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Phytochemical profiling and HPTLC fingerprinting of *Cassia fistula* Leaf extracts using solvents of varying polarity

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Abstract

Cassia fistula L. is a medicinally important plant known to contain a wide range of secondary metabolites; however, systematic evaluation of solvent-dependent phytochemical composition and chromatographic fingerprints of its leaf extracts remains limited. The present study aimed to compare the qualitative phytochemical profiles and High-Performance Thin Layer Chromatography (HPTLC) fingerprints of aqueous, methanolic, and acetone extracts of *C. fistula* leaves. Shade-dried and defatted leaf powder was subjected to solvent extraction using water, methanol, and acetone. Qualitative phytochemical screening was performed for major metabolite classes including alkaloids, flavonoids, tannins, carbohydrates, reducing sugars, proteins, and lipids. HPTLC fingerprinting was carried out on silica gel plates using a toluene:ethyl acetate (7:3) mobile phase, with visualization at 254 nm and 366 nm before and after derivatization. Phytochemical tests revealed the presence of tannins, flavonoids, and alkaloids in all extracts, while carbohydrates, reducing sugars, and proteins were predominantly detected in the aqueous extract. Methanolic and acetone extracts showed stronger reactions for phenolic and flavonoid constituents. HPTLC analysis demonstrated solvent-specific chromatographic patterns, with the methanolic extract exhibiting the highest number and diversity of bands across a wide R_f range, followed by the aqueous extract, while the acetone extract showed comparatively fewer bands. Common bands indicated shared core constituents, whereas solvent-specific bands reflected differential extraction of metabolite subsets. The combined qualitative and chromatographic data confirm the chemically diverse nature of *C. fistula* leaves and highlight the strong influence of solvent polarity on extract composition. The generated HPTLC fingerprints provide reference profiles useful for authentication, quality control, and future marker-based standardization of *C. fistula* leaf extracts.

Keywords: *Cassia fistula*, phytochemical screening, HPTLC fingerprinting, solvent polarity, herbal standardization

Introduction

Medicinal plants represent rich reservoirs of structurally diverse secondary metabolites with important pharmacological and biochemical significance. Phytochemicals such as phenolics, flavonoids, tannins, alkaloids, and glycosides contribute to antioxidant, anti-inflammatory, antimicrobial, and protective functions in both plants and derived herbal preparations [1-3]. Systematic phytochemical characterization is therefore essential for understanding chemical diversity, ensuring quality control, and supporting the development of standardized herbal products [4, 5].

Cassia fistula L. (Fabaceae), commonly known as the golden shower tree, is widely distributed in tropical regions and is traditionally used in several medicinal systems. Previous studies have reported the presence of diverse secondary metabolites in different parts of the plant, including leaves, bark, pods, and flowers, suggesting that extraction conditions and solvent systems strongly influence phytochemical recovery [2, 6]. Despite its medicinal importance, comparative information on solvent-dependent phytochemical profiles and chromatographic fingerprints of *C. fistula* leaf extracts remains inadequate.

High-Performance Thin Layer Chromatography (HPTLC) has emerged as a robust and cost-effective analytical technique for phytochemical profiling of complex plant extracts. HPTLC enables simultaneous qualitative and semi-quantitative assessment of multiple constituents and is widely used for authentication, detection of adulteration, and standardization of herbal materials [4, 7, 8].

Solvent polarity plays a critical role in determining the diversity and abundance of compounds extracted, with polar organic solvents such as methanol often yielding richer chromatographic profiles than water or less polar solvents [9, 10].

The present study was therefore undertaken to (i) perform qualitative phytochemical screening of aqueous, methanolic, and acetone extracts of *Cassia fistula* leaves, and (ii) develop and compare HPTLC fingerprints of these extracts under different detection conditions. The resulting solvent-specific profiles are intended to serve as baseline reference data for quality assessment and future phytochemical investigations of *C. fistula*.

2. Materials and Methods

2.1 Plant material and extraction

Fresh, healthy leaves of *Cassia fistula* were collected from Satna district, Madhya Pradesh, India, taxonomically identified, and authenticated against herbarium reference material. Leaves were washed, shade-dried at room temperature, defatted, powdered, and stored in airtight containers.

Extraction was carried out using three solvents of differing polarity: water (aqueous), methanol, and acetone. Methanol and Acetone extract were obtained through Soxhlet. Leaf powder was extracted until the siphon solvent became colourless, indicating exhaustive extraction. Extracts were

filtered, concentrated, dried to constant weight, and stored under refrigeration until analysis. Aqueous extract was prepared through maceration.

