

Detailed cyto-anatomy and cytometry of *Cassia mimosoides* L. leaf - An unexplored plant

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Abstract

Background: *Cassia mimosoides* L. is one of the nonclassical folklore plants of Gujarat, known as *Aval ni jaat*. Root and leaves of this plant are used in diarrhea, wound healing and mouth ulcers by tribal people. **Aims and Objectives:** Review revealed that the leaf part of this plant are unexplored from the pharmacognostical aspects. Hence, the present study focuses on the pharmacognostical study of leaf along with its powder. **Materials and Methods:** Collection, macroscopy and microscopy have been carried out as per standard protocol. **Observation and Results:** The leaves are alternate, compound with opposite glabrous leaflets. T. S. of rachis consists of epidermis, pericycle and vascular bundle. T. S. of dorsiventral leaflet shows epidermis and vascular region with 1/5 palisade ratio. Leaf powder is dark green in color. Palisade parenchyma cells, rhomboidal crystals and unicellular warty trichomes are present in the powder of leaf. **Discussion:** Yellow pentamerous flowers and paracytic stomata are key characteristics of genus *Cassia*. The value of stomatal index always remains constant even in different environmental conditions which play a very significant role in the identification of plant. **Conclusion:** Microscopy of leaf and its powder of *Cassia mimosoides* can be helpful in identifying the plant in powder form and the values can be helpful in further standardization.

Keywords: *Aval ni jaat*, caesalpiniaceae, *Cassia mimosoides*, leaf, pharmacognosy

Introduction

In India, many medicinal plants are scientifically unexplored which are used by traditional medicinal practitioners and not mentioned in classical texts or in modern pharmacopoeias. *Cassia mimosoides* L. is one such plant which is known as '*Aval ni jaat*' locally in Gujarat^[1] and Nelloponna in Tamil^[2] belonging to Caesalpiniaceae subfamily. The whole plant is used by tribal to cure colic pain.^[3] This plant is found throughout India, i.e., Himalayas, Dang and also in Pakistan and Bangladesh.^[4-6]

C. mimosoides L. is a diffuse herb or undershrub with curved hairs all over the stem greenish to brown stem with proper nodes and internodes. Leaves are compound, unipinnate, petiole short, rachis with erect or somewhat curled hair, leaflets opposite and sensitive to light, linear, mucronate and glabrous with prominent midrib. Flowering and fruiting occurs in the month of August–October. The flowers are solitary cyme, pentamerous, yellow in color and bisexual. Fruit pods are linear in shape with minute strigose.^[5,7]

The root of this plant is used in diarrhea and spasm of stomach. Pulverized leaves are used for dressing wounds, sores,^[4] mouth ulcers,^[8] spasm of stomach and headache.^[6]

Review revealed that detailed root microscopy and whole plant powder microscopy^[9] have been evaluated, but the leaf of this plant is not scientifically evaluated. Hence, in the present research, an effort has been made to obtain detailed microscopy of leaf along with the leaf powder microscopy and micrometry of *C. mimosoides* L.

Materials and Methods

Collection and authentication

Fresh *C. mimosoides* L. (whole plant) were uprooted from the hedges of the cultivation field from Kharedi, village of

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Jamnagar, Gujarat. The identification and authentication of the collected plant were done in Pharmacognosy laboratory of the Institute, Jamnagar and a specimen of the plant specimen no. Phm/6164/Nov. 2015 is deposited in the pharmacognosy laboratory of the Institute for further references.^[10]

Macroscopic evaluation

Macroscopic characters of *C. mimosoides* L. fresh leaves such as number of leaves, number of leaflets, its size, shape and color were noted.^[11,12]

Microscopic evaluation

Microscopic study includes thin, free-hand transverse section of rachis and leaflet passing through midrib with phloroglucinol and hydrochloric acid staining reagent to assess different cellular structure and lignified elements and slides were studied without staining too.^[13-15]

Leaf surface preparation

For the determination of the type of stomata and stomatal index (SI), epidermis was exposed by scrapping off the other tissues. Wet leaflet was placed on glass slide and tissues were scrapped off with scalpel carefully. Water was slowly added and scrapping was done until transparent epidermis was obtained.^[16]

Quantitative microscopy

Quantitative leaf microscopy was done to determine palisade ratio and SI. The leaf epidermal studies were carried out on fresh specimens. The study specimen was prepared by mechanical peeling off method and SI was calculated. SI is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell. The palisade ratio of lamina was determined according to the standard method.^[10] All determinations were performed five times and the results are presented as mean value. The microphotographs were taken under a Quasmo binocular microscope attached with a camera.

