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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *CASSIA TORA* LINN. (CAESALPINIACEA)

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ABSTRACT

The present communication deals with the pharmacognostical and preliminary phytochemical studies on the stembark of *Cassia tora*. This plant of considerable economic importance has been recommended mainly for its skin diseases, leprosy, ringworm, & ulcer. No study reports are avilable on the microscopic and phytochemical studies of the stembark. Hence, the present attempt has been undertaken to investigate the macroscopical ,microscopic, physico-chemical parameters such as ash value, inorganic element, extractive value, moisture content, behaviour of powder with different chemical reagent, fluorescence analysis, preliminary phytochemical screening, & thin layer chromatography. The outer surface of stembark is greenish brown in colour. The fissures were V shaped. The inner surface is smooth and light yellow coloured. Transverse section of stembark shows the presence of cork, cortex, phloen fibre, xylem parenchyma & pith region. Powder microscopy of stem bark shows the presence of xylem vessel, starch grain, cork cells & parenchymatous cell. Phytochemical screening of the methanol extract and different fractions shows the presence of carbohydrate, glycosides, alkaloids, steroids, flavonoid, tannins & phenols. Thin layer chromatography of different fraction shows number of spots.

Keywords: *Cassia tora,* Stembark, Physicochemical analysis, Transverse section, Chromatography.

INTRODUCTION

A small plant growing on dry soil in Bengal and throughtout the tropical parts of India. An annual herb fetid herb 30-90 cm high. Leaves 7.5-10 cm long; rhachis grooved, more or less pubescent, with a conical gland between each of the 2 lowest pairs of stem barklets; stipules 1.3-2 cm. long, linear-subulate, caducous. Stembarklets 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 costitute a valuable remedy in skin diseases, chiefly for ringworm and itch. In China, the seeds are used extrernally for all sorts o 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, aperients, 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 bark 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 barks 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 stembark was used for chemical analysis. Histochemical study[4], measurement of diameter of starch grains and lenth of phloem fibre[5], physico-chemical studies and preliminary phytochemical screening of the [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[8] and preliminary phytochemical screening of the extract was carried out [9]. Thin layer Chromatography studies of methanol extract & the different fractions (petroleum ether, chloroform, diethyl ether & ethyl acetate) were carried out in various solvents at 30°C using silica gel GF 254 plate as adsorbent [10].

RESULTS AND DISCUSSION

Macroscopical characters

The sample of *Cassia tora* bark was collected and cut into pieces of 3-5 cm long, 1 cm broad. The outer surface is greenish brown in colour. The fissures were V shaped. The inner surface is smooth and light yellow coloured. The taste is bitter and acrid, and characteristic odour. The lenth of fibre is 117.36 µ & diameter of starch grain is 73.35 µ Table-1.

Table 1: Organoleptic character

Colour	Greenish brown
Odour	Characteristic
Taste	Bitter and acrid
Lenth of fibre	117.36µ
Diameter of starch	73.35 µ

Microscopical characters

Transverse section of stem bark Fig-1(a,b)

Cork – 1-2 layered, rectangular cells, with thick wall.

Cortex- Many layers of thin walled cellulosic parenchyma cells with very small intercellular spaces present below the cork with scattered starch grain.

Phloem- Seive tubes are embeded in parenchymatous cells.

Phloem fibre- Thickened walls, cellulosic in the inner part, lignified in the outer part. Below the phloem fibre parenchymatous cells are present.

Xylem parenchyma-The polygonal xylem parenchymatous cell lying side to the xylem vessel

Xylem vessels- Thick, pitted or reticulately thickened walls lignified.

Pith- Large, thin walled, lignified big polygonal parenchymatous cell with very less intercellular spaces

