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SPECTROSCOPIC AND CHROMATOGRAPHIC ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN MEDICINAL PLANT *CAESALPINIA CRISTA*

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ABSTRACT

The present study was undertaken to investigate the phytochemical composition and chromatographic profile of the aqueous extract of *Caesalpinia crista*, a medicinal plant widely used in traditional medicine. The aqueous extract was prepared and evaluated for extractive yield, preliminary phytochemical constituents, total phenolic content, total flavonoid content, and Thin Layer Chromatography (TLC) fingerprinting. The extract yielded 4.92% w/w and appeared as a black-colored residue. Preliminary phytochemical screening revealed the presence of glycosides, flavonoids, saponins, proteins, diterpenes, and phenolic compounds, while alkaloids, carbohydrates, tannins, and sterols were absent. Quantitative estimation showed total phenolic and total flavonoid contents of 0.895 mg/100 mg and 0.742 mg/100 mg of extract, respectively. TLC analysis using gallic acid and quercetin as marker compounds demonstrated characteristic chromatographic profiles. The aqueous extract exhibited spots with Rf values comparable to those of gallic acid and quercetin, indicating the probable presence of phenolic and flavonoid constituents. The results confirmed that *Caesalpinia crista* is a rich source of bioactive phytochemicals and provided valuable chromatographic fingerprints for its identification, authentication, and quality control. The study supports the medicinal significance of the plant and its potential for future pharmaceutical and nutraceutical applications.

Keywords: *Caesalpinia crista*, Phytochemical Screening, Thin Layer Chromatography (TLC), Phenolic Compounds, Flavonoids, Gallic Acid, Quercetin, Bioactive Compounds, Medicinal Plants, Chromatographic Fingerprinting.

INTRODUCTION

Medicinal plants have served as an important source of therapeutic agents since ancient times and continue to play a significant role in modern healthcare systems. The World Health Organization (WHO) estimates that a large proportion of the global population relies on herbal medicines for primary healthcare needs. Medicinal plants are extensively utilized in traditional and modern systems of medicine due to their therapeutic efficacy and relatively lower incidence of adverse effects (Manisha *et al.*, 2025).

The therapeutic efficacy of medicinal plants is attributed to the presence of diverse bioactive phytoconstituents such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, and saponins. Identification and characterization of these bioactive compounds are essential for

establishing the quality, safety, efficacy, and standardization of herbal medicines (Dar *et al.,* 2023; Riaz *et al.,* 2023).

Caesalpinia crista Linn. (Family: Fabaceae), commonly known as Fever Nut, is a well-known medicinal plant widely distributed throughout tropical and subtropical regions. Various parts of the plant have been traditionally used in Ayurveda and folk medicine for the treatment of fever, inflammation, malaria, helminthic infections, liver disorders, rheumatism, and several other ailments (Chan *et al.,* 2018).

Previous pharmacological investigations have reported that *Caesalpinia crista* possesses antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and immunomodulatory activities. These biological activities are mainly associated with the presence of phenolic compounds, flavonoids, diterpenoids, steroids, and other secondary metabolites (Upadhyay *et al.,* 2019; Sodhi *et al.,* 2023).

The increasing interest in plant-based therapeutics has highlighted the need for detailed phytochemical investigations using advanced analytical techniques. Spectroscopic and chromatographic methods play an essential role in the qualitative and quantitative analysis of phytoconstituents. Spectroscopic techniques such as UV-Visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy provide valuable information regarding the presence of chromophoric groups and functional groups present in plant extracts. Similarly, chromatographic techniques including Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), and High Performance Liquid Chromatography (HPLC) are widely employed for fingerprint profiling, identification, separation, and quantification of bioactive compounds (Kumar *et al.,* 2024).

Among the important phytoconstituents present in medicinal plants, phenolic compounds and flavonoids are recognized for their strong antioxidant and therapeutic properties. Gallic acid and quercetin are commonly used marker compounds for the identification of phenolic and flavonoid constituents, respectively (Nwozo *et al.,* 2023). Chromatographic fingerprinting using these markers provides an effective approach for assessing the phytochemical composition and quality of herbal extracts (Mukherjee *et al.,* 2011).

Therefore, the present study was undertaken to perform spectroscopic and chromatographic analysis of bioactive compounds present in *Caesalpinia crista*. The study involved phytochemical characterization using spectroscopic techniques and chromatographic profiling through TLC analysis for the identification of phenolic and flavonoid constituents. The findings of this investigation will contribute to the standardization, authentication, and scientific validation of *Caesalpinia crista* as a valuable medicinal plant and may support its future therapeutic and pharmaceutical applications.

