

Pharmacognostic Investigation of Leaves and Bark of *Cochlospermum Religiosum* Linn.

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Abstract: *Cochlospermum religiosum* (Linn.) Alston. (Bixaceae) otherwise known as Yellow silk cotton tree. Traditionally, the plant is used in the treatment of cough, asthma, jaundice, tuberculosis, inflammation, gonorrhea, fever and dysentery. In the present study micromorphological investigations and the physicochemical analysis of *Cochlospermum religiosum* leaves and stem bark were carried out. The macromorphological examination indicated simple, palmately lobed, alternate distichous leaves possessing the prominent parallel venation, acute apices and crenate margin. Similarly, smooth, fibrous, ash coloured bark containing an orange-coloured gummy exudate observed. The microscopy of leaves presented a dorsiventral lamina, long filamentous unicellular uniseriate covering trichomes, anomocytic stomata, annulated lignified xylem vessel, mucilaginous brown matter, starch grains and prisms of calcium oxalate whereas the microscopic examination of the bark indicated lignified cork cells, thick-walled lignified sclereids, bundles of lignified fibres, rounded starch grains, elongated cellulosic medullary rays, tetragonal and rosette crystals of calcium oxalate. The qualitative analysis of inorganic elements exhibited the presence of aluminium, sodium, calcium, chlorides and iron in leaves and the bark of *Cochlospermum religiosum*. The present investigation on pharmacognostic characters and analytical standards of *Cochlospermum religiosum* will provide a diagnostic tool in the authentication and the assessment of its quality.

Keywords: *Cochlospermum religiosum*, Macroscopic, Microscopic, Physicochemical.

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1. Introduction

Cochlospermum religiosum (Linn.) Alston. (Bixaceae) otherwise known as Yellow silk cotton tree owing to its conspicuous brilliant yellow flowers and fluffy cotton-like silky floss surrounding the seeds. It is a deciduous tree endemic to India, Burma and Thailand. Traditionally, the plant is used in the treatment of cough, asthma, jaundice, tuberculosis, inflammation, gonorrhea, and dysentery [1-7]. Besides, the dried leaves and flowers are used as stimulant, laxative, antipyretic, and sedative [8]. Paste prepared from stem bark is plastered over for the treatment of bone fractures [9]. Phytochemical screening indicated many phytoconstituents are present in the plant including alkaloids, glycosides, flavonoids, phenolic, saponins, steroids, coumarins, Leucoanthocyanins [10,11]. Isorhamnetin-3-glucosides (methylated quercetin) and myricetin are the phytoconstituents

isolated from *Cochlospermum religiosum* [12,13]. Many of its virtues have been scientifically demonstrated as antibacterial, antifungal, insecticidal, antioxidant, and hepatoprotective [14-21].

Detailed pharmacognostic examination of medicinal plant provides valuable information that could be useful in its identification and standardization. Since no scientific reports are available on *C. religiosum* with respect to its pharmacognostic evaluation, the present study was carried out to establish quality parameters for *C. religiosum*.

2. Materials and methods

2.1. Collection and Authentication of plant

The plant material was collected during the flowering stage from Katraj Ghat, Pune (Maharashtra), India. Taxonomic authentication was made by Dr. S.R. Yadav, Head, Department of Botany, Shivaji University, Kolhapur, India. A voucher specimen of *Cochlospermum religiosum* (L.) Alston. (Bixaceae) has been preserved in the herbarium with reference no. PRG-2 for future reference. Leaves and bark of *Cochlospermum religiosum* were collected and thoroughly washed with water. The cleaned fresh leaves and bark were subjected to macroscopic and microscopic studies. The leaves and bark were dried followed by finely powdered with the help of mechanical grinder and stored in an airtight container for further experimental studies.

2.2. Macroscopic examination

The fresh leaves and bark of *Cochlospermum religiosum* were visually examined to study sensory and morphological characteristics such as colour, odour, taste, size, shape, surface characteristic, texture, and fracture. The leaves were also examined for macroscopic characteristics viz. type of margin, apex, base, lamina, petiole and venation etc.

2.3. Microscopic examination

The anatomical characteristics were studied by taking a very thin cross-section of leaf and bark with freehand sectioning. The distinctive histological characteristics of leaf and bark were studied by following standard staining procedures [22].

