

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *CASSIA SOPHERA* LINN.

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ABSTRACT

Pharmacognostical parameters for the all three parts of *Cassia sophera* L were studied with the aim of drawing the pharmacopoeial standards for this species. Macroscopical and microscopical characters, physicochemical constants, extractive values of dry powder and its reaction after treatment with chemical reagents were studied. The determination of these characters will aid future investigators in their Pharmacological analyses of this species.

KEYWORDS: *Cassia sophera* L., Caesalpinaceae, Chromatogram, Standardization.

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INTRODUCTION

Cassia sophera Linn (Family: Caesalpinaceae) is commonly known as "Kasodia" and grows abundantly in India, most of the tropical. It is an important medicinal plant and considered to have expectorant property. The infusion of bark and the seeds along with honey used for diabetes. The bark, leaves and seeds are used as a cathartic. The juice of leaves is specific for ringworm, especially when made into a plaster in combination with sandal wood. The root is administered internally with black pepper for snake bite.¹ The leaves are used in asthma², ulceration³, bronchitis. Infusion of leaves is useful in gonorrhoea and syphilitic sores. In ethno botanical literature, it is mentioned to be effective in the treatment of psoriasis. *Cassia sophera* L is useful in ascites, dyscrasia of liver, piles, jaundice, fever and palpitation. The chemical analysis of the seed of *Cassia sophera* L revealed the presence of ascorbic acid, dehydroascorbic acid and β -sitosterol.⁴ *Cassia sophera* L has been reported for anti inflammatory and analgesic activity.⁵ Aqueous extract of leaves of *CASSIA SOPHERA* showed significant hepatoprotective activity against ranitidine induced hepatic damage as depicted by functional, physical, biochemical and histological changes in liver.⁶ *CASSIA SOPHERA* has been reported to possess antioxidant potential with health benefits.⁷

MATERIAL AND METHODS

The whole plant of *Cassia sophera* L was collected from Junner, Dist. Pune, India. It was identified and

authenticated by the scientists of Botanical Survey of India, Pune, India. The voucher specimen no. is SSDH-1. Collected parts were washed and used for study of organoleptic and microscopic characteristic *Cassia sophera*. All chemicals and reagents used for testing were of analytical grade obtained from SD Fine Chemicals. Microscope, Camera Lucida, Digital camera (Nikon- coolpix) used for morphological study.

Morphological study

Morphological study was carried out for organoleptic evaluation. In that study color, structure, shape and size were visually observed.

Microscopy

The leaves, seeds and root were selected for the microscopical study. Microscopic sections were cut by using microtome and free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaves specimen were made and examined.⁸ A powder characteristic, preliminary examination was carried out.⁹

Physicochemical parameters

The total ash, acid-insoluble ash and water soluble ash were calculated as per the Indian Pharmacopoeia.¹⁰ Different extracts of the all parts were prepared for the evaluation of extractive values.

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 5g of powder of each part were extracted with 50% Methanol. The extracts were dried and weighed. The presence or

absences of different phytoconstituents viz. alkaloids, flavonoids, saponins, tannins, glycosides, mucilage and coumarin were detected by usual prescribed methods^{11,12}

Chromatographic Study

Chromatographic Conditions

The sample was spotted in the form of band of width 6 mm with CAMAG microlitre syringe on precoated silica gel aluminium Plate 60F₂₅₄ (20 cm x 10 cm with 0.2 mm thickness Merck, Germany) using CAMAG Linomat 5 applicator (Switzerland) fitted with a 100 µL syringe. The Linear ascending development was carried out in solvent system (20 ml) Toluene: Ethyl acetate: Methanol: Formic acid (7:2.5:1:1.5 v/v/v/v) in a glass twin through chamber (20x10 cm) previously saturated with mobile phase for 30 min. The TLC plate was allowed to run up to 80 mm from the point of application. TLC plate was dried in hot air oven at 60°C. Densitometric scanning was performed using CAMAG TLC scanner 3 in the absorbance mode at 280 nm and operated by winCATS software (V 1.4.3.6336). The slit dimension was 5×0.45 mm with the scanning speed of 20 mm s⁻¹. Evaluation was done via peak area with linear regression.

Preparation of Sample Solution

An accurately weighed 100 mg of 50 % methanol extract of root, leaves and seeds were transferred to 25 ml of volumetric flask separately and about 15 ml of methanol was added to the flask. The mixture was sonicated for 20 minutes in an ultrasonic water bath and diluted up to the mark with methanol. The solution was filtered through whatman no. 41 filter paper and used for further chromatographic analysis.

