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Comparative Pharmacognostical Studies of Leaves of Three *Cassia species*

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Abstract

The genus Cassia belongs to family Caesalpiniaceae consists of about 400 species of which 45 are found in India and widely used for medicinal purposes. This genus contains mostly trees, shrubs or under-shrubs and contains pharmacologically active chemical constituents and is used in therapeutic. In present study leaves of three species of Cassia viz. C. fistula, C. occidentalis and C. tora are selected for comparative pharmacognostic studies. The main aim of this study is to compare pharmacognostic parameters of leaves of these three species for establishment of pharmacognostic profile of the leaves that will help in sample identification and standardization of quality and purity. The study has been carried out as per standard procedure of Ayurvedic pharmacopoeia of India and WHO guidelines of quality control of medicinal plants to establish the diagnostic keys based on the macroscopic and microscopic characters and physicochemical constants like loss on drying, ash value, extractive value, volatile oil contents, foaming index and swelling index. The result of these studies showed that all these species differ in macro and microscopic characters and the physicochemical constants.

Keywords: *Cassia*, macroscopy, microscopy, pharmacognosy, leaves, caesalpiniaceae.

Introduction

Cassia a genus of ornamental herbs, shrubs & trees, belongs to family Caesalpiniaceae[1]. It is widely distributed predominantely in tropical & warm temperate regions. This genus consists of about 400 species and only 45 are found in India [2]. The three species viz. *C. fistula* Linn (CFL), *C. occidentalis* Linn (COL) and *C. tora* Linn. (CTL) were selected for comparative pharmacognostical studies. These three species are traditionally used as laxative, purgatives, hepatoprotective, antidiabetic, antioxidant, antimicrobial and in wound healing [3]. The taxonomic classification is given below:

Taxonomic Classification:

***K*ingdom**	**Plantae - Plants**
Subkingdom	Tracheobionta --Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida -- Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae – Pea family
Sub family	Caesalpiniaceae
Genus	*Cassia*

Cassia fistula Linn. (Common name - Amaltas) is a moderate sized deciduous tree up to 15 m. in height and distributed throughout India. Flowers are bright yellow and blooms in the month of April to June when it is leafless. Traditionally it is used as laxative, astringent, digestant, antipyretic, hepatoprotective, antidiabetic, antiacne[4] and in skin disease.

Cassia occidentalis Linn. (Common name: Badi Kasondi) is an erect, foetid, annual herb, 60 to 150 cm. in height, found as a weed on waste places along roadside throughout India up to an altitude of 1500 m. Flowers are yellow in colour in short racemes and bloom in the month of July to August. Traditionally it is used as purgative, wound healing, diuretic, antipyretic, hepatoprotective, antidiabetic and in asthma and scorpion sting,

Cassia tora Linn. (Common name: Foetid Cassia, Pawar) is an annual foetid, erect herb or under-shrub 30 to 90 cm. tall, found as a weed on waste places along roadside throughout India up to an altitude of 1550 m. Flowers are yellow, usually in subsessile pairs on the short axillary stalks and blooming in the month of August to September. Traditionally it is used as bitter tonic, mild laxative, anthelmintic, antidiabetic and in liver disorders, skin and eye diseases.

Till date no scientific comparative pharmacognostic study of these three species has been reported, hence this study was undertaken. The main objective is to study and compare pharmacognosy on the basis of morphology, microscopy and physiochemical constants of the selected species.

Material and Methods

The fresh leaves of *C. fistula* were collected from the botanical garden of Hindu college of Pharmacy, Sonepat, Haryana and leaves of *C. occidentalis* and *C. tora* were collected from healthy plants along roadside from Sonepat district. The leaves were authenticated by Dr. H.B. Singh, Scientist F and Head,

Raw Materials Herbarium and Museum, NISCAIR, Delhi, under a voucher specimen number- NISCAIR/RHMD/Consult/-2012-13/2044/53 dated July 09, 2012 and NISCAIR/RHMD/Consult/-2012-13/2082/89 dated September 5, 2012. The specimen of the each plant has been submitted in the Department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonepat, Haryana, (India). The leaves of all the three plants were separated from twigs and shade dried. The leaves were crushed into coarse powder (sieve no. 10/44) and kept in properly labelled air tight containers.

