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Evaluation of Pharmacognostical & Physico-chemical Standards of the Leaf of *Cassia tora* Linn

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ABSTRACT

Pharmacognostic investigation of the fresh, powdered and anatomical sections of the leaves of *Cassia tora* Linn was carried out to determine its macro- and microscopical characters and also some of its physical constants. Leaves 7.5-10 cm long; rachis grooved, more or less pubescent, with a conical gland between each of the 2 lowest pairs of leaflets; stipules 1.3-2 cm. long, linear-subulate, caducous. Leaflets 3 pairs, opposite, 2.5-4.5 by 1.3-2.5 cm. Externally, the leaves possess somewhat oblique, usually rounded base. Internally, its shows the presences of paracytic stomata, unicellular, uniseriated-covering trichomes with swollen base and an acute apex, prism crystals of calcium oxalate and fiber elements. The chemo-microscopy revealed the presences of Starch polyphenol lignins, steroid, flavonoid, alkaloid. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

INTRODUCTION

A small plant growing on dry soil in Bengal and throughtout the tropical parts of India. An annual herb 30-90 cm high. Leaves 7.5-10 cm long; rachis grooved, more or less pubescent, with a conical gland between each of the 2 lowest pairs of leaflets; stipules 1.3-2 cm. long, linear-subulate, caducous. Leaflets 3 pairs, opposite, 2.5-4.5 by 1.3-2.5 cm. (the lowest pair the smallest), obovate-oblong, glaucous, membranous, glabrous or more or less pubescent, base somewhat oblique, usually rounded; main nerves 8-10 pairs; petiolules 2.5 mm. long, pubescent. The leaves are used as laxatives in the form of decotion. Both leaves and seeds constitute a valuable remedy in skin diseases, chiefly for ringworm and itch. In China, the seeds are used externally for all sorts of eye diseases; preparation are also given for liver complaints and boils. In Indo China, the pods are used in dysentery and diseases of the eye. In Nigeria, the leaves are as a mild laxative. The weed is used in various Gold Coast medicines, chiefly as a purgative. In Madagascar and La Reunion, the root is considered bitter, tonic, stomachic. The leaves are used as an antiperiodic, aperient, anthelmintic; they are given to children with intestinal troubles. The root is not an antidote to either snake-venom or scorpion-venom [1].

MATERIALS AND METHODS

The leaf material was collected from the fully grown trees found in Barpali, Bargarh, Odisha, in the month of February. For microscopical studies free hand sections of fresh leafs were cut cleared with chloral hydrate solution and water, stained with safranin according to the prescribed methods[2]. A drop of HCL and Phloroglucinol was used to detect the lignified cells in the powder drug [3]. Photomicrographs were taken by Sony digital camera. Powder of the dried leaf was used for chemical analysis. Histochemical study [4], Measurement of diameter of starch grains and length of phloem fibre [5], Physico-chemical studies and preliminary phytochemical screening of the drug was carried out [6], behaviour of powder drug towards different chemical reagent [7], fluorescence behaviour of the powder drug in different solutions towards the ordinary and ultraviolet light was carried out [8].

RESULT & DISCUSSION

Macroscopical Character

Leaves 7.5-10 cm long; rachis grooved, more or less pubescent, with a conical gland between each of the 2 lowest pairs of leaflets; stipules 1.3-2 cm. long, linear-subulate, caducous. Leaflets 3 pairs, opposite, 2.5-4.5 by 1.3-2.5 cm. (the lowest pair the smallest), obovate-oblong, glaucous, membranous, glabrous or more or less pubescent, base somewhat oblique, usually rounded; main nerves 8-10 pairs; petiolules 2.5 mm. long, pubescent. Colour is green, characteristic odour and bitter in taste. Texture is smooth. (Table No.1)

Table No.1: Organoleptic character of *Cassia tora* leaf

Colour	Green
Odour	Characteristic
Taste	Bitter
Texture	Smooth

Microscopical Character

Transvers Section of Leaf Mid Rib and Lamina

Upper epidermis-Single layered, rectangular cells, wavy anticlinical walls and cuticularized.