RESULTS

Morphology (Figure 1)

➤ Leaves

- Leaves are 6-10 in pairs, lanceolate, acute.
- Base of leaves are generally rounded.
- Its petioles are 1.5-2 mm long, glabrous.
- Main nerves 10-12 pairs.
- Color of leaves is green.

➤ Seed

- Seeds are 30-40 in no.
- Broadly ovoid, acute compressed
- Dark brown
- Thickness 2mm
- Seeds are generally found in a size of 6 by 4 mm

➤ Root

- Root having a length near about 15-20 cm
- Color is dark brown
- Diameter is 20mm 50mm

Microscopic Features

Powder microscopy

The powder microscopy reveals the presence of different types of trichomes, trichome base, fibres, stone cells, laticifers with adjacent parenchyma. Spiral thickenings vascular bundles.

Microscopy of leaves

The transverse section of the leaf showed following characters: (Fig: 2, 3, 4)

Description of microscopy of the leaves

1. Palisade cell layer is single
2. Sclerenchymatous sheath contains lignified thick walled cell covering the vascular bundle.
3. Vascular bundle comprises with xylem and phloem.
4. Xylem is lignified and present at ventral surface.
5. Phloem having non-lignified cells and present at dorsal surface.
6. Upper epidermis is single layered, polygonal and covered with the cuticle
7. Trichomes are conical, Unicellular and Thick walled,
8. Stomata are Paracytic.

Microscopy of Root

The transverse section of the root showed following characters: (Fig: 5, 6)

Description of microscopy of the root

1. It has well developed Periderm and vascular bundles. Periderm is Continuous around the circumference and has shallow, irregular tissue. It consists of seven layers of tabular, thick walled and homogenous Phelloderm. Periderm is of narrow cortex and spread all over the root.
2. The cortical tissue consists of polygonal, thick walled and compact parenchyma cells.
3. The gelatinous fibers have thick primary wall and gelatinous secondary wall which has move away from the primary wall forming a ring in the center of lumen.
4. Secondary phloem consists of clusters of sieve-elements and phloem parenchyma. The sieve-elements are 20 µm in diameter.
5. Secondary xylem is a wide solid and dense cylindrical comprising of vessels and fibers. The vessels are diffused, in distribution. They are circular, mostly solitary and thick walled. The narrow vessel is 50µm in diameter.
6. Xylem fibers include narrow and wide cells. The narrow fiber have thick wall and some of them are gelatinous type.

Microscopy of seed

The section of the seed showed following characters: (Fig. 7, 8, 9)

Description of microscopy of the seed

1. The seeds have thick testa, seed coat, copious endosperm and prominent Embryo.
2. The embryo has thick, solid radical and two folded cotyledon. The embryo is surrounded by dense mass of endosperm.
3. Seed coat is thick or micropylar end and become slightly thinner towards opposite ends. The seed coat contains cuticle, epidermal layer and inner seed coat
4. Cuticle is the outermost waxy layer; it is extremely thick, measuring nearly 20 μ m thickness.
5. Epidermal layer is also known as Palisade layer. It is 50 μ m thick. It consists of vertically elongated, compact, and columnar salaried. It has thick lignified call wall and narrow lumen.
6. Inner seed coat is an innermost part of the seed coat consists of four layers of polygonal, compact, thick walled cells. This inner seed coat is 80 μ m thick.

Physicochemical Parameters**Proximate chemical analysis****Ash value**

The results revealed that the seeds showed highest total ash and acid insoluble ash value. The root showed more water soluble ash value as compare to other parts of *Cassia sophera* L. (Table 1)

Extractive value

The results revealed the highest extractive value for all the parts was from water. However only for leaves the ethanol soluble extractive value was greater than any other solvent. (Table 2)

Preliminary Phytochemical Evaluation

The 50% methanol extract of root, leaves and seeds were subjected for the preliminary phytochemical analysis for their presence of the constituents. It showed the presence of glycosides, flavonoids, tannins and Mucilage in all the extract except in petroleum ether.^{13, 14} (Table 4)

Chromatographical Fingerprinting

The extract was subjected for HPTLC development. The composition of mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity like and the best results were obtained by using Toluene: Ethyl acetate: Methanol: Formic acid (7:2.5:1:1.5 v/v/v/v). The scanning wavelength selected was 280 nm. The selected mobile phase produced highly symmetrical peaks showing good resolution between each other. (Fig. 10, 11, 12)

DISCUSSION

Morphological study gives details about physicochemical constants which help in standardization of crude drugs. Microscopical inspection of crude drugs from plant origin is essential for the identification of the grounded or powdered materials.