2.3.1. Histology of leaf and bark

Transverse section of leaf and bark were examined using the digital microscope and results were recorded by taking photographs. Various diagnostic histological characteristics were noted with or without staining.

2.3.2. Microscopic powder analysis

Microscopic examination of powder was carried out to ascertain the identifying characteristics of *Cochlospermum religiosum*. Sufficient quantity of dried finely powder sample of leaves and bark were mounted uniformly on glass slides and treated with specific staining reagent individually to examine different cellular characteristics and diagnostic cell inclusions [23].

2.4. Determination of analytical standards

The specified quantity of dried powder of leaves and bark was subjected to physicochemical analysis for the assessment of various physicochemical constants and

analytical standards including foreign organic matter, loss on drying, ash value, extractive value, moisture content, swelling index, foaming index through standard methods. [24, 25]

2.5. Analysis of inorganic elements

Elemental analysis was performed to investigate the inorganic elements present in the plant. For this purpose, qualitative elemental analysis of *Cochlospermum religiosum* was attempted by performing various qualitative chemical tests on the ash of *Cochlospermum religiosum* leaves and bark [22,26].

3. Results and discussion

3.1. Macroscopic examination

The simple, palmately lobed, alternate distichous leaves were noticed in *c. religiosum*. Leaves were divided into three to five lobes and articulated with around 5 to 20 cm long brown petiole. Freshly collected leaves were glabrous above, pubescent beneath, intensely glossy green on the upper surface, whereas, densely brownish on the lower surface with a faint odour and bitter taste. The leaves were larger (5 to 15 and 7 to 20 cm). The leaves were elliptic, cordate at the base possessing a prominent parallel venation, acute apices and crenate margin. The bark of *c. religiosum* was smooth, fibrous, ash-coloured, deeply grooved containing an orange-coloured gummy exudate. The bark was flat to curve having varying length and width with the average thickness about 20-25 mm (Figure 1).

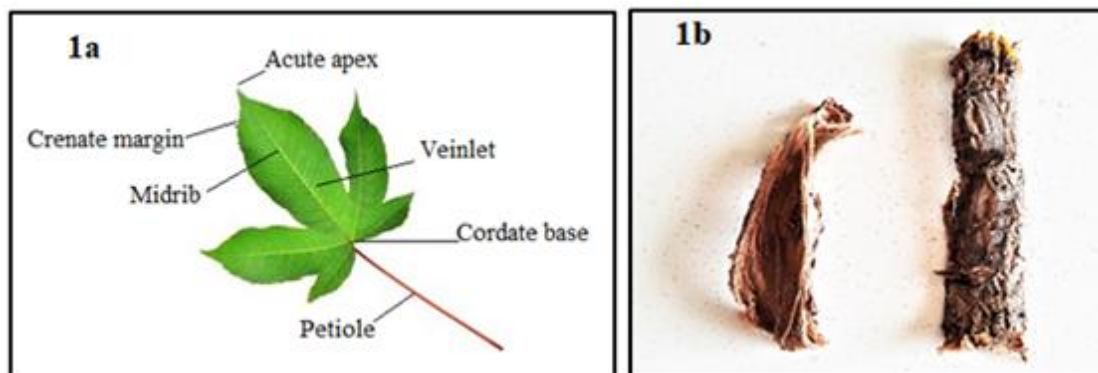


Figure 1. (A) Palmately lobed leaf of *C. religiosum* (B) Bark of *C. religiosum*

3.2. Microscopic examination

3.2.1. Histology of leaf:

The transverse section of *C. religiosum* leaf indicated a dorsiventrallamina. It possessed single-layered epidermis; adaxial epidermis consisted of thick-walled epidermal cells compared to the abaxial epidermis. Adaxial epidermis comprised of rounded cells, covered by a thick cuticle. The long filamentous unicellular uniseriate covering trichomes; abundant numbers are present at a dorsal side of the leaf compared to the ventral surface. Mesophyll composed of single-layered palisade cells and several layers of spongy parenchyma. An amphistomatic leaf was observed containing the anomocytic stomata distributed mostly on the upper epidermis. The large parenchymatous midrib contained a vascular bundle comprising lignified xylem and unlignified phloem. The large solitary mucilaginous brown matter scattered in spongy parenchymatous cells of midrib region. Collenchymatous tissues were present beneath the upper epidermis and above the lower epidermis in the midrib region (Figure 2,3 and 4).

