

**HPTLC FINGERPRINT PROFILE AND PHARMACOGNOSTIC INVESTIGATION OF
CASSIA FISTULA LINN.**

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ABSTRACT

Cassia fistula (family – Leguminosae) is an important drug used in the Indian system of medicine and also known as 'Amaltash'. It is popularly grown in Thailand, it is native to India & distributed in Asia, South Africa, China, West Indies and Brazil. All parts of this plant has been described to be useful against skin disease, liver troubles, tuberculosis gland, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy. Its latex is aphrodisiac, tonic, lessens, piles, nose diseases, gonorrhoea etc. The present study was carried out to investigate the macro and microscopic study, physico-chemical study, phyto-chemical analysis, fluorescence study and HPTLC fingerprints profile of fruits of *Cassia fistula* L. Presence of various phyto- constituents and standardized data can serve as basis for screening of different pharmacological activities, investigation and further research.

KEYWORDS: *Cassia fistula*, Physicochemical analysis, Phyto-chemical studies, Microscopy, HPTLC fingerprints profile.

INTRODUCTION

Cassia fistula (family – Leguminosae) is deciduous and mixed -Monsoon forests throughout greater part of India, ascending to 1300 m in outer Himalaya, is widely used in traditional system of India. It is 8- 15 m to 24 m in height, with greenish grey smooth brown when mature. Trunk straight, bark smooth and pale grey when young, rough and dark when old, branches spreading, slender. Leaves 23 -40 cm long, main rachis pubescent, stipules minute, linear -oblong, acute, 5 - 12.5 by 3.8 - 9.5 cm, bright green and glabrous above. Leaflets 8 -12 pairs flowers- yellow, with long drooping racemes. Fruit is cylindrical pod and seeds many in black, oval shaped attach with sticky brown pulp, sweet pulp separated by transverse partitions. Pulp is dark brown in colour, sticky, sweet and mucilaginous, odour characteristic and somewhat disagreeable. Ayurvedic medicine recognizes the seeds as antibilious aperitif combinative and laxative. Its main medicinal property being that of a mild laxative suitable for children & pregnant women. It is also a purgative due to the wax aloin & a tonic. In the Indian literature, this plant has been described to be useful against skin disease, liver troubles, tuberculosis gland and its use in the treatment of leucoderma & diabetic.^[1-8] The present study was carried out to investigate the macro and microscopic study, physico-chemical study, phyto-chemical analysis, fluorescence study and HPTLC fingerprints profile of fruits of *Cassia fistula* L. Presence of various phyto-constituents and standardized data can

serve as basis for screening of different pharmacological activities, investigation and further research.

MATERIALS AND METHODS

Collection and identification of plant

Fruits of *Cassia fistula* L. was collected from university campus at MGCGV, Chitrakoot, Satna, MP, India. The plant material was identified and authenticated with the help of Herbarium specimen at Herbarium of Research Lab, Chitrakoot. All chemicals and reagents used including the solvents were of analytical grade.

Macroscopically studies

Fruits powder was studied for its organoleptic characters such as colour, odour and taste.^[9]

Microscopically studies

About 1g powdered drug was treated with Chloral hydrate and 2% potassium hydroxide, boiled and cooled. Washed the treated sample with distilled water 5-6 times for Chloral hydrate and 1- 2 times for 2% potassium hydroxide. The treated sample were stained with Sudan III and iodine water, mounted with glycerin and observed under the compound microscope at 40 X 10X magnification of the trinocular research microscope fitted with digital Camera Lucida.^[10]

Physico-chemical analysis

Physicochemical studies are includes extractive values

(alcohol soluble & water soluble), total ash value, acid insoluble ash value and loss on drying (at 105°C.^[11]

Fluorescence Analysis

The color change of the powdered samples with respect to different chemical reagents on the basis of different chemical constituents was observed in daylight and ultraviolet as per the methods described.^[11]

Preliminary phytochemical analysis

Preliminary qualitative phytochemical analysis of aqueous and alcoholic extracts of fruit of *Cassia fistula* L. was carried out by employing standard protocols 4-5 for determining the presence and/or absence of phytochemical.^[12]

Alkaloids

1. Dragendorff's test

Dissolved a few ml of alcoholic or aqueous extracts of drug in 5 ml of distilled water, added 2 ml HCL until an acid reaction occurs, then added 1ml of dragendorff reagent, an orange or orange red precipitate is produced immediately.

2. Wagner test

Acidified 1 ml of alcoholic extract of the drug with 1.5% v/v of HCl and added a few drops of Wagner reagents, a yellow or brown precipitate is formed.

Flavonoid (Shinoda test)

Added 5-10 drops of dil HCL followed by small pieces of mg in a test tube containing 0.5 ml of alcoholic extract of the drug. In the presence of flavonoid, pink, reddish pink or brown color is produced.