The quality and purity of crude drugs depends upon all ash values. The total ash usually consists of inorganic radicals like carbonates, phosphates, silicates and silica of sodium, potassium, magnesium and calcium. Inorganic parts like calcium oxalate, silica, carbonate content of crude drug affects "total ash" values, such variables are then removed by treating with acid (Hydrochloric acid) and then acid-insoluble ash value is determined. The results for total ash, acid-insoluble ash and water-soluble ash have reported first time in this paper. Extractive values are useful for evaluation of crude drugs and gives an idea about the nature of chemical constituents present in them. All the standardization parameter of the plant in this paper has been reported first time.

The extractive value of drug reported as w/w. This yield in given solvent is directly related to amount of a constituent or complex of constituents present in the drug. In some cases the amount of drug soluble in a given solvent is also represents its purity. The solvent used for extraction should be in a position to dissolve appreciable quantities of substances desired.

The qualitative chemical tests were carried out for the identification of the nature of phytoconstituents present in root, leaves and seeds of *Cassia sophera* L. The 50% Methanol extract of *Cassia sophera* L was chosen for present study because of this solvent supposed to have ability to extract maximum amount of desire phytoconstituents.

Results obtained from all phytochemical screening showed mainly the presence glycosides, flavonoids, tannins and mucilage. The chromatographical fingerprinting of leaves, seeds and root of *Cassia sophera* L is important for identification and quality of crude material. The present study will be proved fruitful for the standardization and evaluation of leaves, seed and root of *Cassia sophera* L.

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Table 1: Ash values

Sr. no.	Particulars	Observed values (% w/w)		
		Leaves	Seed	Root
1.	Total ash	NMT 12.27	NMT 13.94	NMT 10.88
2.	Acid insoluble ash	NMT 2.48	NMT 9.62	NMT 7.43
3.	Water soluble ash	NMT 0.53	NMT 1.17	NMT 3.91

Table 2: Extractive Values with Different Solvents

Sr. no	Particulars	Observed values(% w/w)		
		Leaves	Seed	Root
1.	Ether soluble extractive value	2.47	1.93	2.81
2.	Ethyl acetate soluble value	10.42	3.66	5.77
3.	Chloroform soluble extractive value	16.67	2.56	4.83
4.	Ethanol soluble extractive value	19.63	11.98	13.46
5.	Water soluble extractive value	15.82	16.78	14.21

Table 3: Leaf constants

Sr. no.	Particulars	Values
1.	Palisade ratio (Upper epidermis) (lower epidermis)	7.8 9.2
2.	Vein-islet no	38-44
3.	Vein termination no	12-20
4.	Stomatal no	32-45
5.	Stomatal index	12.9

Table 4: Preliminary phytochemical evaluation

S.N.	Chemical tests	Leaves	Seed	Root
1.	Alkaloids			
	Dragendroff's test	-	-	-
	Mayer's test	-	-	-
	Hager's test	-	-	-
	Wagner's test	-	-	-
2.	Glycoside			
	Anthraquinone glycosides	+	+	+
	Cynogenic glycoside	-	-	-
	Cardiac glycoside	-	-	-
3.	Flavonoids	+	+	+
4.	Coumarin	-	-	-
5.	Tannins	+	+	+
6.	Saponins	-	+	-
7.	Mucilage	+	+	+

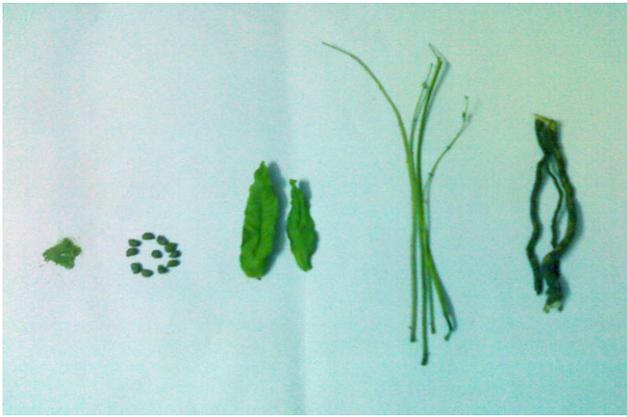


Fig. 1 Powder, seeds, leaves and roots of C.S.