Macroscopic evaluation[5,6]: The macroscopic evaluation of leaves of CFL, COL and CTL was done by observing the external characters like, shape, size, texture, surface characteristics and fractured surface with the help of magnifying lens. The organoleptic features like colour, odour, taste, feel and fracture of the crude drug were observed with sensory organs and compared.

Microscopic evaluation[5,6,7]: Microscopical studies were carried out from transverse sections of fresh leaflets and fine powder (#60) of dried leaves.

Transverse section: Thin free hand fine transverse sections (T.S.) of fresh leaves of CFL, COL and CTL were cut with the help of sharp razor blade. The sections were treated with chloral hydrate solution and warmed gently. The cleared sections were stained with phloroglucinol and concentrated hydrochloric acid and mounted in 50% glycerine and observed under microscope for the identification of various tissues and their arrangement. The microphotographs of sections were taken using Labomed ATC-2000 microscope attached with Sony digital camera for the identification of various tissues and their arrangement. Characteristic features of leaves of CFL, COL and CTL were noted for comparison.

Powder microscopy: For powder microscopy, air dried fine powder (#60) of leaves of CFL, COL and CTL were used. The powder was treated with chloral hydrate and stained with phloroglucinol and hydrochloric acid and mounted in 50% glycerine and observed under microscope for the identification of various tissues.. The microphotographs of different tissues and structures observed under microscope were taken using Labomed ATC-2000 microscope attached with Sony digital camera

Determination of Physicochemical Parameters[6, 7, 8]: Following physicochemical parameters were determined in coarsely powdered leaves of CFL, COL and CTL as per standard procedures.

Ash Value: Total ash, acid insoluble ash and water soluble ash were determined in the powdered leaves of *C. fistula, C. occidentalis and C. tora* according to the standard procedure. These values are used in determining the quality and purity of crude drug in powdered form

Total Ash: About 2g (accurately weighed) each of the air dried powdered leaves of CFL, COL and CTL were taken in previously ignited and tarred silica crucibles. The material was spreaded uniformly and incinerated in an incinerator at a temperature not more than 450°C until free from carbon. The crucibles were cooled in desiccators and weighed. The procedure was repeated till constant weight was obtained. The percentage of the total ash was calculated using the expression given below:

Total ash (% w/w) = (Weight of ash/Weight of sample) × 100

Acid insoluble ash: The total ash was boiled with 25 ml of dil. hydrochloric acid for five minutes, filtered through ash less filter paper and insoluble ash was washed with hot distilled water until the filtrate was neutral. The insoluble ash along with ashless filter paper was taken in tarred silica crucible, incinerated at 450°C, cooled and weighed. The percentage of acid insoluble ash so obtained was calculated as follow:

Acid insoluble ash (% w/w) = (Weight of acid insoluble ash/ Weight of sample) × 100

Water soluble ash: The total ash was boiled with 25 ml of distilled water for 5 minutes, filtered through an ash less filter paper. The insoluble ash along with ash less filter paper was transferred into tarred silica crucible and incinerated at 450°C and cooled in desiccator and weighed.. The weight of water soluble ash was obtained by subtracting the weight of the ash so obtained and weight of the total ash. The percentage of water soluble ash was calculated using the expression given below:

Water soluble ash (% w/w) = (Weight of water soluble ash/ Weight of sample) × 100

Extractive values: About 5 g of dried coarsely powdered leaves of CFL, COL and CTL were accurately weighed and macerated for 24 hours with 100 ml of solvents (ethanol 95% and water) in a separate glass stopper flasks. The flasks were shaked frequently during first six hours and allowed to stand for next 18 hours. Extracts were filtered rapidly, 25 ml of extract was transferred in tarred flat-bottom shallow dish and evaporated to dryness on water bath. The dried extract was further dried in hot air oven to constant weight at 105°C, cooled in desiccator and weighed. The percentage of extractive values for different solvents was calculated using formula given below:

Extractive Value (% w/w) = [(Weight of residue ×100) / (25×weight of sample)] × 100