Carbohydrate

1. Anthrone test

To 2ml of anthrone test solution added 0.5 ml of aqueous extract of the drug, a green or blue color indicates the presence of carbohydrate.

2. Fehling test: To 2 ml of aqueous extract of drug added 1 ml of mix of equal part of Fehling solution A and B and boiled the content of the test tube for few minute, a red or brick red ppt. is formed.

Protein (Biurate test)

To 1 ml of hot aqueous extract of drug, added 5-8 drops of 10% w/v NaOH solution followed by 1-2 drops of 3% w/v CuSO₄ solution, a red or violet color is obtained.

Resin

Dissolved the aqueous extract in 1 ml of acetone and poured the solution into 5 ml of distilled water, turbidity indicates the presence of resin.

Saponins (Foam tests)

In test tube containing about 5 ml of an aqueous extracts of drug, add drops of sodium bicarbonate, shaken it vigorously and left for few minutes, honey comb like

structure is formed.

Tannins

To 1-2 ml of extracts of the drug, added few drops of 5% FeCl₃ solution, a greenish color indicate the presence of gallotannin, and white brown color for tannin.

Steroids (Salkowski tests)

Added 1 ml of conc. H₂SO₄ to 2 ml of chloroform extracts of the drug carefully, from the side of test tube a red color is produced in the chloroform layer.

HPTLC (High Performance Thin Layer Chromatography)

For HPTLC, 2 gm of sample was extracted with 25 ml of ethanol on boiling water bath for 20 min. Filtered through Whatman filter paper No. 1 and concentrated. TLC of methanolic extracts of all the samples was carried out on silica gel 60 F254 percolated plates (0.2 mm thickness; from Merck India Limited Mumbai). An applicator from Camag Linomat-5 Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used Toluene: Ethyl acetate (7:3). The plate was developed over a distance of 8 cm in a saturated development chamber (Twin through chamber 20X10 cm with SS lid and visualized under 254nm, 366nm and visible light. After spraying with 5% methanolic sulphuric acid followed by heating at 110°C for 10 min.^[13-14]

RESULT AND DISCUSSION

Macroscopic characters

Cassia fistula L. fruit powder colour is brown, taste astringent and odour characteristics.

Powder microscopic characters

Under microscope examined powder shows Single and compound starch grains in various shapes and sizes, prismatic and rod shape crystals of calcium oxalate, single and groups of stone cells embedded with simple pits and sclereids, parenchymatous cells embedded with prismatic crystals, starch grains and oil cell, epidermal cells of testa from side view, hypodermal cells of testa in surface view, fragment of cotyledon filled with aleurone grains in surface view, epidermis of testa in surface view, fibres, endosperm cells filled with oil globules and aleurone grains in surface view and crystal fibres. (Figs. 1 to 11).

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table 1).

Fluorescence Analysis

Fluorescence study was done and results are given in (Table 2).

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. Phyto-chemical results of the drug are given in (Table 3).

High performance thin layer chromatography

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the Thin Layer Chromatography plate. Major spots R_f values with colour were recorded under, 254nm, 366nm, after derivatization 366nm.

Chromatogram profile and R_f values are given (Fig.12-15 & Table 4).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Cassia fistula* L. fruit. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. High performance thin layer chromatography finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The High performance thin layer chromatography profile also helps to identify and isolate's important phyto-constituents. These finding could be helpful in identification and authentication.

Table 1 - Physicochemical tests of *Cassia fistula* (fruit)

S. No.	Tests	Results
1	Loss on drying at 105 ^o C (% w/w)	7.9%
2	Water soluble extractive value (% w/w)	47.87 %
3	Alcohol soluble extractive value (% w/w)	16.9645%
4	Total ash value (% w/w)	5.7%
5	Acid-insoluble ash value (% w/w)	0.056%

Table 2- Fluorescence Study of *Cassia fistula* (fruit)

S. No.	Drug powder + Chemical	Observation in day light	Observation in 366nm
1.	Drug powder	Light brown	Yellow
2.	Powder +1 N HCl	Light brown	Brown
3.	Powder +1N NaOH (meth.)	Black	Dark brown
4.	Powder +1 N NaOH (water)	Black	Dark brown
5.	Powder + 50% KOH	Black	Black
6.	Powder + 50% HNO ₃	Brown	Brown
7.	Powder + 50% H ₂ SO ₄	Brown	Green
8.	Powder + Con. H ₂ SO ₄	Black	Green
9.	Powder + Iodine water	Brown	Green
10.	Powder + Con.HNO ₃	Dark brown	Brown

Table 3- Preliminary Phytochemical analysis of *Cassia fistula* (fruit)

S.No	Name of experiment	Observation	Result
1	Alkaloids	Pale yellow colour Appear	Present
2	Carbohydrates	Dark bluish colour appear	Present
3	Protein	Dark red colour Appear	Absent
4	Saponin	Honey comblike structure are formed	present
5	Phytosteroids	Golden yellow colour	Present
	Resin	Turbidity are appear	Present
6	Tannins	Reddish brown bulky ppt.	Present
7	Flavonoids	Reddish brown bulky ppt.	Present