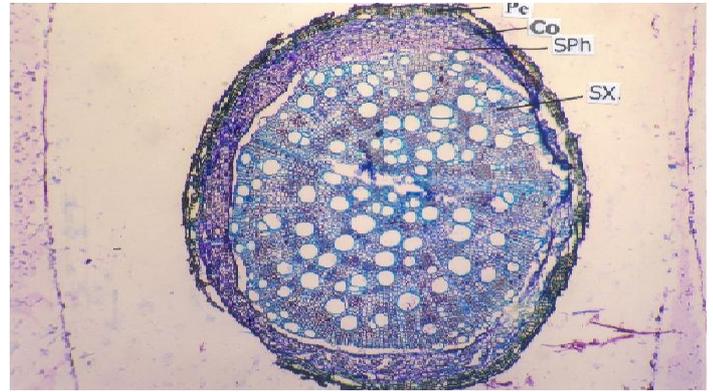


Fig. 5 Entire T.S. of Root



Fig. 2 Entire T.S of leaves

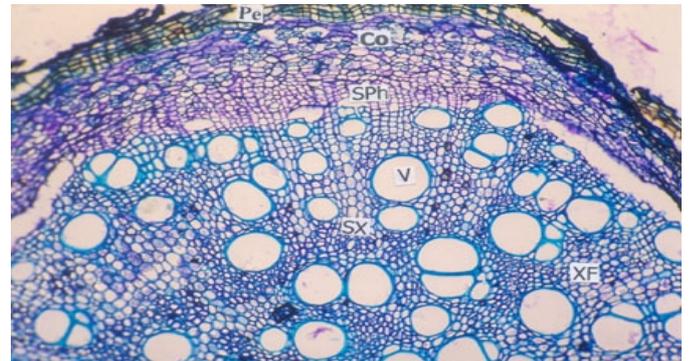


Fig. 6 T.S. of root

Co- cortex; Pe- Pediderm; SPH- Secondary Phloem; Sx- Secondary Xylem
V- Vessels; XF- Xylem fiber

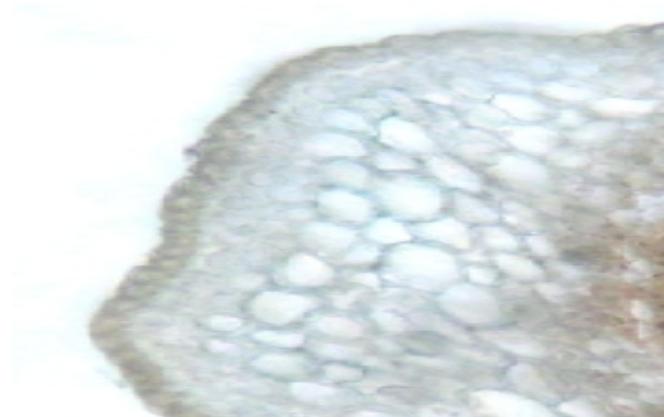


Fig. 3 Upper epidermis, palisade cells and spongy parenchyma

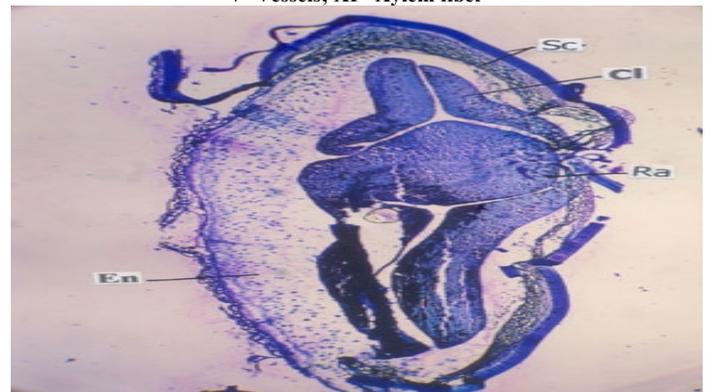


Fig. 7 Vertical longitudinal section of the seed
Cl- Cotyledon; En- Endosperm; Ra- Radical



Fig. 4 Vascular bundles



Fig. 8 Upper portion of Seed
Cl- Cotyledon; En- Endosperm; Pa- Palisade layer; IS- Inner seeds coat
OS- Outer seed coat