Moisture content: 1g air-dried coarse powder of leaves of CFL, COL and CTL were accurately weighed in previously tarred crucible and dried at 105°C in hot air oven to constant weight and cooled in desiccator. Percentage of Moisture content was calculated using the expression given below:

$$\text{Moisture content (\%w/w)} = \frac{\text{Difference in weight before and after drying}}{\text{Weight of the sample before drying}} \times 100$$

Crude fiber content: About 2 gm of accurately weighed coarse powder of leaves of CFL, COL and CTL was extracted with petroleum ether, filtered and marc was air dried. Then 200 ml of 1.25%v/v of H_2SO_4 was added to the marc and boiled for 30 minutes under reflux. This mixture was filtered and residue so obtained was washed with boiling water until free from acid. The residue was again boiled with 200 ml of 1.25%w/v of NaOH for 30 minutes under reflux. This mixture was filtered through ash less filter paper and washed with boiling water until neutral and dried in a hot air oven at 110°C to constant weight and then incinerated to constant weight at temperature 450°C in incinerator.. The crude fiber content is the difference between the weight of the dried residue and incinerated residue. The percentage of crude fiber content was calculated using the expression given below:

$$\text{\%w/w of crude fiber} = \frac{\text{Difference in wt. of dried and incinerated residue}}{\text{Wt. of the air dried crude drug}} \times 100$$

Volatile oil content [9]: The volatile oil content was determined in the fresh leaves of CFL, COL and CTL by steam distillation using.

25g of fresh leaves were distilled in distillation flask using glycerine - water mixture. The distillate was collected in the graduated tube of Clevenger's apparatus. The temperature was adjusted in such a way that graduated tube remains cool. The aqueous portion was allowed to separate automatically and return to the distilling flask of Hydro distillation apparatus. The volatile oil was collected and percentage yield (%v/w) was calculated as follow:

$$\text{Volatile oil (\% v/w)} = \frac{\text{Volume of volatile oil collected}}{\text{Weight of crude drug}} \times 100$$

Swelling index: Swelling index gives an idea of the mucilage content in the crude drug**.** 1g of coarse powder of leaves of CFL, COL and CTL was taken in 25 ml glass- stopper measuring cylinder and water was added up to 25ml marking and shaked occasionally for 1 hour after every 10 minutes and kept aside at room temperature for 3 hours. Swelling index was calculated by measuring the volume in ml occupied by the 1 g swollen drug.

Foaming index: The foaming index measured the foaming ability of an aqueous decoction of plant material.
1g coarse powder of leaves of each plant was accurately weighed and transferred to a 500 ml conical flask containing 100 ml of boiling distilled water and moderate boiling was maintained for 30 minutes, cooled and filtered into 100 ml volumetric flask and the volume was made up to 100 ml with distilled water. The decoction was transferred into 10 labelled stopper test tubes in successive portions of 1 ml, 2 ml, 3 ml and up to 10 ml and volume was made up to 10 ml in each tube with distilled water. Tubes were shaken in length wise motion for 15 seconds with two shakes per second and were allowed to stand for 15 minutes and the height of foam in each tube was measured.

Results and Discussion

In the present study, the leaves of *C. fistula, C. occidentalis* and *C. tora* Linn. were evaluated for their pharmacognostic studies which includes morphology, microscopy and physicochemical evaluation. This study is used for comparison and standardization of herbal drugs and adulteration, if any, can be identified on the basis of these parameters. The results given below:

Macroscopic evaluation: The leaves of CFL, COL and CTL were differentiating on the basis of size, shape, apex, base and presence or absence of gland and flexible spine. The leaves of CFL have symmetric base and the gland is absent; whereas leaves of COL and CTL have asymmetric base and glands are present. The COL has ovoid dark purple coloured gland at the base of leaf and in CTL the main rachis has conical gland between the last two pairs of leaflets. The another differentiating character was presence of flexible spine on the dorsal surface near uppermost pair of leaflets in COL but in CTL it was present near lowermost pair of leaflets and absent in CFL. The other differentiating morphological characters of leaves and leaflets of three species are shown in table -1 and figure – 1A and B.