MATERIALS AND METHODS

Material

Fresh plant material of *Caesalpinia crista* was collected and authenticated for the study. Analytical-grade chemicals and reagents including distilled water, methanol, toluene, ethyl acetate, formic acid, ferric chloride, Folin–Ciocalteu reagent, sodium carbonate,

lead acetate, Wagner's reagent, Hager's reagent, concentrated sulfuric acid, Fehling's solution, Benedict's reagent, gelatin solution, copper acetate, and other reagents required for phytochemical screening were used. Standard compounds such as gallic acid and quercetin were procured for chromatographic and quantitative analysis. Pre-coated silica gel 60 F254 TLC plates were used for chromatographic fingerprinting. All chemicals and solvents used were of analytical reagent (AR) grade.

Methods

Extraction by maceration process

The extract was prepared from the shade-dried and powdered leaves of *Caesalpinia crista* following the maceration method described by Mukherjee (2007). Briefly, 50 g of the dried leaf powder was macerated with a sufficient quantity of distilled water for 48 hours at room temperature with occasional stirring. After completion of the extraction process, the mixture was filtered through muslin cloth followed by Whatman filter paper to remove insoluble materials. The filtrate was then concentrated under reduced pressure using a vacuum evaporator at 40°C to obtain the aqueous extract. The concentrated extract was further dried and stored in an airtight container for subsequent phytochemical and pharmacological studies.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Qualitative chromatographic analysis by thin layer chromatography

Thin Layer Chromatography (TLC) was performed to identify the presence of phenolic and flavonoid compounds in the aqueous extract of *Caesalpinia crista* by comparing the chromatographic behavior of the extract with standard compounds. Pre-coated silica gel 60 F254 TLC plates were used as the stationary phase. The aqueous extract of *Caesalpinia crista* and standard compounds (Gallic acid for phenolic compounds and Quercetin for flavonoids) were separately dissolved in suitable solvents to obtain clear solutions. Using a capillary tube, small spots of the standard and extract solutions were carefully applied approximately 1 cm above the lower edge of the TLC plate.

For phenolic compound analysis, the TLC plate was developed in a mobile phase consisting of Toluene: Ethyl acetate: Formic acid (7:5:1, v/v/v). For flavonoid analysis, a mobile phase of Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v) was used. The developing chamber was saturated with the respective mobile phase for about 20–30 minutes before chromatographic development. The spotted TLC plates were placed in the saturated chamber and allowed to develop until the solvent front traveled approximately 5 cm from the point of

application. The plates were then removed, and the solvent front was immediately marked. After drying at room temperature, the chromatograms were examined under long-wave UV light (365 nm), short-wave UV light (254 nm), and normal daylight.

The distance traveled by each spot and the solvent front was measured, and the retention factor (Rf) values were calculated using the formula:

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Quantitative estimation of bioactive compounds

Estimation of total phenol content

The total phenolic content of the *Caesalpinia crista* extract was determined using the modified Folin–Ciocalteu method as described by Mishra et al. (2017). A standard stock solution was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol, and different concentrations ranging from 10–50 µg/mL were prepared from this stock solution. For sample analysis, 10 mg of the dried extract was dissolved in 10 mL of methanol and filtered. An aliquot of 2 mL of the extract solution (1 mg/mL) was taken for the estimation of total phenolic content. To this, 1 mL of folin–ciocalteu reagent, previously diluted with distilled water in the ratio of 1:10 (v/v), and 1 mL of sodium carbonate solution (7.5 g/L) were added. The reaction mixture was vortexed for 15 seconds and allowed to stand at room temperature for 10 minutes for color development. The absorbance of the resulting blue-colored complex was measured at 765 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract.

4.6.2 Estimation of total flavonoids content

The total flavonoid content of the *Caesalpinia crista* extract was determined using the aluminum chloride colorimetric method as described by Mishra et al. (2017). A standard stock solution of quercetin was prepared by dissolving 10 mg of quercetin in 10 mL of methanol. From this stock solution, various concentrations ranging from 5–25 µg/mL were prepared. For sample analysis, 10 mg of the dried extract was dissolved in 10 mL of methanol and filtered. An aliquot of 3 mL of the extract solution (1 mg/mL) was taken for the estimation of total flavonoid content. To this, 1 mL of 2% aluminum chloride ($AlCl_3$) solution was added and the mixture was allowed to stand at room temperature for 15 minutes to facilitate color development. The absorbance of the resulting yellow-colored complex was measured at 420 nm using a UV–Visible spectrophotometer. The total flavonoid content was calculated using the quercetin calibration curve and expressed as milligrams of quercetin equivalents (QE) per gram of dried extract.

RESULTS AND DISCUSSION

The present study was carried out to investigate the phytochemical composition and chromatographic profile of the aqueous extract of *Caesalpinia crista*. The extraction process yielded a black-colored extract with a percentage yield of 4.92% w/w, indicating the presence of water-soluble phytoconstituents. The yield obtained suggests efficient extraction of polar bioactive compounds using water as the extraction solvent.