Vascular bundles- These are arc shaped, xylem is lignified and phloem is nonlignified.

Sclerenchyma- 4 to 5 layers, below upper epidermis and above lower epidermis; covering the vascular bundle.

Spongy parenchyma- thin narrow loosely arranged between the upper and lower palisade layer.

Ground tissue- Consists of thin layer parenchymatous cell, covering totally the rest part of midrib.

Plate-I: Microscopic character of midrib of *Cassia tora*

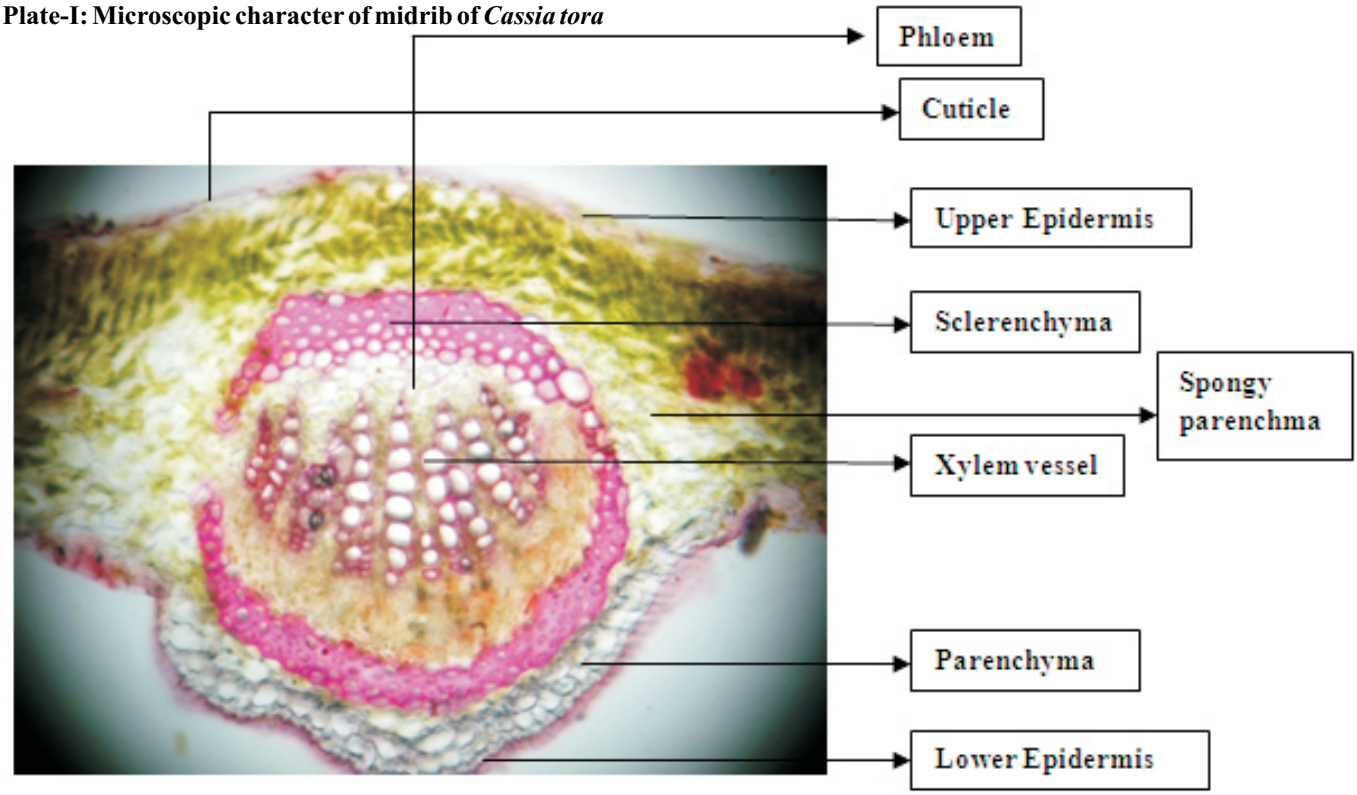


Fig. 1: Transverse section of leaf midrib.