Microscopic Evaluation: Microscopic evaluation of the plant is essential to identify the adulterants and for the correct identification of the plant.

The results of microscopic evaluation are given below-

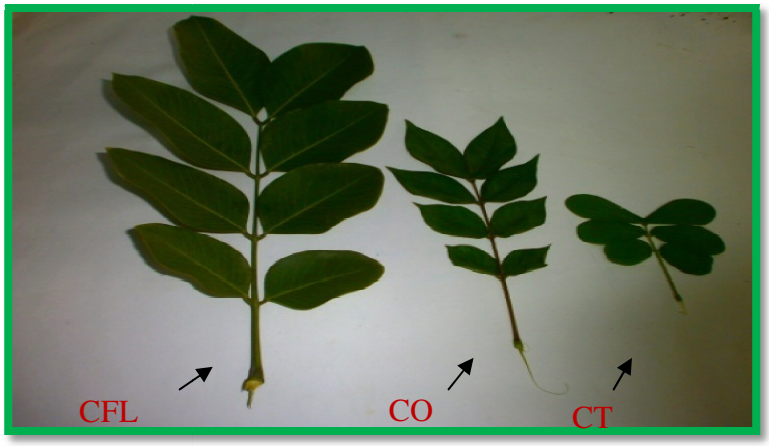
Transverse sections of leaflet: Microphotographs of transverse section of fresh leaflets of CFL, COL and CTL are shown in figure 2 (A2, A3), 3 (B2, B3) and 4 (C2, C3) respectively. T.S of leaflet through midrib and lamina region is dorsiventral in structure and shows two main regions lamina and midrib.

Lamina region in transverse section shows the upper and lower epidermis covered by cuticle. Epidermis exhibits unicellular covering trichomes in CFL, multicellular covering and glandular trichomes in COL and uni- to multicellular uniseriate covering trichomes with constricted uppermost cell in CTL on both the surfaces. Under the upper epidermis single layer of elongated palisade cells which is followed by 3-4 layers of loosely arranged spongy parenchymatous cells are present in all the three species. Paracytic stomata are present on both the surfaces

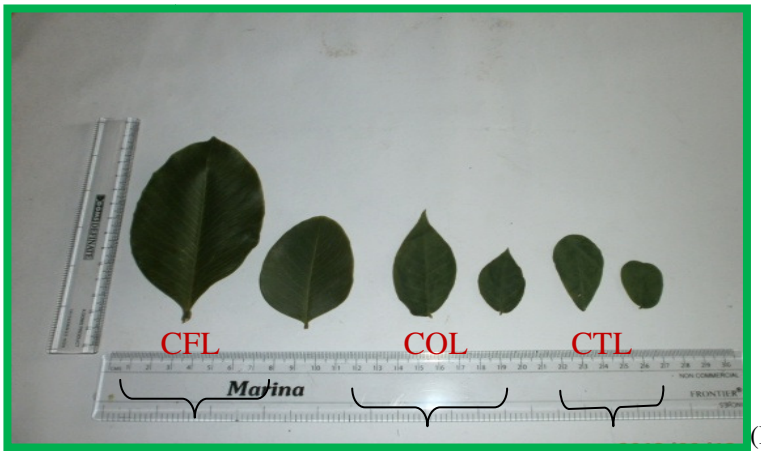
The midrib is biconvex in CFL and CTL whereas concavo-convex in COL. The epidermal cells covered with cuticle are present in the midrib region. The palisade parenchyma is continuous in the midrib region below the upper epidermis in COL and CTL but not in CFL. In CFL below the upper epidermis 3 to 4 layers of collenchymatous cells are present (figure 1, A3).

Table-1
Morphology of leaves and leaflets of *C. fistula, C. occidentalis* and *C. tora* Linn.