PLATE-I

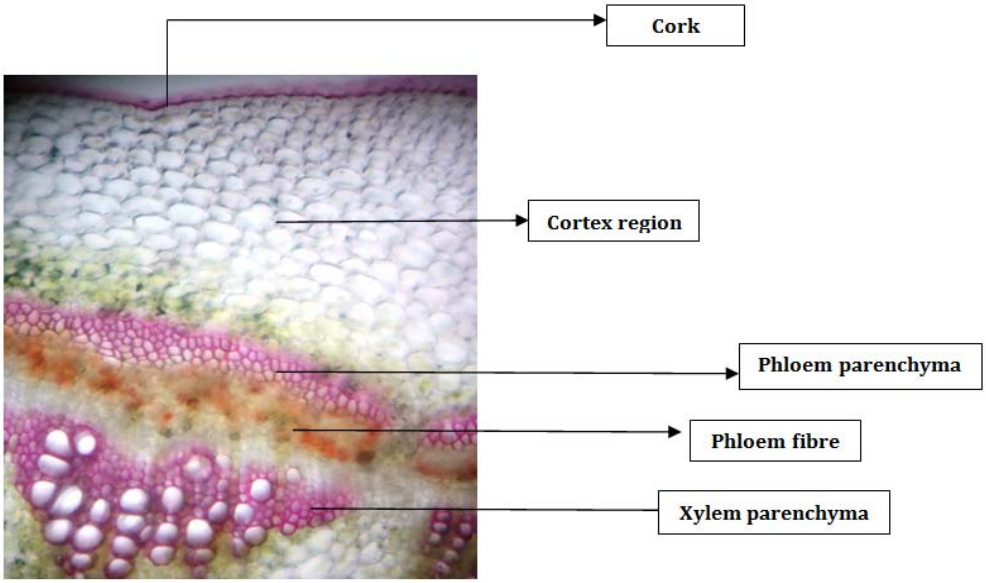

Fig. 1.a: T.S of stembark of *Cassia tora*

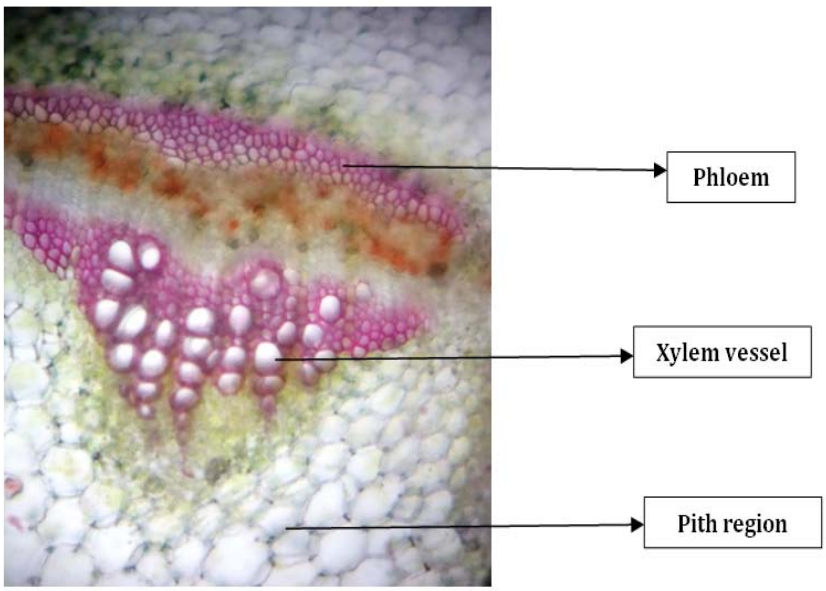

Fig. 1.b: T.S of stembark of *Cassia tora*

Plate I: Microscopic character of stembark of *Cassia tora*

Table 2: Histochemical test of stembark of *Cssia tora*

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 + Liberman	Steroid	+
9	Section + 5% KOH	Flavonoid	+
10	Section + Dragendorff's reagent	Alkaloid	+

+ Present, - Absent

Physico-chemical and Preliminary phytochemical analysis.

Powder microscopy of stem bark

Xylem vessel- Reticulate and spiral xylem vessel

Starch grain- Starch grains are spherical in shape. Found in group.

Cork cells- Rectangular parechymatous cell. Colour less.

Parenchyma- Broken pieces of parechymatous cells are found in the powder.