Powder Microscopy

Stomata- Fragments stomata are found.

Covering trichomes- Unicellular with blunt tips.

Starch grains- Spherical in shape abudantly found throughout the powder.

Sclerenchyma- Pitted walls, narrow lumen longitudinally elongated. Fibrous sclereides from the stalk. The very occasional group of small, lignified spirally or annulary thickened vessels from the vascular strand. Groups of large, fibrous occur frequently.

Vascular strand- Fibro-vascular tissue. The fragments of lignified fibro-vascular tissue composed of small, thin walled fibres and vessels with spiral and annular thickening.

Fibre- Part of a group of fibrous with calcium oxalate prism sheath. Part of a group of fobres and prism crystal sheath, showing end of a fibre.

Pollen grain- Mature pollen grains. The abundant pollen grains which are small rounded. A number of immature pollen grains occur and these may be found in closely packed masses, frequently enclosed in the pollen sacs.

Calcium oxalate crystal- Abundant in the powder. Large prismatic crystals are found scattered in the powder and larger ones may be broken.

QUANTITATIVE MICROSCOPY

Stomata- Paracytic stomata. (Long axis of two subsidiary cells is parallel to that of stoma. The lenth of stomata are & width of stomata are 102.69 & 78.35 micron. The stomatal index was found to be 51.66 (Table No.2).

Epidermal cells. Straight walled polygonal cells.

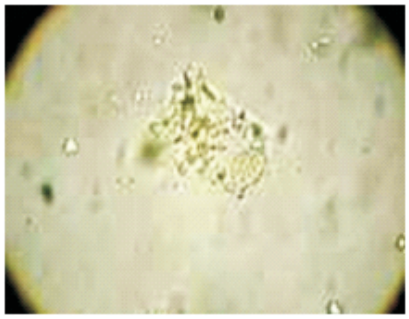


Fig. 2(a): Fragment of stomata.

Fig. 2(b): Covering trichomes.

Fig. 2(c): Fibre with calcium oxalate crystal

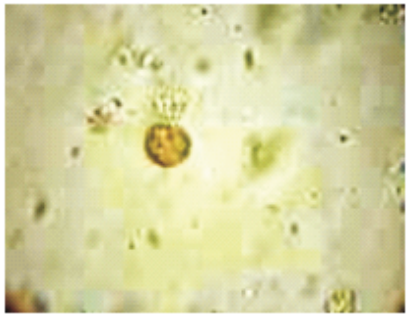
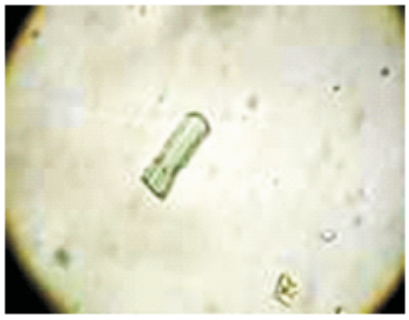


Fig. 2(d): Pollen grains



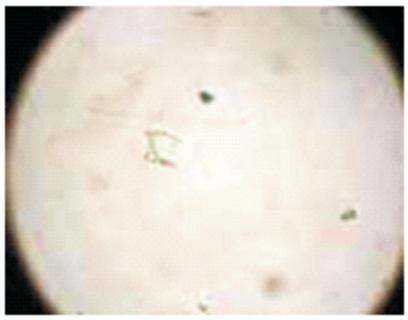


Fig. 2(e,f): Calcium oxalate crystals.