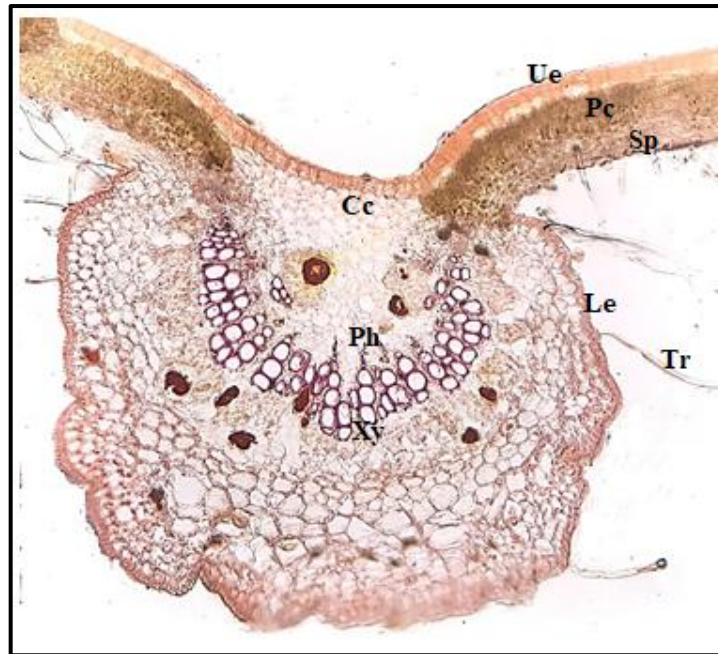


Figure 2. Transverse section of *C. religiosum* Leaf.

Ue: Upper epidermis; Pc: Palisade cells; Sp: Spongy parenchyma; Le: Lower epidermis; Tr: Trichomes; Cc: Collenchyma; Ph: Phloem; Xy: Xylem.

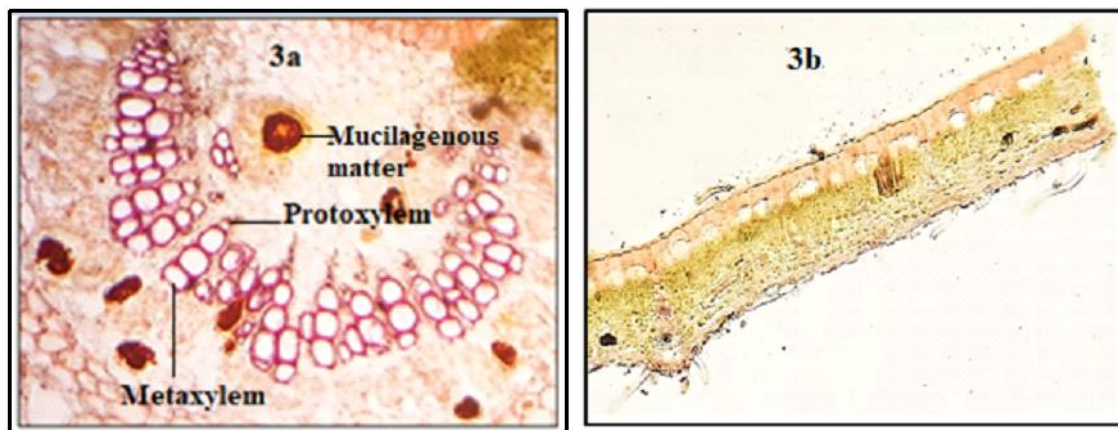


Figure 3. Cross section of *C. religiosum* Leaf showing midrib(3a) and lamina (3b).

