

**COMPARATIVE PHYTOCHEMICAL STUDY OF STEM BARK  
VERSUS SMALL BRANCHES OF *CASSIA FISTULA* LINN. USING  
HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC  
ULTRA VIOLET DETECTION METHOD**

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**ABSTRACT**

*Cassia fistula* belonging to the family Fabaceae commonly known as Golden Shower. In Ayurvedic medicine, Golden Shower Tree is known as "disease killer". In literature every part of this plant has been described to be useful for its medicinal properties. The stem bark is anti tubercular, anthelmintic, diuretic, depurative, emetic, febrifuge, laxative and useful in treatment of boils, colic, constipation, cardiac problems, dyspepsia, diabetic, fever, leprosy, ring worm, and pustules. It is effective in suppressing blood glucose levels and in prevention and management of coronary artery disease. The unique patterns of the chromatographic fingerprint were validated by analyzing stem bark and small branches of *C. fistula*. Our results revealed that the chromatographic fingerprint combined with similarity measurement

could efficiently identify and distinguish *C. fistula* stem bark and small branches. The phytochemical fingerprint profiling of stem bark and small branches of *C. fistula* were found similar as an official part of *C. fistula* plant i.e. stem bark, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The method can also be used for identification of different *C. fistula* species and adulterants.

**KEYWORDS:** *Cassia fistula*, HPTLC–UV detection, phytochemical fingerprint profiling analysis.

## INTRODUCTION

*Cassia fistula* [Fig.1] belonging to the family Fabaceae commonly known as Amaltas a semi-wild Indian Labernum, (also famous as the Golden Shower).<sup>[1]</sup> It is a moderate sized deciduous tree<sup>[2]</sup>, distributed in various countries including Asia, Brazil, East Africa, South Africa, China, Mexico, West Indies<sup>[3]</sup>, also grows throughout in Bangladesh and in many other Asian countries such as India, Hong Kong, Philippines, Malaysia, Indonesia and Thailand.<sup>[4]</sup> It is native to southern Asia, southern Pakistan, Myanmar and Sri Lanka. It is the national tree of Thailand and its flower is Thailand's national flower. It is also the state tree and state flower of Kerala in India and of immense importance amongst the Malayali population<sup>[5,6]</sup> Its common names in Hindi are Bendra lathi (or bandarlauri), Dhanbahar (or dhanbohar) and Girimaloah.<sup>[5]</sup> This plant is also known as yellow shower because of its characteristic yellow flowers in pendulous raceme and with typical branches.<sup>[7]</sup> It is 8-15m to 24m in height with greenish grey smooth bark when young, but became rough and dark brown at maturity. The leaves are deciduous or semi-evergreen, 15-60 cm long, pinnate, leaflets 8-12 pair, each leaflet 7-21 cm long and 49 cm broad. The flowers are yellow coloured with long drooping racemes, 20-40 cm long; each flower is 4-7 cm in diameter with five yellow petals of equal size and shape. Pods are cylindrical in shape (30-60 cm long and 1.5-2.5 cm broad), with a pungent odour, pulpy and containing several seeds. Seeds are light brown in colour hard & shiny.<sup>[2, 8]</sup> It is a popular ornamental plant and herbal medicine. It blooms in late spring. Flowering is profuse with trees, being covered with yellow flowers, many times with almost no leaf being seen. It grows well in dry climate.<sup>[4]</sup> The phytochemical screening revealed the presence of various phytoconstituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins in the plant.<sup>[9]</sup> This species of Cassia are considered as rich sources of flavonoids, anthraquinones and polysaccharides.<sup>[2]</sup> The stem bark commonly known as "Tanner's bark of Cassia", because it is rich in tannin and produce a bright red dye, 1,8-Dihydroxy-6-Methoxy-3-Me anthraquinone, also contains 5, 7, 31, 41, tetrahydroxy-6, 8-dimethoxyflavone-3-O- $\alpha$ -arabinopyranoside and 5, 7, 41-trihydroxy-6,8,31 trimethoxyflavone-3-O-  $\alpha$  -L-rhamnosyl (1  $\rightarrow$ 2)-O- $\beta$  -D-glucopyranoside, fistucacidin, a leuco anthocynidin, racemic or meso-3, 4, 41, 7, 8-pentahydroxyflavan.<sup>[10]</sup> 1, 4, 5-trihydroxy-6, 7-dimethoxy-3-methyl-9, 10-dioxo 9, 10-dihydroanthracene-2-carboxylic acid [fistulic acid], 4,5-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid [rhein], 4, 5-dihydroxy-9, 10-dioxoanthracene-6-methyl-2-carboxylic acid [6-methylrhein], (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H