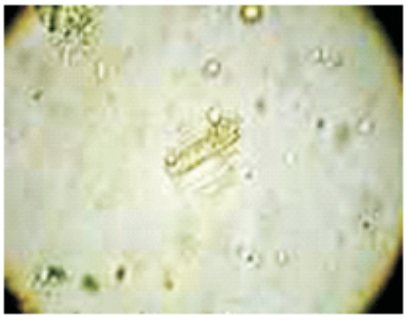


Fig. 2(g): Vascular strands

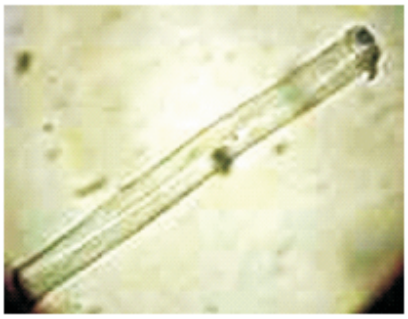


Fig. 2(h): Sclereids

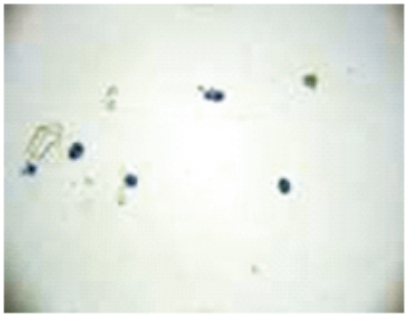


Fig. 2(i): Starch grain.

Table No.2 Quantitative microscopy study of *Cassia tora* leaf

Sl. No	Parameter	Range	Mean
1	No. of stomata	30-40/sq.mm	35/sq.mm
2	No. of epidermal cell	40-80/sq.mm	60/sq.mm
3	Stomatal index	51.66 µ	
4	Length of stomata	88.02-117.36µ	102.69µ
5	Width of stomata	58.68-88.02µ	78.35µ
6	Vein-islet no.	20-30/sq.mm	25/sq.mm
7	Vein-termination no.	50-90/sq.mm	70/sq.mm
8	No. of palisade cell	11-27	19
9	Length of fibre	102.69-117.36µ	110.02µ
10	Diameter of starch grain	13.46-26.92µ	20.19µ

µ- micron; sq.mm-per square millimeter

Venation pattern- The veins are uniformly thick and distinct scattered on the epidermal layer. No of vein-islet per square mm was found to be 25. Number of vein-termination per square mm was found to be 70. The length of fibre was found to be 110.02µ. The diameter of starch grain was found to be 20.19µ. (Table No.2). Average number of palisade cells were found to be 19. (Table No.2).

Histochemical tests

Transverse sections leaf of *Cassia tora* were treated with routinely used chemicals and reagents, gave positive tests for starch, polyphenol, lignin, steroid, flavonoid and alkaloid. (Table No.3).

Physico-chemical Standards

Ash values

The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Cassia tora* leaf were found to be 18.5 w/w, 6 w/w, 1w/w, 22.5 w/w. Total ash of *Cassia tora* leaf was found to be more than water soluble ash and acid insoluble ash. Acid insoluble ash was found to be very less as compared to total ash and acid

Table No. 3 Histochemical test of *Cassia tora* leaf

SL. No	Reagent	Test for	Inference
1	Section + Iodine solution	Starch	+
2	Section + IKI	Starch	-
3	Section + Sudan Red	Oil globules	-
4	Section + Ferric chloride	Tannin/Phenol	-
5	Section + Lugol's iodine	Tannin	-
6	Section + Toluidine blue	Polyphenol	+
7	Section + Phloroglucinol & HCL	Lignins	+
8	Section + Libermann	Steroid	+
9	Section + 5% KOH	Flavonoid	+
10	Section + Dragendorff's reagent	Alkaloid	+

+ Present, - Absent

Table No.5: Test for inorganic elements in *Cassia tora* leaf

SL.NO	Test for	Inference
1	Calcium	+
2	Magnesium	-
3	Sodium	-
4	Potassium	-
5	Iron	+
6	Sulphate	+
7	Phosphate	+
8	Chloride	+
9	Carbonate	+
10	Nitrate	-

+ Present, - Absent

Table No.6: Behaviour of *Cassia tora* powder leaf with different chemical reagents