Preparation of leaf powder

Leaves were separated, shade dried, powdered by mechanical mixer grinder, sieved through 80# sieve and stored in air-tight glass container for further powder microscopy.^[13]

Organoleptic evaluation

Evaluation of the powder sample was done by their various characters, i.e. color, texture, odor and taste.^[13]

Powder microscopy

For powder microscopy, slides were prepared using water and stained with phloroglucinol and concentrated HCl for lignified tissues and iodine for starch grains.^[10]

Micrometric evaluation

Measurement of the length and width of different cell layers along with their contents including powder characteristics were noted by a preloaded micrometric scale.^[13]

Observation and Results

Morphology of *Cassia mimosoides* L.

In natural habit, the plant is found as erect or diffuse herb or under shrub having curved hair on stem and greenish to brown with proper node and internodes. Leaves are compound, alternate, petiole short, rachis with erect or somewhat curled hair, leaflets opposite and sensitive to light, 20–24 pairs and glabrous with prominent midrib. The flowers are pentamerous, yellow in color, pedicles very slender and hairy. Fruit pods are linear, minutely strigose, 8–12 seeded. Flowering and fruiting occurs in August–October [Figure 1].

Floral Formula: Br, %, ♀ K_s, C_s, A_s, G₁

Leaf macroscopy

Leaves are compound, unipinnate, paripinnate, alternate, petiole short, bearing a sessile gland at the top, stipules 7.5 mm, subulate with a filiform tip and a broad sometimes subcordate base, strongly nerved, rachis with erect or somewhat curled hair, leaflets opposite and sensitive to light, linear, subfalcate, 3.5–5 mm long, mucronate apex, glabrous with prominent midrib, leaflets upper side dark green and lower side light green in color and oblong. Length of leaf is 3.7 cm and width is 1.26 cm. Each leaf consists of 20–24 pairs of leaflets, measuring about 0.83 mm × 0.16 mm [Figure 2].

Transverse section of rachis

Diagrammatic section of rachis is irregular-to-circular in shape. Epidermis is single layered with unicellular warty trichomes followed by hypodermis filled with brown content. In the T. S., phloem is situated just below the ring of lignified pericyclic region followed by the xylem; medullary rays are uniseriate to biseriate. Ground tissue is made up of parenchyma cells with oil globules and starch grains. Diameter of T. S. of rachis measures about 4.8 μm², region of epidermis is about 0.7 μm, cortical zone is about 1.3 μm and diameter of vascular bundle measures about 1.2 μm² [Figure 3].

Transverse section of leaflet through midrib

Leaflet is dorsiventral type and differentiated into upper palisade parenchyma and lower mesophyll tissue. Upper and lower epidermis single layered with thin cuticle is rarely interrupted by paracytic stomata. Transverse section through



Figure 1: *Cassia mimosoides* L. (a) Herbarium of plant. (b) Natural habitat of plant

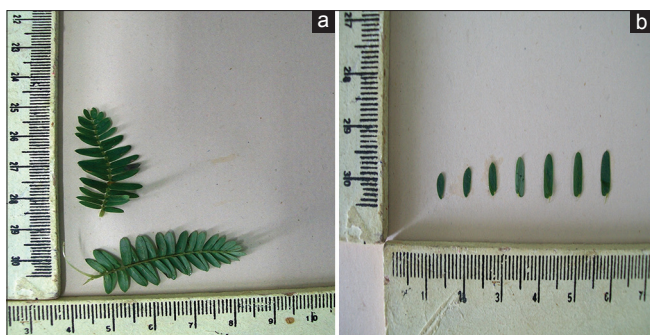


Figure 2: Macroscopy of leaf. (a) Measurement of leaf. (b) Measurement of leaflet

midrib shows main vascular bundle present at the center. Width of lamina of leaflet T. S. measures about $4 \mu\text{m}$ [Figure 3].

Epidermis

Upper epidermis consists of single-layered barrel-shaped compactly arranged cells. Some of the cells lead to form unicellular warty trichomes. Lower epidermis also consists of epidermal cells as in upper epidermis. Stomata are mainly paracytic in nature. Both the epidermises are covered with thin cuticle and brown content. Thickness of cuticle is $0.1 \mu\text{m}$ and the upper epidermal cell measures about $0.5 \mu\text{m} \times 1 \mu\text{m}$.

Mesophyll

Mesophyll is differentiated into upper palisade and lower spongy parenchymatous layers. Palisade consists of one or two layers of elongated compactly arranged cells, below the upper epidermis. Lower spongy parenchyma is filled with chloroplast and oil globules. Spongy parenchyma is made up of 2 or 3 layers.

Vascular bundle

Vascular bundle is situated at the center through the midrib section of the leaflet. Vascular bundle is collateral and of open type. Metaxylem is found toward lower epidermis and protoxylem toward upper epidermis. Xylem is composed of its parenchyma and fibers. Phloem is situated beneath the xylem toward lower epidermis and is composed of some sieve elements and fibers. Vascular bundle is surrounded by parenchymatous cells forming bundle sheath, extended up to both the epidermises. Length and width of vascular bundle measure about $2.8 \mu\text{m}$ and $1.9 \mu\text{m}$, respectively.