2.2 Qualitative phytochemical screening

Qualitative phytochemical screening of all extracts was conducted using standard colour reactions. Alkaloids were detected using Dragendorff's and Mayer's reagents; flavonoids by Shinoda and alkaline reagent tests; tannins by ferric chloride reaction; carbohydrates and reducing sugars by Molisch's and Benedict's tests; proteins and amino acids by Biuret and xanthoproteic tests; and lipids by Sudan III test. Results were recorded as positive or negative based on colour development or precipitate formation.

2.3 HPTLC fingerprinting

HPTLC analysis was performed on silica gel 60 F₂₅₄ precoated plates. Extract solutions (10 µL each) were applied as 10 mm bands using a CAMAG Linomat-5 applicator. Plates were developed in a toluene: ethyl acetate (7:3) mobile phase to a distance of 9 cm. After development, plates were air-dried and visualized under UV light at 254 nm and 366 nm before derivatization. Plates were then derivatized with 5% methanolic sulphuric acid reagent, heated at 105 °C, and re-visualized under UV and visible light. R_f values, band colours, and intensities were recorded and presented in Plate 1-2.

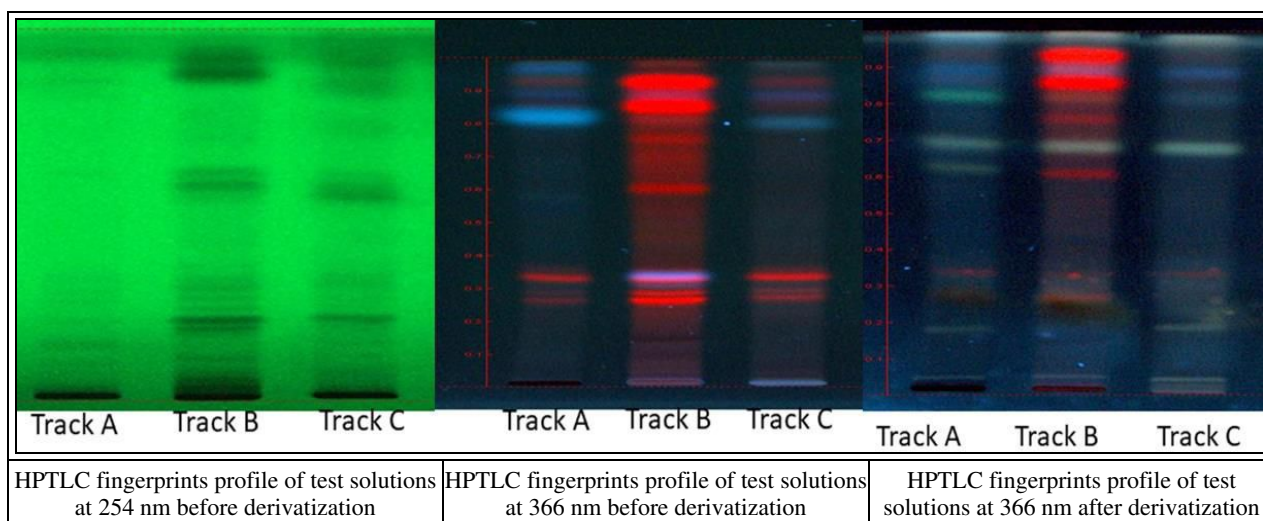


Plate 1: HPTLC Fingerprints at different wavelength.