PLATE-II

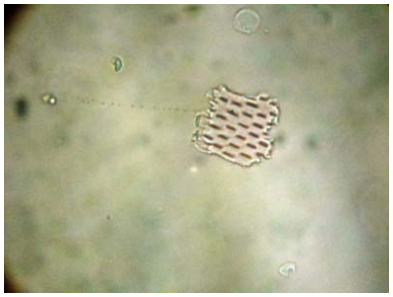

Fig. 2.a: Reticulate xylem vessels

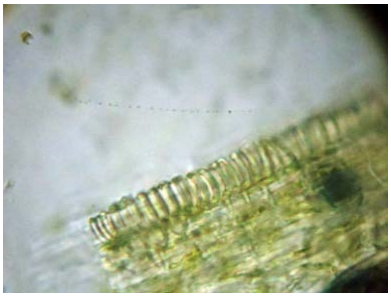

Fig. 2.b: Spiral vessel

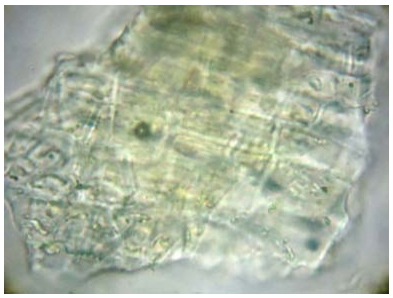

Fig. 2.c: Cork cell

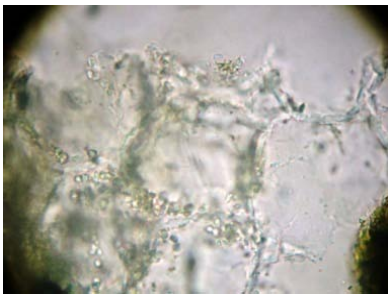

Fig. 2.d: Parenchymatous cell

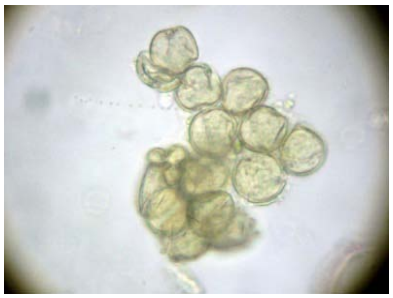

Fig. 2.e: Starch granules

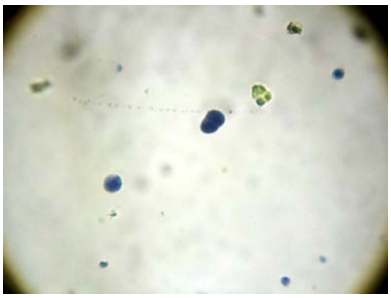

Fig. 2.f: Starch granules

Plate II: Powder Microscopic character of Stembark of Cassia Tora

Histochemical tests

Transverse sections stembark of *Cassia tora* were treated with routinely used chemicals and reagents, gave positive tests for starch, tannin, phenol, polyphone, lignin, steroid, flavonoid and alkaloid Table-2.

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 stembark of *Cassia tora* were found to be 1.18 w/w, 1.66 w/w, 2.86 w/w, & 5.42 w/w. The stem bark showed more amounts of methanol soluble components than petroleum ether, chloroform & ethyl acetate extracts. Table-3.

Ash values

The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Cassia tora* stembark were found to be 9.33 w/w, 4.5 w/w, 1.5 w/w, 14 w/w. The total ash value and water soluble ash value of *Cassia tora* stem bark powder were found to be more in crude drug. Sulphated ash was found to more than total ash and water soluble ash. Acid insoluble ash was found to be very less than total ash, water soluble ash and sulphated ash. Ash value is a measure of the quality and purity of the drug Table-3.

Loss on drying

The Loss on drying of stem bark was found to be 2.6 w/w which was shown in Table 3.

Table 3: Physicochemical analysis of *Cassia tora* stembark

Extractive value in Percentage	
Petroleum ether	1.18 w/w
Chloroform	1.16 w/w
Ethyl acetate	2.86 w/w
Methanol	5.42 w/w
Ash value in percentage	
Total ash	9.33 w/w
Water soluble ash	4.5 w/w
Acid insoluble ash	1.5 w/w
Sulphated ash	14 w/w
Loss on drying	2.6 w/w

Inorganic element

In organic element found in the ash of stembark of *Cassia tora* were calcium, iron, sulphate, carbonate & chloride Table-4.

Table 4: Test for inorganic elements in stem bark

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

Behaviour of powdred materials towards some chemical reagents

The behaviour of the powdered stembark was treated with picric acid, conc.sulphuric acid, con.hydrochloric acid, con.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-5.