SL.No	Acid/Reagent	Observation
1	Powder as such	Light green
2	Powder + Picric acid	Light green
3	Powder + Con.Nitric acid	Brown
4	Powder + Con.HCL	Deep green
5	Powder + Con.H_2SO_4	Black
6	Powder + Glacial acetic acid	Deep green
7	Powder + 5% $FeCl_3$	Green
8	Powder + NaOH(5N)	Green
9	Powder + KOH (5%)	Light green
10	Powder + Iodine/20	Yellowish red

Table No.4: Physicochemical analysis of *Cassia tora* leaf

Extractive value in	Percentage
Petroleum ether	1.68 w/w
Chloroform	2.36 w/w
Ethyl acetate	2.26 w/w
Methanol	10.78 w/w
Ash value in percentage	
Total ash	18.5 w/w
Water soluble ash	6 w/w
Acid insoluble ash	1w/w
Sulphated ash	22.5 w/w.
Loss on drying	7.15 w/w

insoluble ash. Sulphated ash was found to more than total ash and water soluble ash (Table No.4)

Total extractive values

The extractive values were determined to find out the amount of soluble compounds. The petroleum ether, chloroform, ethyl acetate and methanol extractive values of leaf of *Cassia tora* were found to be 1.68 w/w, 2.36 w/w, 2.26 w/w, & 10.78 w/w. The leaf showed more amount of methanol soluble component than petroleum ether, chloroform & ethylacetate extracts(Table No.4).

Inorganic element

In organic element found in the ash of leaf of *Cassia* tora were calcium, iron, sulphate, phosphate, carbonate & chloride. (Table No.5).

Loss on drying

The moisture content of leaf was found to be 7.15 w/w which was shown in (Table No.4).

Behaviour of powdred materials towards some chemical reagents

The behaviour of the powdered leaf was treated with Picric acid, conc.sulphuric acid, con. hydrochloric acid, conc. nitric acid, glacial acetic acid, 5% ferric chloride, sodium hydroxide (5N), potassium hydroxide (5%), Iodine/20 solution were observed and the results are present in (Table No.6).

Fluorescence analysis

Fluorescence analysis of entire leaf has been carried out in daylight and under U.V light. The powders were treated with different organic solvents and solutions were again observed in normal daylight and under U.V. light and the observations are pooled in (Table No.7).

CONCLUSION

The present work focuses on the pharmacognostical characters of *Cassia tora leaf*. As there is no pharmacognostical anatomical work on records for this traditionally much valued drugs, present work is taken up in the view to lay down the macroscopic and microscopic standards, which could be used in

Table No.7: Fluorescence analysis of the powder leaf of *Cassia tora*.

SL.No	Reagent	Day light	Short wave
1	Powder as such	Light green	Green
2	Powder + 1N NaOH in methanol	Green	Green
3	Powder + 1N NaOH	Light green	Green
4	Powder + Ethanol	Green	Green
5	Powder + HNO_3+NH_3 solution	Dark green	Dark green
6	Powder + 50%HNO_3	Brown	Black
7	Powder + 1N HCL	Light green	Green
8	Powder + HCL	Light green	Green
9	Powder + H_2SO_4	Green	Green
10	Powder + 50% H_2SO_4	Green	Green
11	Powder + Glacial acetic acid	Green	Dark green
12	Powder + HNO_3	Brown	Green

deciding the genuineness of the above-described drugs irrespective of their collection from different sources. Macroscopic and microscopic descriptions are provided from diagnostic point of view. The colored photographs of the leaf of the above-mentioned plant might facilitate the researcher for identification. The results of the phytochemical screening, chemomicroscopical tests and fluorescence behaviors of the of the powdered drugs of the leaf can be considered as distinguishing parameters to identify and decide the authenticity of the above mentioned herbal drugs and thus can be used as standards for reference purpose also. The outcome of the quantitative parameters described on the above mentioned plant parts (leaves) might be useful in the determining the authenticity of the drugs. These parameters, which are being reported for the first time, could be useful in the preparation of the herbal section of different Herbal Pharmacopoeia.

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