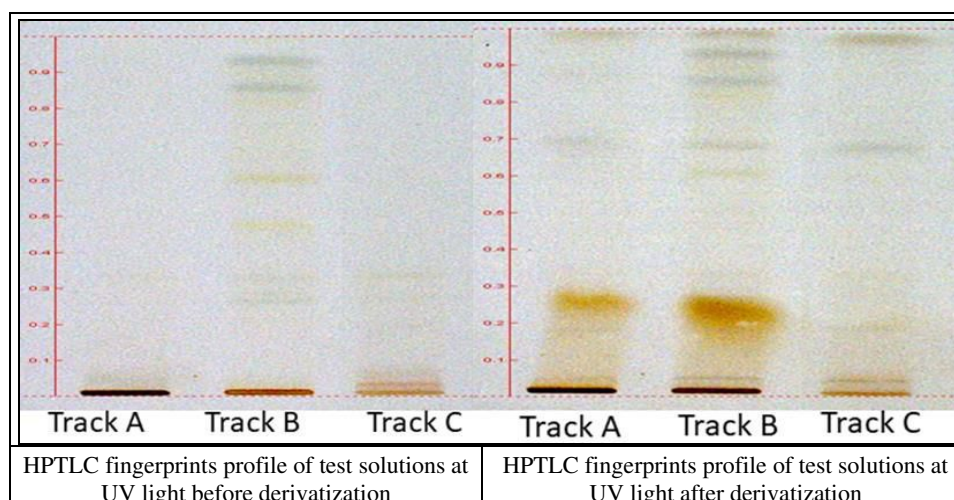


Plate 2: UV Visualization before and after derivatization

3. Results

3.1 Qualitative phytochemical screening

Phytochemical screening revealed solvent-dependent distribution of metabolites. Tannins, flavonoids, and alkaloids were detected in all extracts, indicating their widespread presence in *C. fistula* leaves. Carbohydrates,

reducing sugars, and proteins showed strong positive reactions predominantly in the aqueous extract, reflecting the high polarity of water. Methanolic and acetone extracts exhibited stronger reactions for phenolic and flavonoid constituents, consistent with their ability to solubilize moderately polar secondary metabolites.

Table 1: Phytochemical screening of solvent extracts of aqueous extract of *Cassia fistula*

Name of Test	Target Compound	Aqueous Extract	Methanolic Extract	Acetone Extract
Dragendorff's Test	Alkaloids	Negative	Positive	Positive
Shimoda Test	Flavonoids	Negative	Positive	Positive
Ferric Chloride Test	Tannins	Positive	Positive	Positive
Molisch's Test	Carbohydrates	Positive	Negative	Negative
Benedict's Test	Reducing Sugars	Positive	Negative	Negative
Biuret Test	Protein	Positive	Negative	Negative
Xanthoproteic Test	Protein/Amino acids	Positive	Negative	Negative
Sudan III Test	Lipids	Negative	Negative	Positive

Here, Positive = Present; Negative = Not Present,

3.2 HPTLC fingerprint profiles

At 254 nm before derivatization, methanolic extract exhibited the highest number of well-resolved bands across a broad R_f range, indicating a rich diversity of UV-absorbing compounds. The aqueous extract showed a moderate number of bands, while the acetone extract displayed fewer and less intense bands.

Under 366 nm, methanolic and acetone extracts revealed multiple fluorescent and quenching bands, particularly in mid to high R_f regions, whereas the aqueous extract showed fewer fluorescent zones. After derivatization, all extracts exhibited enhanced band visibility, with methanolic extract again showing the richest and most complex fingerprint pattern.

Common bands at specific R_f values were observed across all extracts, suggesting shared core constituents, while additional solvent-specific bands reflected selective enrichment of distinct metabolite subsets. Measured R_f is presented in Table 2-6.

Table 2: R_f values of HPTLC fingerprints profile of test solutions at 254 nm before derivatization

R _f values	Track A (Aqueous)	Track B (Methanolic)	Track C (Acetone)
Rf1	0.10 black	0.08 black	0.18 black
Rf2	-	0.18 black	0.26 black
Rf3	-	0.26 black	0.64 black
Rf4	-	0.64 black	0.66 black
Rf5	-	0.66 black	0.90 black
Rf6	-	0.90 black	0.92 black
Rf7	-	0.92 black	-

Table 3: R_f values of HPTLC fingerprints profile of test solutions at 366 nm before derivatization

R _f values	Track A (Aqueous)	Track B (Methanolic)	Track C (Acetone)
Rf1	0.26 red	0.08 pink	0.26 red
Rf2	0.35 red	0.12 pink	0.35 pink
Rf3	0.80 sky blue	0.26 red	0.80 blue
Rf4	0.92 red	0.35 pink	0.84 pink
Rf5	0.94 red	0.60 red	0.90 pink
Rf6	-	0.70 red	0.92 blue
Rf7	-	0.84 red	-
Rf8	-	0.90 red	-
Rf9	-	0.92 red	-

Table 4: R_f values of HPTLC fingerprints profile of test solutions at 366 nm after derivatization

R _f values	Track A (Aqueous)	Track B (Methanolic)	Track C (Acetone)
Rf1	0.18 brown	0.26 pink	0.08 brown
Rf2	0.36 pink	0.36 pink	0.20 brown
Rf3	0.64 brown	0.64 pink	0.36 pink
Rf4	0.70 brown	0.70 brown	0.70 brown
Rf5	0.84 brown	0.75 red	0.80 sky blue
Rf6	-	0.80 pink	0.90 sky blue
Rf7	-	0.86 red	0.92 brown
Rf8	-	0.92 red	-