Parameters	***C. fistula* Linn.**	***C. occidentalis* Linn.**	***C. tora* Linn.**
LEAVES			
Type	Paripinnate, Compound having 4-8 pairs of leaflets	Paripinnate, Compound having 3-5 pairs of leaflets	Paripinnate, Compound having 3-4 pairs of leaflets
Colour	Green	Green	Green
Odour	Faint characteristic	Foetid	Faint characteristic
Taste	Slightly bitter	Slightly bitter	Slightly bitter
LEAFLETS			
Size Length(cm.) Breadth(cm.)	 7-15 4-7	 4.5-10.5 3-4.5	 2.5-4.5 1.3-2.5
Apex	Acute	Acuminate	Obtuse to Slightly retuce
Margin	Entire	Entire	Entire
Shape	Oblong	Broadly Lanceolate	Obovate
Base	Symmetric	Asymmetric	Asymmetric
Surface	Pubescent	Pubescent	Pubescent
Texture	Coriaceous & Leathery	Slippery & papery	Smooth
Midrib	Biconvex and more prominent on lower side	Concavo-convex and less prominent on lower side	Biconvex but less prominent on either side
Venations	Reticulate	Reticulate	Reticulate



(A)



(B)

Figure-1
Morphology of (A) Leaves and (B) Leaflets of *C. fistula* (CFL), *C. occidentalis* (COL) and *C. tora* Linn. (CTL)

In the centre of midrib vascular bundles are present which are surrounded by sclerenchymatous pericyclic fibres. Above the lower epidermis 3-4 layers of collenchymatous cells are present.

Powder Microscopy: The powder under microscope shows the presence of trichomes, stomata, epidermal cells in surface view, palisade parenchyma (in transverse and surface view) and vessels. Microphotographs are shown in figure 2(A4 to A9), 3 (B3 to B9) and 4 (C3 to C9).

The microscopic differentiating characters are shown in table-2.

Physicochemical evaluation: The physicochemical evaluation was carried out to set standards, along with morphological and microscopical evaluation, for the plant and to ensure quality and purity of the crude drug. From this study it is observed that CFL has maximum moisture content (7.794%w/w), alcohol soluble extractive value (13.864% w/w) and crude fibre content (5.48%w/w). COL has maximum water soluble extractive value (7.925%w/w) and CTL has maximum ash value (15.76%w/w). In all the three species foaming index is less than 100 and volatile oil are absent and show no swelling. The results of physicochemical analysis are compiled in table-3**.**

Table-2
Microscopic differentiating characters of leaves of three *cassia* species

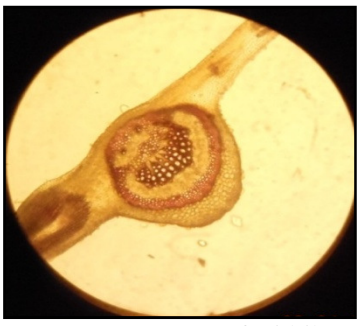
Characters	***C. fistula***	***C. occidentalis***	***C. tora***
Trichomes	Unicellular covering	Multicellular uniseriate covering trichomes, Glandular Multicellular stalk and multicellular head	Unicellular and Multicellular uniseriate covering trichomes and uppermost cell is constricted
Palisade parenchyma in midrib region	Absent	Present	Present
Midrib	Biconvex	Concavo-convex	Biconvex
Stomata Adaxial surface Abaxial surface	 Paracytic Paracytic	 Paracytic Paracytic and Anisocytic	 Paracytic Paracytic and anisocytic

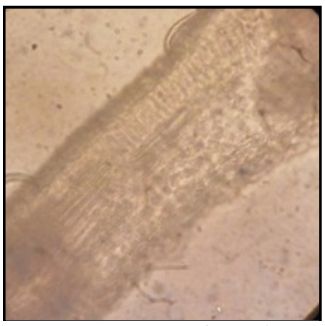
Table-3
Comparative Physicochemical parameters of leaves of three *Cassia* species