Surface study and quantitative microscopy

The surface study shows epidermal cells, wavy parenchyma cells, oil globules and paracytic stomata [Figure 3].

Length and breadth of stomata measure about $0.56 \mu\text{m}$ and $0.46 \mu\text{m}$, respectively. SI measures about 25.15 where stomatal number is $14.33 \mu\text{m}^2$ while epidermal cell count is $42.66 \mu\text{m}^2$. The palisade ratio of the leaflet measures about 1/5.

Powder microscopy

Organoleptic characteristics

The powder of *C. mimosoides* leaves is dark green in color with characteristic odor and coarse in nature with slightly astringent taste.

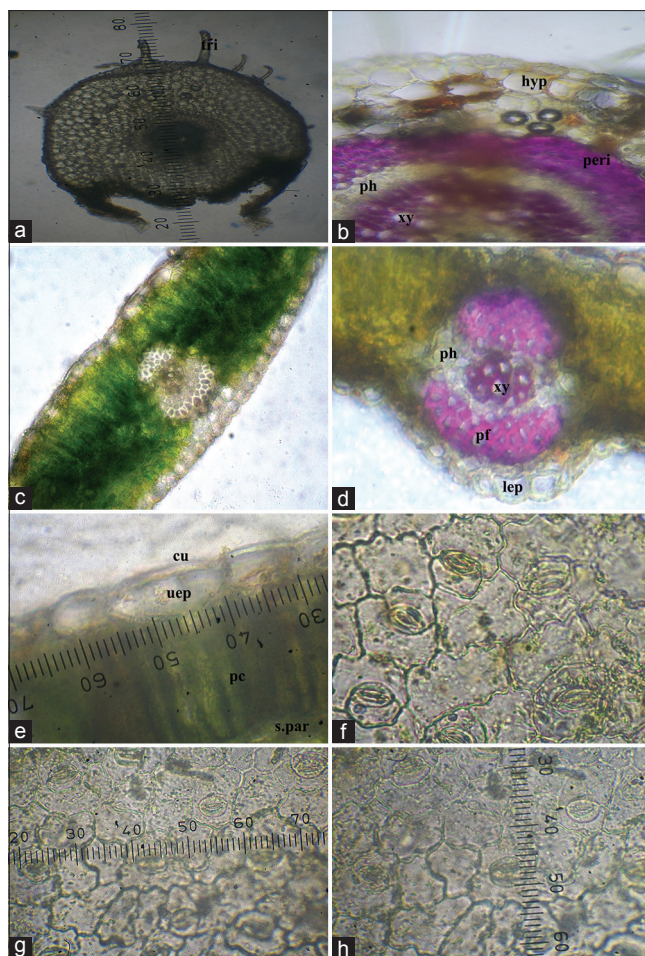


Figure 3: Microscopy. (a) Measurement of rachis T. S. (b) Stained T. S. of rachis. (c) T. S. of leaflet. (d) Stained T. S. of leaflet. (e) Epidermis measurement. (f) Paracytic stomata. (g and h) Stomata measurement. hyp: Hypodermis, peri: Pericycle, ph: Phloem, xy: Xylem, lep: Lower epidermis, pf: Pericyclic fibers, cu: Cuticle, uep: Upper epidermis, pc: Palisade cells, s. par: Spongy parenchyma

Powder characteristics along with micrometry

Diagnostic characteristics of the powder are fragments of palisade parenchyma cells, fragment of annular vessel, unicellular warty trichomes ($3.3 \mu\text{m} \times 0.3 \mu\text{m}$), oil globules, rhomboidal crystals ($0.2 \mu\text{m} \times 0.3 \mu\text{m}$), brown contents, simple fibers ($1.2 \mu\text{m}$ width), green contents, stomata ($0.56 \mu\text{m} \times 0.46 \mu\text{m}$) and group of vessels [Figure 4].

Discussion

Taxonomically, the plant was placed under the family Caesalpiniaceae. The plant is herbaceous with compound leaves, a common specific characteristic of Caesalpiniaceae. T. S. of rachis showed the presence of continuous layer of pericyclic fibers and hypodermal cells filled with tannin are features which are characteristic of rachis.