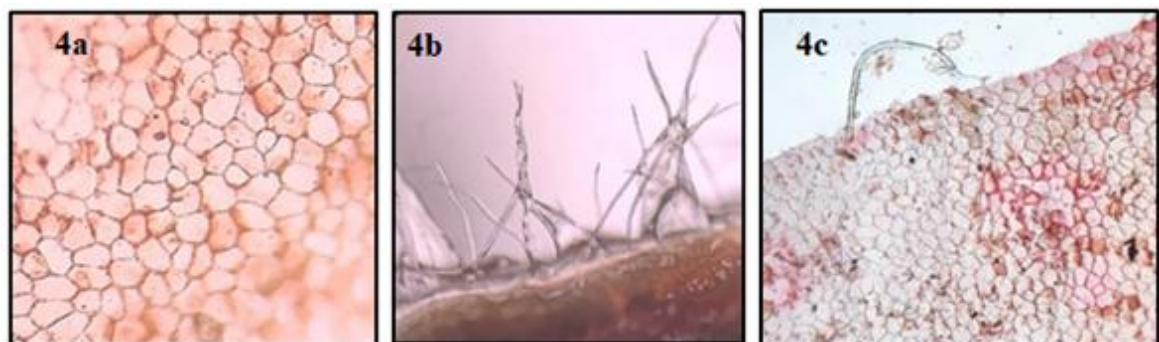


Figure 4. Histochemical characteristics of *C. religiosum* leaves (4a) Anomocytic stomata, (4b and 4c) Epidermal portion showing long filamentous unicellular uniseriate covering trichomes.

3.2.2. Histology of bark

The transverse section of a bark presented several layers of the cork followed by a few layers of the phelloderm. The cork consisted of rounded cells arranged in regular rows, whereas, the phelloderm layer was indistinct. The cortex region of the bark displayed secretory cavities as well as thick-walled lignified sclereids. Phloem region of the bark contained; thick-walled, polygonal, lignified phloem fibers with a narrow lumen in groups of 20-50, present between medullary rays. The large mucilaginous brown cells were noted in the phloem parenchyma. Starch grains, tetragonal and rosette crystals of calcium oxalate were observed throughout the phloem region. Uniseriate to biseriate elongated cellulosic medullary rays were also observed in the transverse section of the bark (Figure 5).