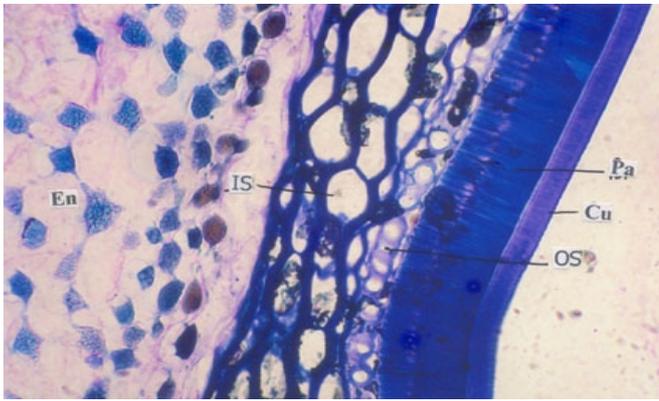


Fig. 9 Outer and Inner Seed Coat

Cl- Cotyledon; En- Endosperm; Pa- Palisade layer; IS- Inner seeds coat; OS- Outer seed coat

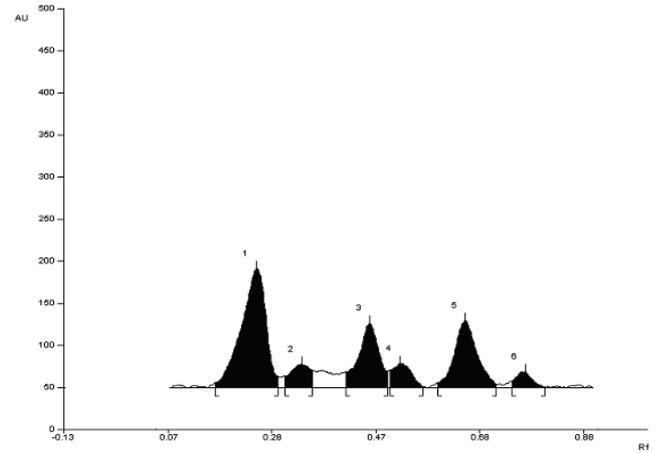


Fig. 11 HPTLC Densitograms of Seed of *Cassia sophera L*

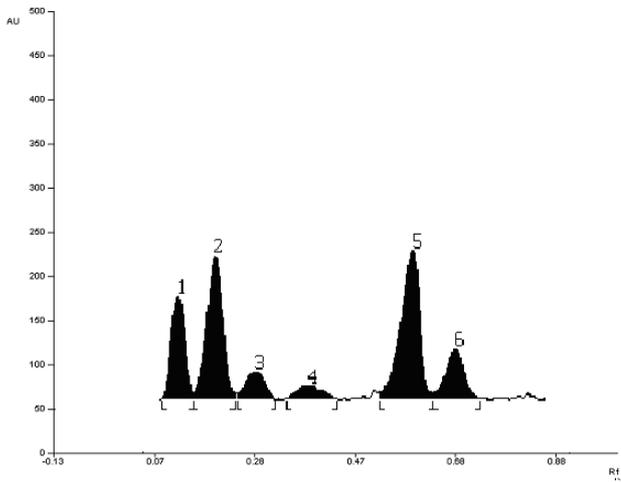


Fig. 10 HPTLC Densitograms of Leaf of *Cassia sophera L*

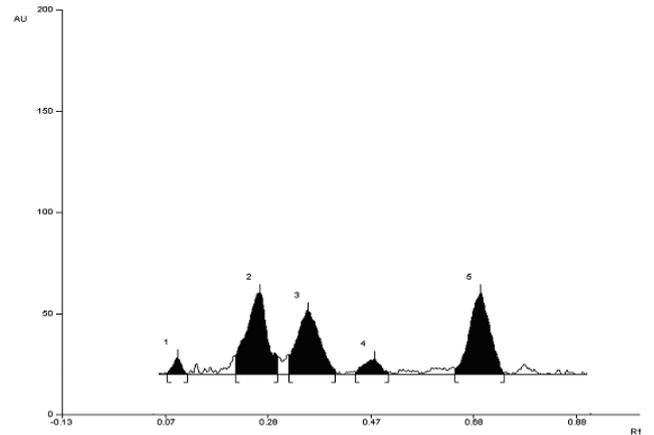


Fig. 12 HPTLC Densitograms of Root of *Cassia sophera L*

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