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Research Article

A Comparative Study on the Isolation of Flavonoids from *Cassia fistula* and its Characterization by TLC and UV-Vis Spectroscopy

Ankur Kumar Singh[1], Himanshu Choudhary[1] and Ankita Raikwar[2]*

[1]*B Pharm scholar, Jaipur National University, Jaipur, India*

[2]*PhD Scholar, Jaipur National University, Jaipur, India*

***Corresponding Author:** Ankita Raikwar, PhD Scholar, Jaipur National University, Jaipur, India.

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Abstract

Introduction: *Cassia fistula* Linn., commonly known as the golden shower tree, is recognized for its rich content of bioactive phytochemicals, especially flavonoids, which are associated with a broad spectrum of pharmacological activities. This study aimed to isolate flavonoids from different parts of *Cassia fistula* and to evaluate the efficiency of various extraction methods in yielding high-quality flavonoid compounds.

Methodology: Crude extracts of *Cassia fistula* were prepared using Soxhlet extraction technique. Preliminary phytochemical screening was conducted to confirm the presence of flavonoids. The extracts were subjected to isolation and purification using Thin Layer Chromatography (TLC), and further characterized by UV-Visible spectroscopy to identify specific absorption maxima indicative of flavonoid chromophores.

Result: The TLC analysis revealed distinct Rf values, confirming successful separation of flavonoid constituents. UV-Visible spectroscopy confirmed the presence of flavonoids through characteristic absorption peaks. The study concludes that soxhlet is a efficient and reproducible technique for isolating flavonoids from *Cassia fistula*, and the findings support the potential use of such flavonoid-rich extracts in pharmacological and nutraceutical applications.

Keywords: *Cassia fistula*; Flavonoids; Thin Layer Chromatography (TLC); UV-Visible Spectroscopy; Phytochemical Screening; Extraction

Introduction

Flavonoids

Flavonoids are a diverse group of naturally occurring polyphenolic compounds widely distributed in the plant kingdom [1-3]. They are characterized by a common 15-carbon skeleton composed of two aromatic rings (A and B) connected by a three-carbon bridge, typically forming a heterocyclic ring (C), resulting in a general C_6-C_3-C_6 structure [2,5]. Over 8,000 different flavonoids have been identified, making them the most common group of polyphenolic compounds in the human diet [2,3].

Flavonoids are secondary metabolites in plants, where they play crucial roles such as providing pigmentation to flowers and fruits, attracting pollinators, protecting against ultraviolet radiation, and defending against pathogens and environmental stressors [1,2,4,8]. They are almost always water-soluble and are responsible for a wide range of colours in nature, including the yellows of flavonols and chalcones, and the reds, blues, and purples of anthocyanins [4,5].

Cassia fistula

Cassia fistula, also known as the golden shower tree, Indian laburnum, or "amaltas," is a medium-sized, deciduous flowering tree, being used as a mild laxative to treat constipation, skin diseases, wound healing, fever, arthritis, and gastrointestinal issues [7, 9]. Containing phytochemical constituents that include anthraquinone glycosides including rhein, sennosides A and B, fistulic acids, flavonoids like Kaempferol, quercetin [10,11], tannins, saponins, glycosides, triterpenoids, alkaloids, essential oils, phenolic compounds, phytosterol, carbohydrates, proteins, amino acids, miscellaneous compounds including fistulin, volatile compounds, fatty acids like linoleic and oleic acids [12,13]. Ethanolic extraction is a widely used method to obtain flavonoids and other phytochemicals from *Cassia fistula* plant flowers by Soxhlet apparatus, often at strengths around 95% [14]. Preliminary phytochemical screening (using methods like the alkaline reagent test) confirms the presence of flavonoids along with other bioactive compounds (phenolics, saponins, tannins, alkaloids) [15,17]. These tests are standard in phytochemistry and involve colorimetric detection or reactivity with specific reagents [14,16]. Yield of extract and flavonoid content can vary with part of the plant used and the exact extraction conditions [18]. Such extracts have been used in studies for various biological activities (antioxidant, hepatoprotective, nephroprotective, anti-solar, and anti-thrombocyte effects), supporting their value in traditional medicine and potential pharmaceutical applications [19]. This methodology is consistent across studies and is supported by standard phytochemical practices for isolating flavonoids from *Cassia fistula* [20].