Table 5: R_f values of HPTLC fingerprints profile of test solutions at UV light before derivatization

R _f values	Track A (Aqueous)	Track B (Methanolic)	Track C (Acetone)
Rf1	-	0.26 green	0.04 brown
Rf2	-	0.50 yellow	-
Rf3	-	0.60 yellow	-
Rf4	-	0.86 green	-
Rf5	-	0.92 green	-

Table 6: R_f values of HPTLC fingerprints profile of test solutions at UV light after derivatization

R _f values	Track A (Aqueous)	Track B (Methanolic)	Track C (Acetone)
Rf1	0.28 yellow	0.28 yellow	0.06 brownish blue
Rf2	0.70 black	0.70 black	0.70 black
Rf3	-	0.84 black	0.92 black
Rf4	-	0.92 black	-
Rf5	-	-	-

4. Discussion

The present study clearly demonstrates that solvent polarity exerts a strong influence on the phytochemical composition and chromatographic fingerprints of *Cassia fistula* leaf extracts. Among the solvents evaluated, methanol produced the richest qualitative phytochemical profile and the most complex HPTLC fingerprint, followed by aqueous extract, while acetone yielded comparatively fewer chromatographic bands. Similar solvent-dependent extraction patterns have been reported for several medicinal plants, where polar organic solvents such as methanol were shown to extract a broader spectrum of secondary metabolites compared to water or less polar solvents [6, 9, 10].

The predominance of flavonoids, tannins, and alkaloids observed in methanolic and acetone extracts is consistent with earlier phytochemical investigations on *C. fistula* and related Fabaceae members, which reported efficient recovery of phenolic compounds using methanol-based extraction systems [2, 10, 11]. Phenolics and flavonoids are known to exhibit strong UV absorbance, which explains the higher number of detectable bands in methanolic extracts under 254 nm and 366 nm during HPTLC analysis [7, 8]. In contrast, the aqueous extract showed stronger reactions for carbohydrates and proteins but generated simpler chromatographic patterns, a trend that aligns with previous reports highlighting the limited ability of water to extract structurally diverse secondary metabolites [4, 9].

The HPTLC fingerprint profiles generated in this study revealed both common and solvent-specific bands. The presence of common bands across all extracts suggests the existence of core phytoconstituents inherent to *C. fistula* leaves, irrespective of solvent system. Similar observations have been reported in HPTLC-based studies of medicinal plants, where shared bands were proposed as indicators of species-specific chemical identity, while solvent-specific bands reflected selective enrichment of distinct metabolite classes [7, 12]. Such solvent-driven differentiation has been widely acknowledged as a critical factor in phytochemical standardization protocols [4, 8].

The strong concordance observed between qualitative phytochemical screening and HPTLC fingerprinting in the present study supports the reliability of combining these approaches for comprehensive phytochemical characterization. Previous studies have emphasized that preliminary phytochemical tests, when complemented with chromatographic fingerprinting, enhance interpretability and reproducibility of herbal profiling data [5, 7]. The richer and more resolved HPTLC patterns obtained for methanolic extracts further corroborate earlier findings that identify methanol as a preferred solvent for developing reference fingerprints for quality control of herbal raw materials [8, 10]. From a phytochemical and biochemistry perspective, the solvent-specific HPTLC fingerprints established in this study have important practical implications. HPTLC profiles are increasingly recognized as effective tools for authentication, detection of adulteration, and batch-to-batch consistency assessment of medicinal plant materials [4, 7]. The characteristic bands identified, particularly in the methanolic extract, may serve as potential marker peaks for future standardization and formulation development involving *C. fistula* leaf extracts. Comparable applications of HPTLC fingerprinting for herbal standardization have been reported for several pharmacologically important plant species [6, 8, 12].

Overall, the present findings are in strong agreement with earlier phytochemical and chromatographic studies on *Cassia fistula* and other medicinal plants, reinforcing the critical role of solvent selection in phytochemical investigations and highlighting the suitability of methanol-based extracts for detailed phytochemical profiling and quality assessment.

5. Conclusion

This study provides a comprehensive phytochemical and HPTLC-based characterization of aqueous, methanolic, and acetone leaf extracts of *Cassia fistula*. The findings confirm that *C. fistula* leaves possess a chemically diverse secondary

metabolite profile, strongly influenced by solvent polarity. Methanolic extract exhibited the most complex and information-rich HPTLC fingerprint, followed by aqueous and acetone extracts. The established solvent-specific fingerprints offer valuable reference profiles for authentication, quality control, and future marker-guided standardization of *C. fistula*-derived herbal products.

6. References

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