chromene-3,5,7-triol [+ catechin], 1,3-dihydroxy-2-methyl-5,6dimethoxyanthraquinone<sup>[11]</sup>, lupeol,  $\beta$ -sitosterol and hexacosanol.<sup>[12]</sup> Leaf contains (-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B<sub>2</sub>, rhein, rhein glucoside, sennoside A & B, chrysophanol, physcion.<sup>[13]</sup> Heartwood contains fistucacidin (3,4,7,8,4 -pentahydroxyflavan). The ethanol extracts of *C. fistula* flowers reported contains bianthraquinone glycoside, fistulin together with kaempferol and rhein. Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in the flowers.<sup>[14, 15]</sup> The fruits, stem bark, and leaves of this plant contain a variety of biologically active compounds such as anthraquinones, flavonoids, flavon-3-ol derivatives, alkaloid, glycosides, tannin, saponin, terpenoids, reducing sugar and steroids those have various medicinal properties.<sup>[3]</sup> Traces of triterpenes have been observed in both flowers and fruits.<sup>[14, 15]</sup> A compound 3B-hydroxy-17-norpimar-8(9)-en-15-one was isolated from the pods.<sup>[14]</sup> The compounds reported in seed are 5-(2-hydroxyphenoxymethyl) furfural, (2'S)-7hydroxy-5-hydroxymethyl-2-(2'-hydroxypropyl) chromone, benzyl-2 hydroxy-3, 6-dimethoxybenzoate, benzyl 2 $\beta$ -O-D-glucopyranosyl 3,6-dimethoxy benzoate, 5-hydroxymethylfurfural, (2'S)-7-hydroxy-2-(2'-hydroxypropyl)-5methylchromone, oxyanthraquinones, chrysophanol, chrysophanein, glycoside 5, 3', 4'-trihydroxy-6-methoxy-7-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- O-  $\beta$ -D-galactopyranoside. The seeds are also rich in glycerides with linoleic, oleic, stearic and palmitic acids as major fatty acids together with traces of caprylic and myristic acids.<sup>[12]</sup>

In Ayurvedic medicine, Golden Shower Tree is known as "disease killer". In literature every part of this plant has been described to be useful for its medicinal properties. The plant is being considered as a firewood source in Mexico. The reddish wood, hard and heavy, strong and durable is suited for cabinetwork and farm implements.<sup>[3]</sup> The main medicinal property of *C. fistula* is as a mild laxative which is suitable for children and pregnant women.<sup>[1, 5, 11]</sup> Root is prescribed as a diuretic, tonic, astringent, febrifuge and strong purgative.<sup>[3]</sup> It is used for curing adenopathy, burning sensations, leprosy, skin diseases, syphilis, tubercular glands, in chest pain, joint pain, cardiac disorders biliousness, rheumatic condition, haemorrhages, wounds, ulcers and boil, migraine and blood dysentery. The extract of the root lowered the blood sugar level up to 30 %.<sup>[3, 16]</sup> It is useful in fever, retained excretions and biliousness. The aqueous extract of the root bark exhibits anti-inflammatory activity. Extract of the root bark with alcohol can be used for backwart fever.<sup>[16]</sup> The stem bark is anti tubercular, anthelmintic, diuretic, depurative, emetic, febrifuge, laxative and useful in treatment of boils, colic, constipation, cardiac problems, dyspepsia, diabetic, fever, leprosy, ring worm, and