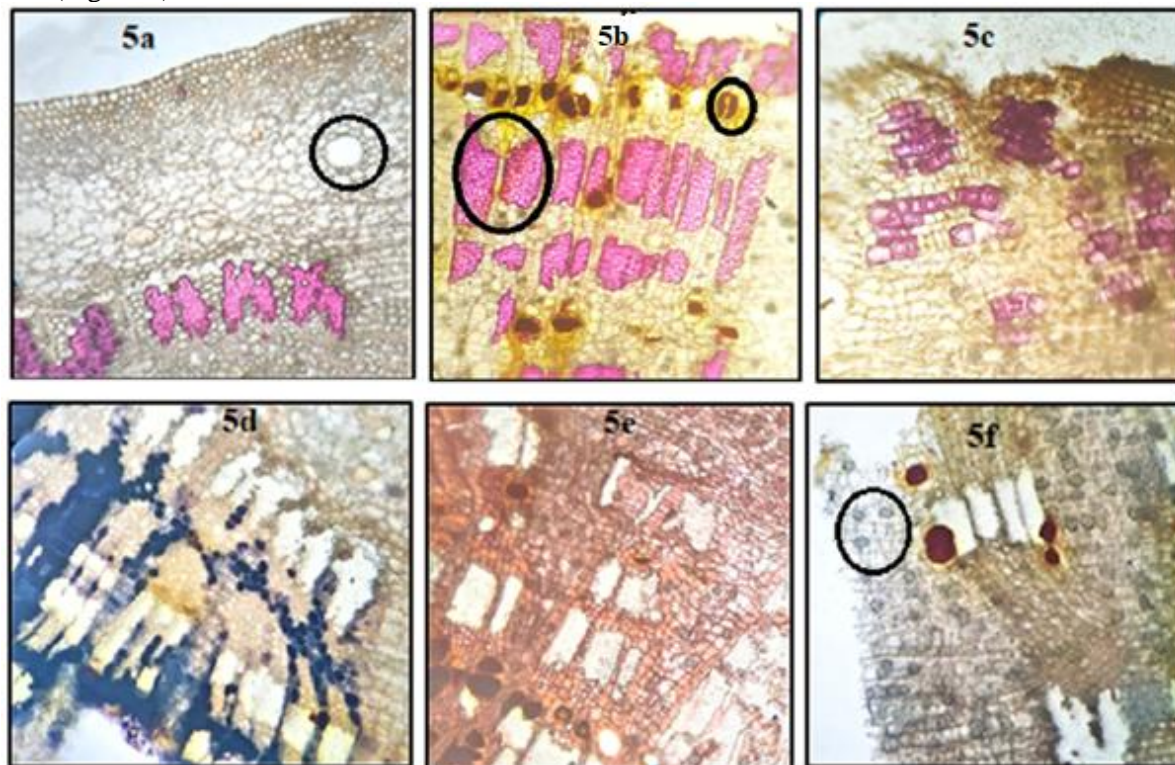


Figure 5. Histochemical characteristics of *C. religiosum* bark (5a) Secretory cavity in the cortex of bark, (5b) Lignified phloem fibers and mucilaginous brown cells, (5c) Thick-walled lignified sclereids, (5d) Starch grains, (5e) Medullary rays and (5f) Rosette calcium oxalate crystals.

3.2.3. Microscopic powder analysis of leaf

The microscopic powder analysis of *Cochlospermum religiosum* leaf indicated the presence of various cellular characteristics and diagnostic cell inclusions including long filamentous unicellular uniseriate covering trichomes, annulated lignified xylem vessel, simple prisms of calcium oxalate and rounded starch grains (Figure 6).

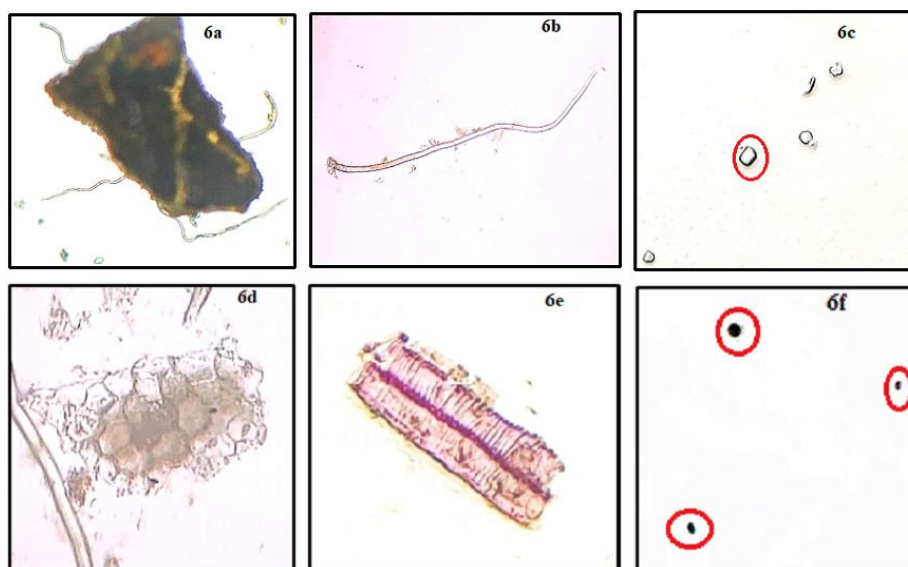


Figure 6. Powder characteristics of *C. religiosum* leaves (6a) Epidermal fragments with stomata, (6b) Unicellular uniseriate trichomes, (6c) Prisms of calcium oxalate, (6d) Epidermis with stomata (6e) Lignified spiral xylem vessel (6f) Starch grains.

3.2.4. Microscopic powder analysis of bark

The microscopic powder analysis of the bark presented lignified cork cells, thick-walled lignified sclereids, bundles of lignified phloem fibres, fragments of parenchyma, mucilaginous brownish matter and rounded starch grains. The bark powder also presented rosette as well as tetragonal crystals of calcium oxalate (Figure 7).