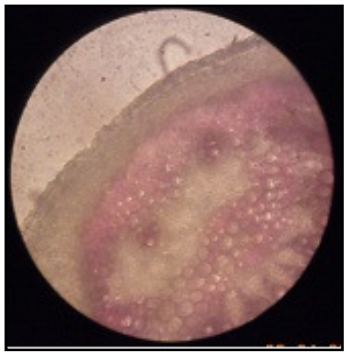
S. No.	**Parameters**	***C. fistula* Linn.**	***C. occidentalis* Linn.**	***C. tora* Linn.**
1	Moisture content (%w/w)	7.794	6.323	5.985
2	Extractive value(% w/w) Water soluble Alcohol soluble	 17.44 13.864	 30.80 12.089	 25.66 9.266
3	Ash value (% w/w) Water soluble(%w/w) Acid insoluble(% w/w)	9.699 1.390 2.48	12.616 3.750 2.750	15.76 7.925 3.708
4	Crude fibre content(%w/w)	5.48	3.84	5.10
5	Foaming index(ml)	<100	<100	<100
6	Volatile oil content	Nil	Nil	Nil
7.	Swelling index	Nil	Nil	Nil

Values are mean of three determinations

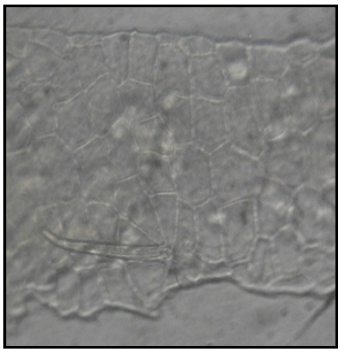

A1: *C. fistula* leaflets (CFL)


A2: T.S. of midrib

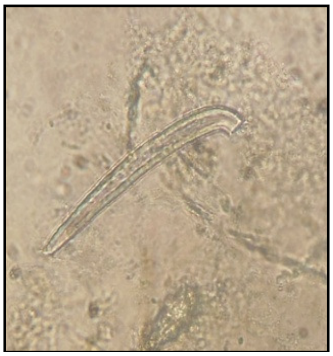

A3: T.S. of Lamina



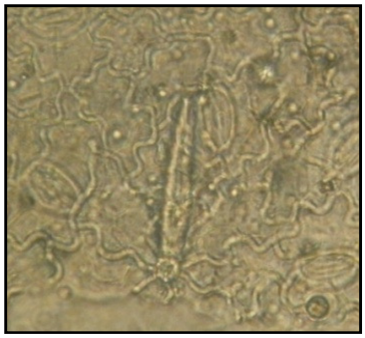
A4: T.S. of midrib with upper Epidermis and collenchymatous cells below it



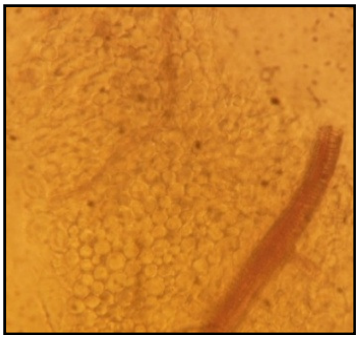
A5: Upper epidermis polygonal Cells



A6: Unicellular covering trichome



A7: Lower epidermis wavy Cells with paracytic Stomata



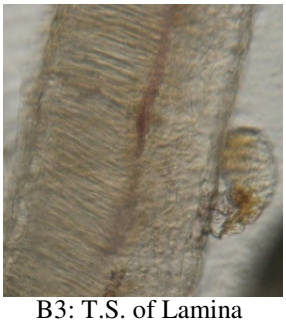
A8: Palisade cells in surface view

A9: Palisade cells in transverse view

Figure-2
T.S. and powder microscopy of leaves of *Cassia fistula* Linn

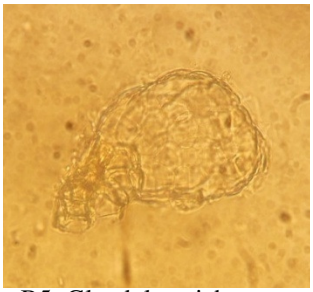
B1: *C. occidentalis* leaflets (CFL)