pustules. In Cambodia, the bark is used in dysentery. It is effective in suppressing blood glucose levels and in prevention and management of coronary artery disease. It has antioxidant activity, inhibition of peroxidation, O<sub>2</sub><sup>-</sup> and DPPH radical scavenging ability. Fallen cow and buff hides are tanned by East India tanning process using stem bark.<sup>[10]</sup> The leaves are laxative and used externally as emollient, a poultice is used for chilblains, in insect bites, swelling, rheumatism, facial paralysis and for erysipelas, malaria, ulcers.<sup>[3,16]</sup> Leaves possess anti periodic and laxative properties, used in jaundice, piles, rheumatism ulcers and also externally skin eruptions, ring worms, eczema. The leaves and bark mixed with oil and are applied to pustules and insect bites. Juice of leaves is useful as dressing for ringworm, relieving irritation, in skin diseases and relief of dropsical swelling.<sup>[16]</sup> Leaf extract used for its anti-tussive, wound healing properties and in the treatment of hypercholesterolaemia.<sup>[11]</sup> The extract of the flower inhibits the ovarian function and stimulate the uterine function in albino rats.<sup>[16]</sup> Flowers used for fever.<sup>[3]</sup> Flowers and fruits are used to treat skin diseases, fever, abdominal pain, leprosy by traditional people.<sup>[14]</sup> Fruits are used in the treatment of diabetes, antipyretic, abortifacient, demulcent lessens inflammation and heat of the body; useful in chest complaints, throat troubles, liver complaints, diseases of eye, in snake bite and gripping. The fruit pulp is used for constipation, colic, chlorosis and urinary disorders.<sup>[16]</sup> It is also used as mild laxative as well as in cardiac conditions and stomach problems such as acid reflux.<sup>[3]</sup> The seeds are emetic, used in constipation and have cathartic properties. They are slightly sweet and possess laxative, carminative, cooling, improve the appetite and antipyretic activity. They are useful in jaundice, biliousness, skin disease and in swollen throat. A dried seed produces marked hypoglycaemic activity. Seed powder is used in amoebiasis.<sup>[16]</sup> It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolaemia partially due to their fibre and mucilage content. It has been reported to possess antitumor, hepatoprotective, antifertility, antioxidant properties, to cure leprosy, skin diseases and syphilis. In addition its action on the central nervous and inhibitory effect on leukotriene biosynthesis has also been suggested.<sup>[1,18]</sup>

Golden-Shower is ideal for use as a specimen planting. It can look a bit coarse and unkempt for short periods when the leaves drop but the vibrant flower display more than makes up for this. Some communities have planted this as a street tree where it has held up quite well. Trees will need occasionally pruning when they are young to control shape and develop a uniform crown.<sup>[17]</sup>



**Figure 1: *Cassia fistula* Linn. Plant.**



**Figure 2: Small Branches**



**Figure 3: Stem bark**

#### **Taxonomic Classification<sup>[3]</sup>**

<b>Kingdom</b>	<b>Plantae</b>
<b>Subkingdom</b>	Tracheobinota
<b>Super Division</b>	Spermatophyta
<b>Division</b>	Mangoliophyta
<b>Class</b>	Magnoliopsida
<b>Sub Class</b>	Rosidae
<b>Order</b>	Fabales
<b>Family</b>	Fabaceae
<b>Genus</b>	<i>Cassia</i>
<b>Species</b>	<i>fistula</i>



## MATERIALS AND METHODS

### Plant Materials and Chemicals

Plant materials i.e small branches of stem (Fig.2) and stem barks (Fig. 3) of *C. fistula* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer, Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

### Sample preparation

The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature  $30 \pm 2^{\circ}\text{C}$  and relative humidity  $50 \pm 5\%$ ) and powdered in an electric grinder.