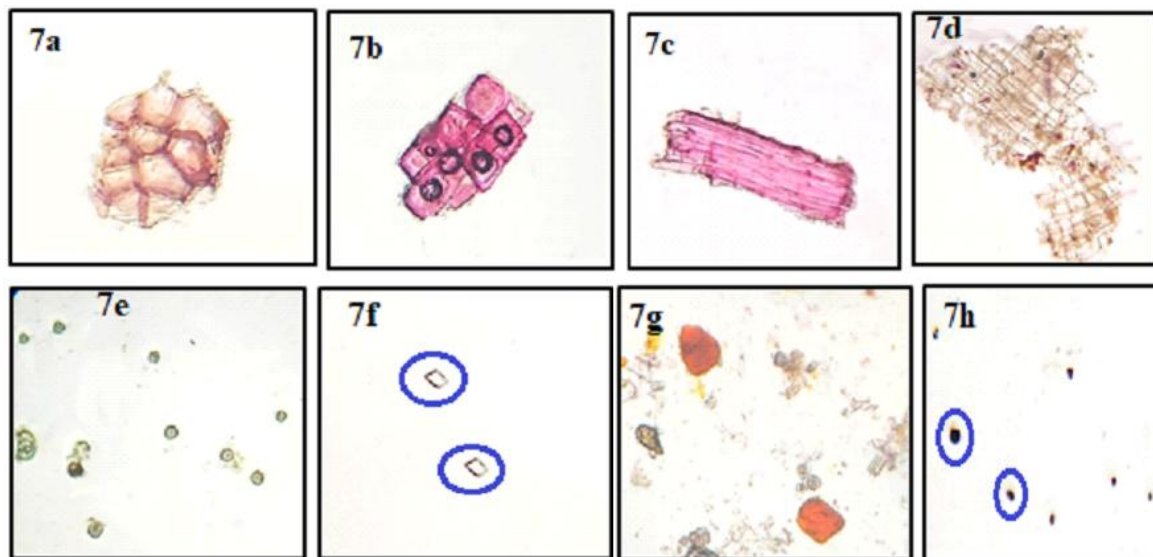


Figure 7. Powder characteristics of *C. religiosum* bark (7a) Lignified cork, (7b) Thick-walled lignified sclereids, (7c) Lignified phloem fibres, (7d) Parenchyma (7e) Rosette calcium oxalate crystals (7f) Tetragonal crystals of calcium oxalate, (7g) Mucilaginous brownish matter, (7h) Starch grains.

3.3. Determination of analytical standards

The outcomes of the physicochemical analysis are mentioned in Table 1 and 2.

Table 1. Physicochemical Analysis of *Cochlospermum Religiosum*

Parameters	Leaves	Bark
FOM (% w/w)	0.35	0.45
LOD (% w/w)	7.25	4.65
Total ash (% w/w)	9.45	11.45
Acid Insoluble ash (% w/w)	1.65	2.35
Water soluble ash (% w/w)	2.53	5.55
Swelling index (mL)	0.45	1.35
Foaming index	Less than 100	Less than 100

Table 2. Extractive Value of *Cochlospermum Religiosum*

Parameters	Extractive value (%w/w)	
	Leaves	Bark
Ether soluble	4.31	3.45
Benzene soluble	2.56	2.10
Chloroform soluble	7.15	5.80
Ethyl acetate soluble	10.24	8.70
Ethanol soluble	25.42	21.25
Methanol soluble	27.94	23.42
Water soluble	18.42	15.23

3.4. Analysis of inorganic elements

The ash of *Cochlospermum religiosum* leaves and bark was undertaken for qualitative elemental analysis exhibited the presence of aluminium, sodium, calcium, chlorides and iron (Table 3).

Table 3. Qualitative Elemental Analysis of *Cochlospermum Religiosum* Ash

Element	Result	
	Leaves	Bark
Aluminium	Present	Present
Magnesium	Absent	Absent
Calcium	Present	Present
Sodium	Present	Present
Potassium	Absent	Absent
Chlorides	Present	Present
Carbonates	Absent	Absent
Iron	Present	Present

4. Conclusion

Herbal medicine requires well established experimental and clinical data to meet the quality requirement of the modern system of medicine hence the standardization of herbal medicine is highly recommended. The pharmacognostic study of an unexplored plant generates the scientific profile for proper authentication of the plant and hence the detailed analysis of pharmacognostic parameters is helpful to establish quality standards for crude drugs. In the present study macroscopic, microscopic and physicochemical evaluation was carried out to establish the standard for *Cochlospermum religiosum*. The pharmacognostic parameters of *Cochlospermum religiosum* leaves and the bark noted in this study will help as a diagnostic tool in the authentication and the assessment of the quality of this medicinally useful plant.

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