Leaflet dorsiventral, hairs non-glandular, unicellular, warty type. Mesophyll is distinguished into upper palisade and lower spongy parenchyma and central vascular bundle. Solitary

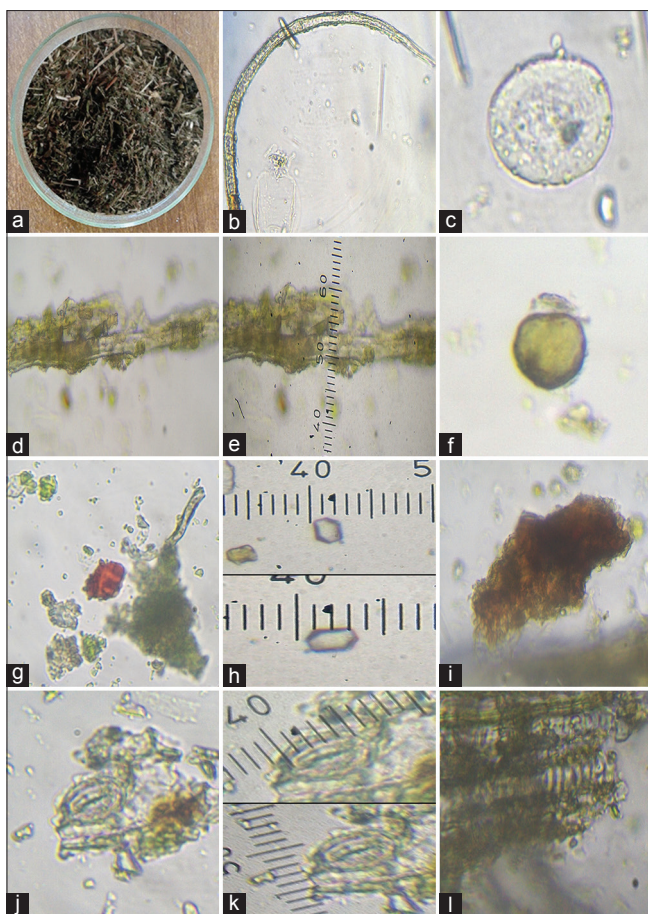


Figure 4: Powder microscopy. (a) Leaf powder. (b) Unicellular warty trichome. (c) Oil globule. (d) Simple fiber. (e) Measurement of simple fiber. (f) Greenish brown content. (g) Dark brown content. (h) Measurement of rhomboidal crystal. (i) Brown content. (j) Stomata. (k) Measurement of stomata. (l) Group of vessel

prismatic and rhomboidal crystals were very commonly observed in the mesophyll tissue and also at the junction of vascular bundle, which is dominant characteristic of Caesalpiaceae family.^[17]

The epidermal cells are capable of division of radial plane and also tangential enlargement. This characteristic enables epidermis to withstand the stress during increase in thickness of the rachis. The primary function of hypodermis is to provide mechanical strength to the young plant against compression and pulling due to wind. Xylem is composed of tracheary elements, xylem fibers and xylem parenchyma in which xylem fibers retain their living contents for a long time and are concerned with storage of reserve materials such as starch and oil. In general, the term pericycle is applied for the tissue of nonphloic origin, occurring between the vascular tissue and cortical zone. Pericyclic fibers (sclerotic parenchyma) have a great tensile strength, flexibility and elasticity which enable plant organs to withstand a variety of strains and tensions resulting from the action of gravity, wind etc. The mechanical strength of these cells is largely due to their arrangement in overlapping and interlocking groups.

In the lamina, the parenchyma cells are modified to spongy parenchyma to function as water storage tissue. They are arranged in rows and are elongated like palisade cells with thin layer of parietal cytoplasm.^[18]

Leaf powder was green as it contains chlorophyll pigments and having astringent taste. Diagnostic characteristics are fragments of palisade parenchyma cells, unicellular warty trichomes, rhomboidal crystals, fibers, stomata and group of vessels.

The results obtained in surface study showed the presence of paracytic stomata, hence supporting the reference.^[17]

SI of the *C. mimosoides* is 25.15, palisade ratio is 1/5 and length and breadth of stomata is 0.56 μm and 0.46 μm , respectively. These values are always constant even in different environmental conditions and hence they play a very significant role in the identification of plant even up to the species level.

Conclusion

Morphology of the plant *Cassia mimosoides* is much similar to the plant “*Aaval*,” i.e., *Cassia auriculata*, resembling the arrangement of leaves and the other parts of the plant. Hence, the plant is known as “*Aaval ni jaat*” in Gujarati. While the leaves of *C. mimosoides* resemble with that of *Lajjalu* (*Mimosa pudica*), belonging to the same family, this may be the reason for its scientific name *C. mimosoides*.

The plant had been systematically identified on the basis of its morphological characteristics such as corolla not Papilionaceous, stamens usually free denoting characteristics of Caesalpiaceae (subfamily). Leaves are abruptly pinnate and petals are five, denoting the characteristic of *Cassia* genus. Midrib was near the upper than the lower margin of the subfalcate leaflet, denoting the species *C. mimosoides*. Microscopy of leaf and its powder can be helpful in identifying the plant in powder form. The values reported in this article can be helpful in further standardization and monograph preparation.

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Conflicts of interest

There are no conflicts of interest.

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