Conventional extraction of stem bark and small branches of stem of *C. fistula* were performed at room temperature ( $28^{\circ} \pm 3^{\circ}\text{C}$ ) with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, ethyl acetate and ethanol. Dried and powdered parts of *C. fistula* (10 g each) were extracted three times ( $3 \times 50\text{ mL}$ ) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no.1 and the solvents were removed under vacuum at  $50^{\circ}\text{C}$ , separately and concentrated up to 10 mL to get the sample solution of  $100\text{ mg mL}^{-1}$ . 5  $\mu\text{L}$  of each sample was applied separately to TLC plate for the development of fingerprints.

### HPTLC-UV detection Method

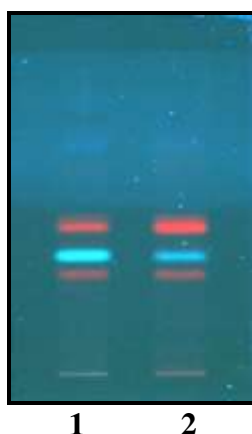
High Performance Thin Layer Chromatography was performed on  $10\text{ cm} \times 10\text{ cm}$  TLC plates pre-coated with  $0.25\text{ }\mu\text{m}$  thin layers of silica gel 60 F<sub>254</sub> (E. Merck). Both samples (stem bark and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100  $\mu\text{L}$  syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate: 9: 1 (v/v)* and as mobile phase for both *n*-hexane extract was performed in a twin-trough glass chamber ( $20\text{ cm} \times 10\text{ cm}$ ) previously saturated with vapours of mobile phase for 20 min. The plates were dried in air and visualized under  $\lambda\text{ }254\text{ nm}$  and  $\lambda\text{ }366\text{ nm}$  for ultra violet detection and taken the fingerprints as evident in Figures 4-5. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 6 using CAMAG Reprostar and WinCATs software (V1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs was performed same procedure with the mobile phases of *Toluene: Ethyl acetate 8: 2 (v/v)* and *Toluene: Ethyl acetate: Formic acid 6.5:3.5:0.5 (v/v/v)* respectively and then visualized in  $\lambda\text{ }254\text{ nm}$ ,  $\lambda\text{ }366$

nm and white light using CAMAG Reprostar and WinCATs software as shown in Figure 7-12.



254 nm

Figure 4



366 nm

Figure 5



After derivatization

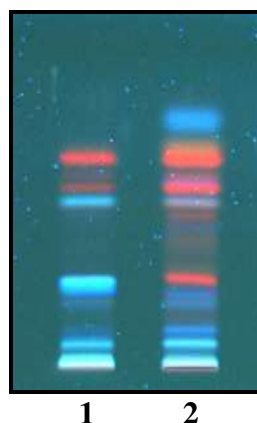
Figure 6

Figure 4-6: TLC fingerprint of *n*-hexane extract of *C. fistula* (1= St. Bk.; 2= Sm. Br.)



254 nm

Figure 7



366 nm

Figure 8



After derivatization

Figures 9

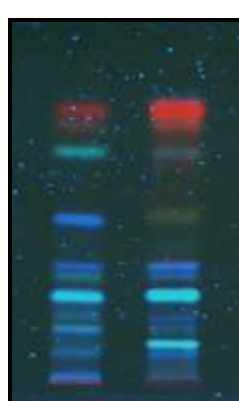
Figure 7-9: TLC fingerprint of ethyl acetate extract of *C. fistula* (1= St. Bk.; 2= Sm. Br.)



1 2

254 nm

Figure 10



1 2

366 nm

Figure 11



1 2

After derivatization

Figures 12

Figure 10-12: TLC fingerprint of ethanol extract of *C. fistula* (1= St. Bk.; 2= Sm. Br.)