B2: T.S. of COL

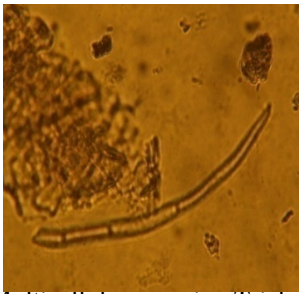


B3: T.S. of Lamina

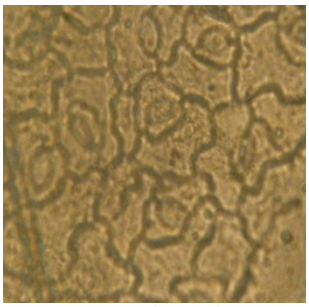
B4: Upper epidermis with paracytic stomata

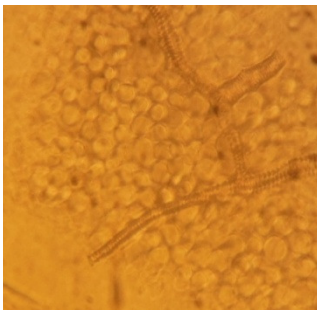


B5: Glandular trichomes



B6: Multicellular covering Trichomes


B7: Lower epidermis wavy Cells with paracytic and Anisocytic stomata


B8: Palisade cells in surface view

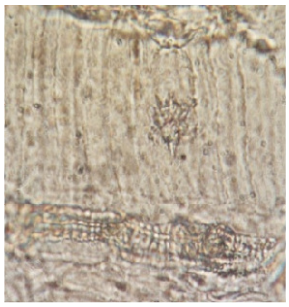
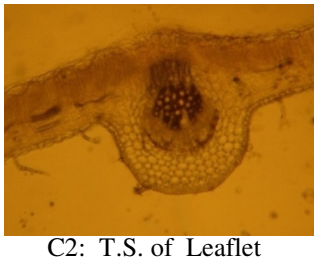
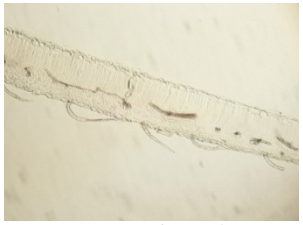
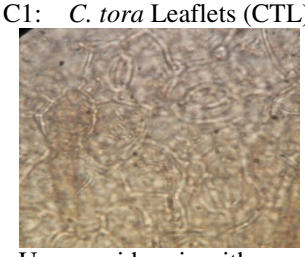

B9: Palisade cells in transverse view

Figure-3
T.S. and powder microscopy of leaves of *Cassia occidentalis* Linn

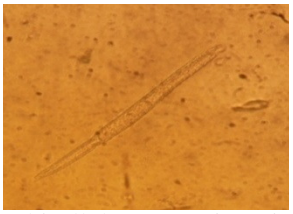
C1: *C. tora* Leaflets (CTL)

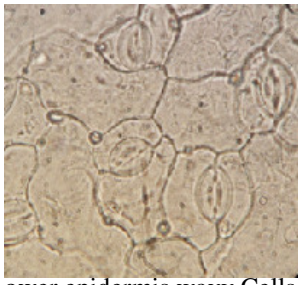

C2: T.S. of Leaflet

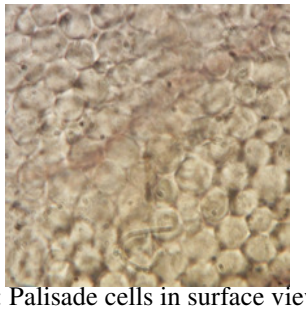

C3: T.S. of Lamina


C4: Upper epidermis with paracytic stomata


C5: Unicellular covering trichomes


C6: Multicellular covering trichome


C7: Lower epidermis wavy Cells with paracytic and Anisocytic stomata


C8: Palisade cells in surface view

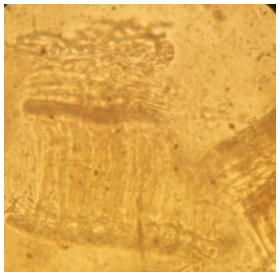

C9: Palisade cells in transverse view

Figure-4
T.S. and powder microscopy of leaves of *Cassia tora* Linn

Conclusion

From the study it has been concluded that comparative pharmacognostical studies, i.e. the macroscopic, microscopic characters and physic-chemical parameters, are helpful in identification and differentiation of three species of *Cassia* based on their morphology, microscopy and physicochemical constants.

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