Preliminary phytochemical screening revealed the presence of several important secondary

metabolites in the aqueous extract. Positive results were obtained for glycosides, flavonoids, saponins, proteins, diterpenes, and phenolic compounds, while alkaloids, carbohydrates, tannins, and sterols were absent. The occurrence of flavonoids and phenolic compounds is of particular significance because these phytoconstituents are known to possess potent antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities. The presence of glycosides and saponins may also contribute to the diverse pharmacological properties previously reported for *Caesalpinia crista*. These findings are in agreement with earlier phytochemical studies that reported the presence of polyphenolic and flavonoid constituents in the plant.

Thin Layer Chromatography (TLC) was performed to further characterize the phenolic and flavonoid constituents of the extract. In the phenolic analysis, gallic acid was used as the reference standard and produced a characteristic Rf value of 0.44 under long UV, short UV, and normal light conditions. The aqueous extract exhibited multiple spots with Rf values ranging from 0.26 to 0.60 under UV light. One of the spots observed at Rf 0.42 was very close to the Rf value of gallic acid, suggesting the possible presence of gallic acid or structurally related phenolic compounds in the extract. The appearance of multiple spots further indicates the presence of a complex mixture of phenolic constituents.

Similarly, TLC analysis for flavonoids was carried out using quercetin as the standard marker compound. Quercetin exhibited an Rf value of 0.58 under all observation conditions. The aqueous extract showed five distinct spots under long UV light, with one prominent spot appearing at Rf 0.58, corresponding closely to the standard quercetin. This observation indicates the probable presence of quercetin or quercetin-like flavonoids in the extract. Additional spots observed at different Rf values suggest the presence of other flavonoid compounds, demonstrating the phytochemical diversity of *Caesalpinia crista*.

Quantitative estimation of total phenolic and total flavonoid contents revealed values of 0.895 mg/100 mg and 0.742 mg/100 mg of extract, respectively. The higher phenolic content compared to flavonoid content indicates that phenolic compounds constitute a major group of bioactive constituents in the aqueous extract. Phenolics and flavonoids are recognized for their free radical scavenging ability and are often responsible for the antioxidant and therapeutic potential of medicinal plants. The appreciable amounts of these compounds support the traditional medicinal use of *Caesalpinia crista* and provide a scientific basis for its pharmacological activities.

Table 1: % Yield of aqueous extract of *Caesalpinia crista*

S. No.	Colour	Extract	% Yield (w/w)
1.	Black	3.45	4.92%

Table 2: Result of phytochemical screening of aqueous extract of *Caesalpinia crista*

S. No.	Constituents	Aqueous extract
1.	**Alkaloids** Wagner's test: Hager's test:	 -Ve -Ve
2.	**Glycosides** Conc. H_2SO_4 test	 +Ve
3.	**Flavonoids** Alkaline reagent test: Lead acetate test:	 +Ve +Ve
4.	**Saponins** Froth test:	 +Ve
5.	**Phenol** Ferric chloride test: Folin ciocalteu test	 -Ve +Ve
6.	**Proteins** Xanthoproteic test:	 +Ve
7.	**Carbohydrate** Fehling's test: Benedict's test:	 -Ve -Ve
8.	**Diterpenes** Copper acetate test:	 +Ve
9.	**Tanins** Gelatin test	 -Ve
10.	**Sterols** Salkowski test	 -Ve

[+Ve= Present; -Ve= Absent]

Table 3: TLC of *Caesalpinia crista* (Phenol)

Aqueous extract of *Caesalpinia crista*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (7:5:1)	***Rf* value**
1.	**(Gallic Acid)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.44 Short- 0.44 Normal- 0.44
2.	**(Aqueous extract)** Dis. Travel by mobile phase= 5cm No. of spot at long UV = 4 No. of spot at short UV = 2 No. of spot at normal light= 0	 Long- 0.26, 0.42,0.52, 0.60 Short- 0.26, 0.42 Normal- 0

Table 4: TLC of *Caesalpinia crista* (Flavonoids)

Aqueous extract of *Caesalpinia crista*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.58 Short- 0.58 Normal- 0.58
2.	**(Aqueous extract)** Dis. Travel by mobile phase=5cm No. of spot at long UV = 5 No. of spot at short UV = 3 No. of spot at normal light= 1	 Long- 0.28, 0.34, 0.56, 0.58, 0.70 Short- 0.28, 0.34, 0.56 Normal- 0.56

Table 5: Total phenol and flavonoid content of *Caesalpinia crista*

S. No.	**Extract**	**Total phenol content**	**Total flavonoid content**
		mg/ 100mg	
1.	Aqueous extract	0.895	0.742

CONCLUSION

The present study confirmed the presence of important bioactive phytoconstituents in the aqueous extract of *Caesalpinia crista*, including flavonoids, phenolic compounds, glycosides, saponins, proteins, and diterpenes. TLC fingerprinting demonstrated the probable presence of gallic acid-like phenolics and quercetin-like flavonoids, while quantitative analysis revealed appreciable total phenolic (0.895 mg/100 mg) and flavonoid (0.742 mg/100 mg) contents. These findings support the medicinal value of *Caesalpinia crista* and provide a scientific basis for its standardization, quality control, and potential pharmaceutical applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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