**Table 1:  $R_f$  value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *C. fistula* (St. Bk. and Sm. Br.) at different wave-lengths.**

Wave-length	<i>n</i> - Hexane extract		Ethyl acetate extract		Ethanol extract	
	Stem bark	Small branches	Stem bark	Small branches	Stem bark	Small branches
<b>254</b>	-	-	-	0.80	0.15, 0.28, 0.33, 0.49	0.15, 0.28, 0.36, 0.49
<b>366</b>	0.32, 0.38, 0.46	0.32, 0.38, 0.46	0.20, 0.22, 0.28, 0.54, 0.58, 0.6, 0.66	0.20, 0.24, 0.29, 0.45, 0.49, 0.52, 0.54, 0.58, 0.61, 0.66, 0.71, 0.79	0.12, 0.17, 0.27, 0.32, 0.36, 0.49, 0.70, 0.77, 0.82	0.12, 0.27, 0.32, 0.33, 0.51, 0.66, 0.70, 0.77, 0.82
<b>Visible light after derivatization</b>	0.16, 0.28, 0.42, 0.82, 0.89	0.16, 0.28, 0.42, 0.89	0.28, 0.41, 0.53, 0.87	0.28, 0.41, 0.53, 0.87	0.16, 0.22, 0.61, 0.68, 0.91	0.16, 0.22, 0.27, 0.35, 0.61, 0.68, 0.91

## RESULTS AND DISCUSSION

No such study was found in literature for comparative phytochemical study of stem bark versus small branches of *C. fistula* Linn by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of stem bark and small branches of *C. fistula* revealed that many similarities in phytochemical fingerprints were found and evident in Table-1 and Fig. 4-12.

Phytochemical fingerprints of *n*-hexane extract of stem bark and small branches showed no band under UV detection at 254 nm. Under 366 nm UV detection, stem bark and small branches showed three similar bands at  $R_f$  0.32 (red) 0.38 (light blue) and 0.46 (red). After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches both were showed five and four bands respectively, out of which four bands at  $R_f$  0.16 (violet), 0.28 (blue), 0.42 (blue) and 0.89 (blue ) were found similar as represented in Table 1 and Fig. 4-6.

There is no band seen in the phytochemical fingerprints of ethyl acetate extract of stem bark under 254 nm detection whereas a single band is observed in small branches. Under 366 nm UV detection, stem bark and small branches showed seven and twelve bands respectively, out of which six bands at  $R_f$  0.20 (blue) 0.21 (blue), 0.54 (blue), 0.58 (red), 0.61 (red) and 0.66 (red) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed four similar bands at  $R_f$  0.28 (blue), 0.41 (blue), 0.53 (dark blue), 0.87 (blue) as showed in Table 1 and Fig. 5-8.



Phytochemical fingerprints of ethanol extract of stem bark and small branches under UV detection at 254 nm, showed four and three bands respectively, out of which two bands were found similar at  $R_f$  0.15 and 0.28 (All were black). While under 366 nm UV detection, stem bark and small branches showed eight and nine bands respectively, out of which seven bands at  $R_f$  0.12 (blue), 0.27(blue), 0.32(blue), 0.36(blue), 0.70(blue), 0.77(red) and 0.82 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed five and six bands respectively, out of which five bands at  $R_f$  0.16 (brown), 0.22 (dark blue), 0.61 (blue), 0.68 (blue) and 0.91 (blue) were found similar in both parts (St. Bk. and Sm. Br.) as evident in Table 1 and Fig.10-12.

## CONCLUSION

The phytochemical fingerprint profiling of stem bark and small branches of *C. fistula* were found similar as an official part of *C. fistula* plant i.e. stem bark, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of stem bark and small branches of *C. fistula* have been given an idea about the presence of various phytochemicals in their reported parts. The TLC spots provided valuable clue regarding presence or absence of various phytochemicals or metabolites of the plants.

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