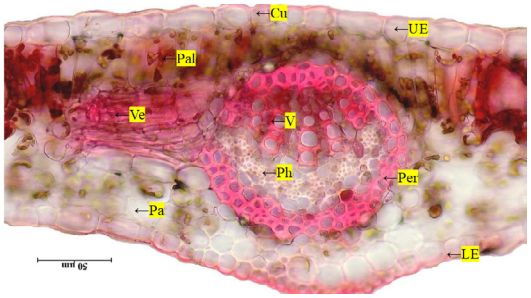



**Figure 3:** (b) TS of *C. sappan* Linn. lamina through midrib

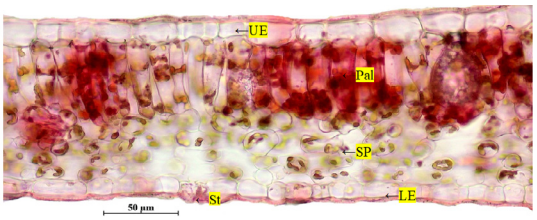



**Figure 3:** (c) TS of lamina

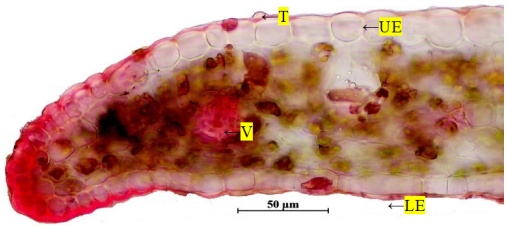
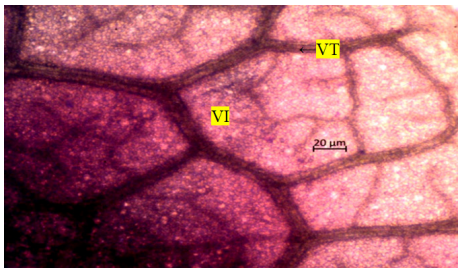
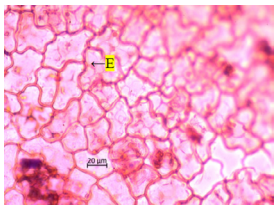


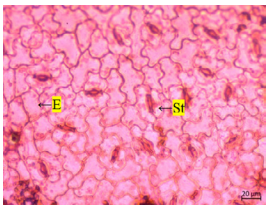

**Figure 3:** (d) TS of lamina margin




Vein islet and vein termination




Upper epidermis

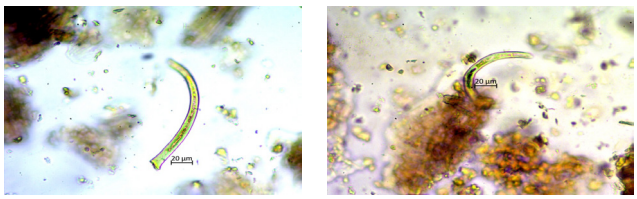



Lower epidermis

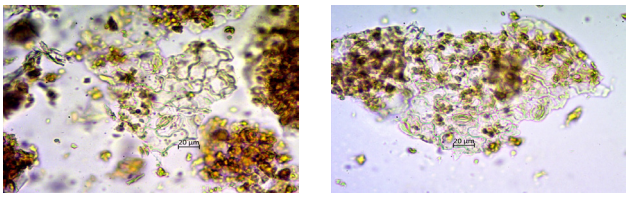
**Figure 4:** Quantitative microscopy of *C. sappan* Linn. leaflet

**Table 1:** Quantitative microscopy of *C. sappan* Linn. leaflet

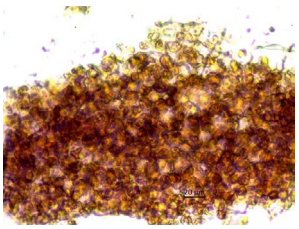
| *Parameters* | *Upper epidermis (/mm²)* | *Lower epidermis (/mm²)* |
|---|---|---|
| Epidermal number | 70–80 | 110–125 |
| Stomatal number | - | 85–95 |
| Stomatal index | - | 43–45 |
| Palisade ratio | 13–18 | |
| Vein islets number | 4–6 | |
| Vein termination number | 10–16 | |



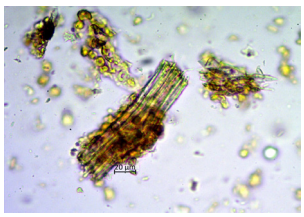

Unicellular trichome




Epidermis with paracytic stomata



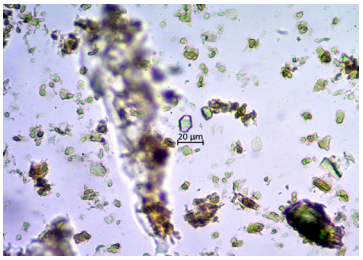

Mesophyll




Fibre bundle



Spiral vessel




Prismatic crystal

**Figure 5:** Powder microscopy of *C. sappan* leaflet

E stands for epidermis, St for stomata, VT for vein islet, and VI for vein terminal.

*Powder microscopy*

It appears as a greyish-greenish powdered material with a distinctive taste with slightly sour and shows unicellular covering trichomes, epidermal fragments with paracytic stomata, mesophyll tissues, fibre bundles, spiral vessels and prismatic crystals (Figure 5).

## CONCLUSION

The leaf of *C. sappan* Linn. is bipinnate, 20 to 45 cm long and 10 to 20 cm wide-ranging. The leaflets are sub-sessile, oblong, oblique at the base and rounded to emarginate at the apex. Dried leaf grayish in color; odor characteristic with slightly sour in taste. The rachilla is nearly oval in shape and a thick cuticle covers the single-layering epidermis and few covering trichomes. When the leaflet goes through the midrib, it reveals a thick cuticle covering a single layer of epidermis and up to 3 layers of cells of collenchyma covering the vascular bund. Phloem is present towards the lower side and xylem toward the upper side. A dorsiventral, hypostatical transverse section of the lamina reveals an epidermis with a single layer and a thick cuticle covering the bottom and top layers; few trichomes are seen in upper epidermis. Veins are seen traversing through the mesophyll region. The quantitative microscopic parameters show numerous paracytic stomata. They are absent on the upper surface. Powder microscopy shows unicellular covering trichomes, epidermal fragments with paracytic stomata, mesophyll tissues, fiber bundles, spiral vessels and prismatic crystals.

The current research used a range of pharmacognostical standardization parameters that might aid in the authenticity of *C. sappan Linn*, including macroscopy, microscopy, quantitative microscopy, and powder microscopy. The herbal monograph will also make use of the current study's results as a source of information.

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