UV analysis of ethanolic extract of *Cassia fistula*

UV analysis of the ethanolic extract of *Cassia fistula* typically involves using UV-Visible spectroscopy, to profile and identify flavonoid compounds in the extract [22]. Flavan-3-ols (such as catechin and epicatechin) show maximum absorbance around 276 nm. Flavanol glycosides (such as kaempferol-3-O-glucoside) display maximum absorption at both 265 nm and 360 nm, which is characteristic for flavanols [23]. After extraction, the extract is either directly analysed or subjected to further purification [24,25]. Flavonoids are identified by comparing their retention time and UV spectra with those of pure standards. Catechin/epicatechin (flavan-3-ols) shows peak at 276 nm, Kaempferol-3-O-glucoside (flavanol glycoside) at 265 nm and 360 nm and Anthocyanins at 520 nm [26]. For total flavonoid content, the aluminium chloride colorimetric method is used, measuring absorbance at 415 nm [27,28]. Total phenolic content is often measured at 765 nm using Folin-Ciocalteu reagent [29].

TLC of ethanolic extract of *Cassia fistula*

Thin Layer Chromatography (TLC) of the ethanolic extract of *Cassia fistula* is a standard method for detecting and profiling flavonoids and other phytochemicals in the extract. Common mobile phases for TLC of *Cassia fistula* extracts include Chloroform : Methanol mixtures (e.g., 90:10 or similar ratios) or Ethyl acetate : Formic acid : Glacial acetic acid : Water (EAFW; 100:11:11:26) [32]. While the detection of plates include analysing under UV light (254 nm and 366 nm), and then post-spraying with reagents such as ammonia vapor, vanillin-sulphuric acid, or anisaldehyde-sulphuric acid, which enhance specific bands associated with flavonoids and other classes [33,35,37].

Salient findings

Soxhlet extraction with ethanol produced a 7% yield, indicating a good recovery of phytoconstituents from flowers. Both Ferric chloride and Shinoda tests confirmed the presence of flavonoids. IR spectral peaks (3432, 1630, 1512 cm^{-1}) matched characteristic flavonoid functional groups. TLC Rf value (0.60) closely matched quercetin standard (0.58). λmax at 424.5 nm confirmed the flavonoid nature of the extract. The protocol used (Soxhlet extraction + TLC + UV–Vis) can be reliably applied for future phytochemical studies.

Justification for the study

Cassia fistula is traditionally used for multiple therapeutic purposes; flavonoids are known for antioxidant, anti-inflammatory, and antimicrobial activities. Limited comparative phytochemical data exists for *Cassia fistula* flowers using standardized TLC and UV–Vis characterization. Establishing a reproducible method for flavonoid isolation is essential for quality control in herbal drug formulation. Results support possible development of flavonoid-rich nutraceuticals and pharmacological agents. Confirms traditional claims through modern analytical approaches.

Material and Methods

Material

Fresh plant material of *Cassia fistula* (commonly known as the Golden Shower Tree) was collected from Nahargarh botanical garden during the peak summer season in the month of May 2024. The parts used for this study were the flowers, known to be rich in flavonoids [7-9].

Method

Extraction by soxhlet apparatus

The collected plant materials were carefully washed with distilled water to remove dust and debris, and then dried under shade at room temperature (25–30°C) for 7–10 days. Once thoroughly dried, the materials were ground into a coarse powder using a mechanical grinder and stored in airtight containers for further extraction.

The 30 g of powdered plant material was packed into a thimble made of Whatman filter paper. A volume of 350–400 ml of ethanol (95%) was poured into the round-bottom flask connected to the extractor. The setup was assembled and heated on a heating mantle at a controlled temperature (30-40 °C) for a period of 54 hours. The solvent vaporized, condensed in the condenser, and dripped into the thimble containing the plant powder. The extraction process was allowed to continue until the siphon tube solvent turned colorless, indicating exhaustive extraction. After completion, the solvent-containing extract in the round-bottom flask was collected and concentrated using a rotary evaporator or water bath to remove the excess ethanol [15].

Concentration and storage

The concentrated extract was allowed to cool and stored in an airtight amber-colored container to protect it from light and oxidation. The crude extract was stored at 4°C until further use in phytochemical screening, TLC [8].