Table 4- Rf values of HPTLC fingerprints profile of *Cassia fistula*(fruit)

Rf values	Before derivatization		After derivatization	
	At 254nm	At 366nm	At 366nm	At UV light
Rf 1	0.17(black)	0.06(sky blue)	0.10(whitish brown)	0.10(brownish pink)
Rf 2	0.33(black)	0.25(light green)	0.18(sky blue)	0.38(brown)
Rf 3	0.40(black)	0.35(florescence)	0.26(florescence)	0.60(yellow)
Rf 4	0.61(black)	0.60(yellow)	0.38(sky blue)	0.66(grey)
Rf 5	-	0.80(red)	0.40(blackish brown)	-
Rf 6	-	-	0.60(yellow)	-
Rf 7	-	-	0.68(white)	-
Rf 8	-	-	0.76(white)	-

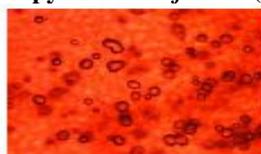
Microscopy of *Cassia fistula* (fruit)

Fig.1



Fig.2



Fig.3



Fig.4



Fig.5



Fig.6



Fig.7



Fig.8



Fig.9



Fig.10



Fig.11

Fig.1: Single and compound starch grains in various shapes and sizes; Fig.2: Prismatic and rod shape crystals of calcium oxalate; Fig.3: Single and groups of stone cells embedded with simple pits and sclereids; Fig.4: Parenchymatous cells embedded with prismatic crystals, starch grains and oil cell; Fig.5: Epidermal cells of testa from side view; Fig.6: Hypodermal cells of testa in surface view; Fig.7: Fragment of cotyledon filled with aleurone grains in surface view ; Fig. 8: Epidermis of testa in surface view; Fig.9: Fibres; Fig. 10: Endosperm cells filled with oil globules and aleurone grains in surface view; Fig.11: Crystal fibres

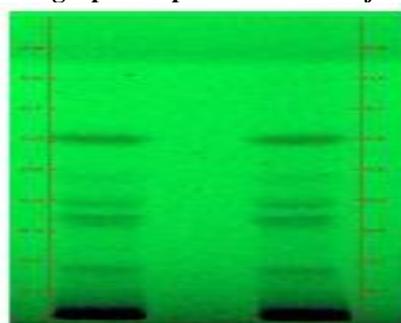
HPTLC fingerprints profile of *Cassia fistula* (fruit)

Fig.12: HPTLC fingerprint at 254nm Before derivatization

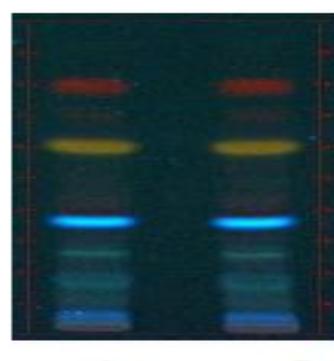


Fig.13: HPTLC fingerprint at 366 nm Before derivatization

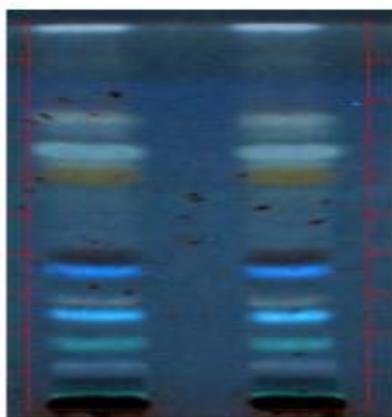


Fig. 14: HPTLC fingerprint at 366nm after derivatization

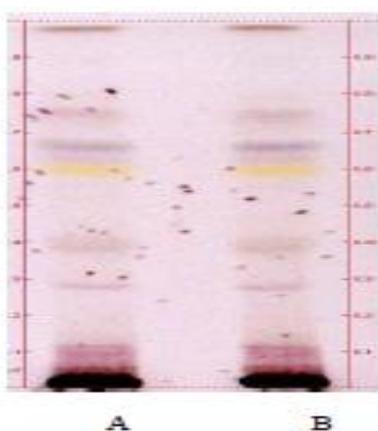


Fig. 15: HPTLC fingerprint at UV light after derivatization

CONCLUSION

Cassia fistula L. has numerous uses in traditional medicine to treat several ailments *viz.* in treating skin disease, liver troubles, tuberculosis gland, ulcers, erysipelas, vomiting, vaginal complaints, fever, inflammations, leprosy. Its latex is aphrodisiac, tonic, lessens, piles, nose diseases, gonorrhoea etc. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals pharmacognostical standardization of drug *Cassia fistula* L. which would be of immense value in botanical identification and authentication of plant drug may help us in preventing its adulteration.

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