Table 5: Behaviour of stembark powder with different chemical reagents

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

Fluorescence analysis

Fluorescence analysis of entire stembark 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-6.

Table 6: Fluorescence analysis of the stembark of *Cssia tora*

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

Extraction and Prelimnary phytochemical test

The dried powder of the material was initially extracted with methanol by decotion. The extract was filtered while hot and solvent removed by distillation. The residue was then suspended in distill water and fractionated with petroleum ether, chloroform, diethyl ether, ethyl acetate, The percentage yield were found to be 12.38 w/w, 0.12 w/w, 0.42 w/w, 0.78 w/w and 1.24 w/w respectively. The preliminary phytochemical studies were carried out to investigate the presence of various phytoconstituents. The methanol extract shown the presence of carbohydrate, protien, steroid, glycoside, flavonoid, alkaloid, tannin & phenols. The petroleum ether fraction shown the presence of carbohydrate, glycoside, & alkaloid. The chloroform fraction shown the presence of carbohydrate, glycoside, & alkaloid. The diethyl acetate shown the presence of carbohydrate, steroid, glycoside, flavonoid, alkaloid, tannin & phenols. The ethyl acetate fraction shown the presence of carbohydrate, glycoside, & alkaloid. The datas were shown in Table-7.

Table 7: Preliminary phytochemical screening of extract and different fractions of stem bark of *Cassia tora*

Test	Methanol	Petroleumether fraction	Chloroform fraction	Diethylether fraction	Ethylacetate fraction
Test forcarbohydrate					
Molish test	+++	+	+	++	+
Test for protien					
Millon's test	+	-	-	-	-
Test for steroid					
Salkowski reaction	+++	-	-	+	-
Liebermann-burchard reaction	+++	-	-	+	-
Test for glycosides					
Baljet test	+	+	+	++	+
Legal test	+	+	+	++	+
Saponin glycosides	++	-	-	++	+
Test for flavonoids					
Shinoda test	+++	-	-	+++	-
Lead acetate test	+++	-	-	+++	-
Test for alkaloids					
Dragendorff'test	++	+	+	+	++
Meyer's test	++	+	+	+	++
Hager'test	++	+	+	+	++
Wagner's test	++	+	+	+	++
Test for tannins & phenols					
5% $fecl_3$	++	-	-	++	-
Leadacetate	++	-	-	++	-

+ Mild, ++ Moderate, +++ Frequent, - Absent

Thin layer chromatographic studies

Thin layer chromatographic studies were carried out in methanol extract, petroleum ether fraction, chloroform fraction, diethyl ether fraction and ethyl acetate fraction. The methanol extract showed maximum three spots on TLC plate. Petroleum ether fraction showed two spots. Chloroform fraction showed one spots. Diethyl ether fraction showed two spots. Ethyl acetate fraction showed one spot. Vanilin sulphuric acid is used as detecting agent. The solvent systems used and Rf values recorded were given in Table-8.

Table 8: Thin layer chromatographic studies of extract & different fractions of the of *Cassia tora*

Extract	Solvent system	Spraying reagent	No. spots	Rf. values
Methanol	Cyclohexane:Acetone:Methanol (2: 4: 4)	Vaniline sulphuric acid	3	0.72 0.83 0.94
	Cyclohexane: Methanol (7: 3)	Vaniline sulphuric acid	1	0.74
	Cyclohexane : Ethylacetate : Methanol : Acetone (1: 2: 4 : 3)	Vaniline sulphuric acid	2	0.58 0.62
Petroleum ether fraction	Cyclohexane:Acetone:Methanol (2: 4: 4)	Vaniline sulphuric acid	2	0.68 0.69
Chloroform fraction	Cyclohexane:Acetone:Methanol (2: 4: 4)	Vaniline sulphuric acid	1	0.74
Diethylether fraction	Cyclohexane:Acetone:Methanol (2: 4: 4)	Vaniline sulphuric acid	2	0.58 0.74
Ethylacetate fraction	Cyclohexane:Acetone:Methanol (2: 4: 4)	Vaniline sulphuric acid	1	0.6

CONCLUSION

The present work focuses on the pharmacognostical of *Cassia tora* stembark of these plants. 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 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 stembark 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 stembark 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 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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