Phytochemical screening

- **Ferric Chloride Test**: Added few drops of 1% ferric chloride solution to the extract.
- **Shinoda Test (Magnesium-HCl Test):** Added small amount of plant extract and few pieces of magnesium ribbon and then added concentrated HCl dropwise [10,11,14].
- **Characterization by IR Spectroscopy:** Calibrated the IR spectrophotometer with background check and then placed sample on the sample holder over diamond crystal, scanned the sample from 4000 cm^{-1} to 400 cm^{-1} [23].
- **Ultraviolet Visible Spectroscopy Analysis:** Sample was prepared with dilute solution plant extract in 95% ethanol, then calibrate the UV-Vis spectrophotometer using the blank solvent as ethanol 95%, placed the sample in the cuvette and record the spectrum from 200–800 nm [27].
- **Isolation of Flavonoids Using Thin Layer Chromatography (TLC):** Extract was dissolved in 95% ethanol, and then applied over pre coated TLC plate with a sealed capillary tube. Then placed the plate in a TLC chamber containing the mobile phase Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (10 : 1.1 : 1.1 : 2.7), after the solvent being absorbed in ascending order at a height of $3/4^{th}$ of the plate, the plate was removed and allowed to dry with hair dryer and then visualized under UV light at 254 nm and 366 nm [31,33].

Result and Discussion

Extraction yield

Plant material used: 30 g of dried *Cassia fistula* (flowers), crude drug extract obtained: 2.1 g.

Percentage Yield = (Weight of Extracted Product/Weight of Initial Sample) ×100

= Practical yield/Theoretical yield x 100

= 2.1/30 x 100 = 7%

This yield falls within the typical range reported for flavonoid-rich plant extractions using ethanol as the solvent, which often ranges from 5–10% depending on the plant part, solvent polarity, and extraction conditions. The 7% yield suggests that ethanol (95%) was effective in solubilizing and recovering a significant portion of the flower's phytoconstituents, particularly polyphenols and flavonoids, due to its intermediate polarity.

Phytochemical screening test of flavonoid

The phytochemical screening of the *Cassia fistula* flower extract revealed positive results for both the Ferric chloride test and the Shinoda test, confirming the presence of flavonoids.

Table 1: Phytochemical screening test.

S. No.	Phytochemical Test	Result
1.	Ferric chloride ($FeCl_3$) test	Positive
2.	Shinoda Test	Positive

The Ferric chloride test produces a blue, green, or black coloration due to the formation of ferric–phenolate complexes, hence confirming phenolic hydroxyl groups, characteristic of flavonoid structures. While in Shinoda test involves the reaction of flavonoids with magnesium turnings and concentrated hydrochloric acid, results in red, orange, or pink coloration due to the reduction of the flavonoid's carbonyl group and subsequent formation of colored flavilium salts, which further supports the presence of flavonoid chromophores in the extract.

IR spectral analysis and interpretation

The wave number obtained at 3432 cm^{-1} represents O–H stretching (broad) indicating Presence of phenolic or alcoholic –OH group, at 2925 cm^{-1}, C–H stretching (alkanes) is observed indicating Aliphatic C–H, common in organic backbones, at 1630 cm^{-1} because of presence of C=O or C=C stretching aromatic ring or flavonoid core is observed, at 1512 cm^{-1} C=C aromatic skeletal vibrations are observed indicating aromatic system which is characteristic property of flavonoid, at 1251 cm^{-1} C–O–C stretching characteristic of ether or phenolic group is seen indicating presence of ether/phenol linkages, and at 1020–1050 cm^{-1} C–O stretching is observed indicating supportive phenolic or glycosidic structures.

These IR spectral features collectively validate the presence of flavonoid structures in the *Cassia fistula* extract, supporting the results from phytochemical screening and aligning with previously reported IR profiles of flavonoid-rich plant extracts. The combination of hydroxyl, aromatic, and ether functional groups is consistent with flavonoids like quercetin and kaempferol, which have been reported in *Cassia fistula* in earlier studies.

UV spectra

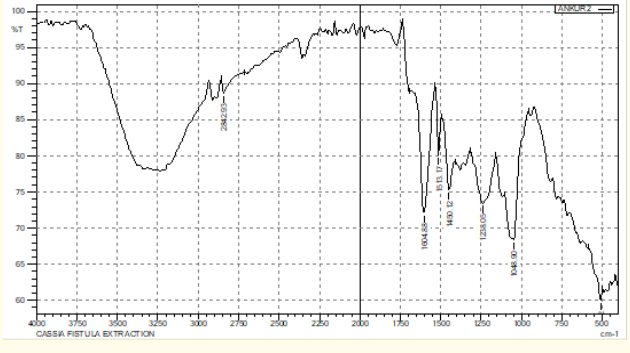

Figure 1: IR Spectrum of Cassia extract.

The UV–Visible spectrum of the *Cassia fistula* flower extract displayed a major absorption peak at 424.5 nm with an absorbance value of 0.570, which is characteristic of flavonoid compounds, particularly quercetin-type flavonols. Quercetin and its glycosides are known to exhibit strong absorption in the range of 350–450 nm due to π→π* transitions in the conjugated aromatic system and C=O chromophores. The absence of significant peaks in the lower UV range (<300 nm) for this sample suggests that the extract is dominated by flavonoid chromophores rather than non-conjugated phenolic acids. A minor absorbance at 651 nm (0.047) was recorded, but this is likely due to background or trace pigments and does not contribute significantly to flavonoid identification. The λmax observed aligns with reported values for quercetin-rich plant extracts, reinforcing that *Cassia fistula* flowers are a source of

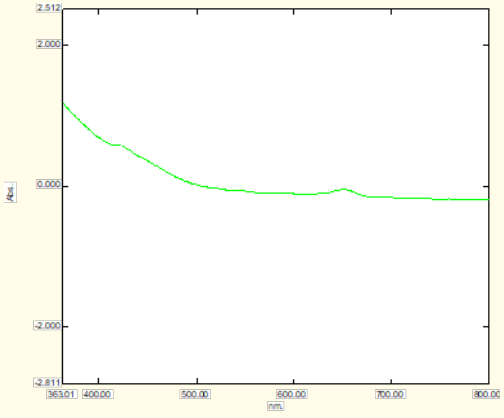

Figure 2: UV Spectra of Cassia extract.

Table 2: UV Spectra.

S. No.	Wavelength (nm.)	Absorbance	Description
1.	651	0.047	-
2.	424.5	0.570	Flavanoid (Quercetin)

flavonoids with potential antioxidant and pharmacological activity. This also validates ethanol as a suitable solvent for extracting light-sensitive flavonoid compounds, as the peaks were sharp and well-defined, indicating minimal degradation.

TLC analysis

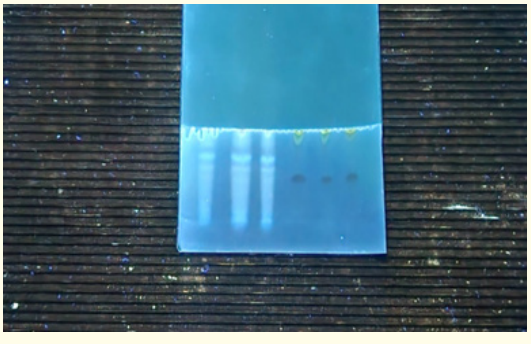

Figure 3: TLC Plate.

Rf Value calculation

Standard (Quercetin)

Rf = Distance travelled by compound/Distance travelled by solvent
Rf = 2.3/3.9 = 0.58

Cassia fistula extract

Rf = Distance travelled by compound/Distance travelled by solvent
Rf = 2.3/3.8 = 0.60

The TLC analysis of the *Cassia fistula* flower extract revealed a major spot with an Rf value of 0.60, which is very close to that of the quercetin standard (Rf = 0.58) when developed in the mobile phase Ethyl acetate : Formic acid : Glacial acetic acid : Water (10:1.1:1.1:2.7). This close similarity strongly suggests the presence of quercetin or quercetin-like flavonoids in the extract. Under UV light at 366 nm, the flavonoid-rich fractions exhibited blue fluorescence, a characteristic feature of many flavonols and flavones due to their conjugated aromatic systems. This observation complements the results obtained from phytochemical screening, IR spectroscopy, and UV–Visible analysis, all of which confirmed the presence of flavonoids.

The use of quercetin as a reference standard provided a reliable basis for comparison, and the minor difference in Rf values may be attributed to the presence of glycosylated derivatives or other flavonoid congeners in the extract. The distinct separation on the TLC plate indicates that the chosen mobile phase system was effective in resolving the flavonoid fraction from other constituents.

Overall, the TLC findings not only confirm the presence of flavonoids in *Cassia fistula* flowers but also suggest that quercetin is likely a major component, supporting the ethnomedicinal use of the plant and highlighting its potential for pharmacological applications.

Conclusion

Flavonoids were effectively extracted from *Cassia fistula* using ethanol as the solvent, yielding a crude extract rich in polyphenolic compounds, whereas IR Spectroscopy result confirms presence of flavonoids. Phytochemical qualitative flavonoid tests produces positive results for presence of flavonoids. Thin Layer Chromatography (TLC) successfully separated flavonoid components where distinct spots with Rf values ranging from 0.32 to 0.75 were observed under UV light, indicating the presence of quercetin flavonoid.

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