

Phytochemical Profiling of *Cassia fistula* Leaf Extract and Evaluation of Its Antioxidant and Antibacterial Potential

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Abstract

The present study examined the phytochemicals, antioxidant activity, and antibacterial activity of the leaf extract of *Cassia fistula* L. Leaves were collected from Jaipur, Rajasthan, and extracted with distilled water using sonication. Phytochemical screening showed the presence of carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins. Alkaloids were not detected. Antioxidant activity was tested using the DPPH assay. The extract showed higher antioxidant activity at higher concentrations, with an IC₅₀ value of 156.74 ± 2.75 mg/L. Antibacterial activity was tested against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* using the agar well diffusion method. The extract inhibited all four bacterial strains. The highest inhibition zone was 25 mm against *B. subtilis* at 100 mg/L, followed by 24 mm against *S. aureus*, 22 mm against *P. aeruginosa*, and 21 mm against *E. coli*. These results show that *Cassia fistula* leaf extract contains several phytochemicals and has antioxidant and antibacterial potential. Further studies are needed to identify the active compounds and explore their biological applications.

Keywords: *Cassia fistula*, phytochemicals, antioxidant activity, antibacterial activity

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1. Introduction

Cassia fistula L., commonly known as Amaltas or the golden shower tree, belongs to the family Fabaceae. It is a medicinal plant used in traditional medicine for various health conditions. Different parts of the plant, including the leaves, flowers, bark, and fruits, have been studied for their biological properties. Previous studies have reported antioxidant, anti-inflammatory, and antibacterial activities of *C. fistula* extracts (Panda et al., 2011; Bhalodia & Shukla, 2011).

Plants are important sources of diverse bioactive compounds, and phytochemicals such as phenols, flavonoids, terpenoids, alkaloids, and saponins can contribute to the biological and functional properties of plant-derived materials (Saraf et al., 2026). Phytochemicals such as carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins have been reported in *C. fistula* leaves. These compounds may contribute to the biological activity of plant extracts (Panda et al., 2011; Bhalodia & Shukla, 2011). Therefore, phytochemical screening is useful for identifying the major compound classes present in plant extracts.

Oxidative stress occurs when free radical production exceeds the body's antioxidant defense system.

Plant-derived antioxidants are being studied as natural sources of compounds that may help reduce oxidative damage. The DPPH radical scavenging assay is commonly used to evaluate the antioxidant activity of plant extracts. It measures an extract's ability to reduce DPPH radicals. The IC₅₀ value indicates the concentration required to achieve 50% radical scavenging activity (Panda et al., 2011). Previous studies have reported antioxidant activity in *C. fistula* extracts, supporting further investigation of *C. fistula* leaves (Panda et al., 2011; Bhalodia & Shukla, 2011).

Antibacterial activity is another important area of medicinal plant research. Plant extracts contain several compounds that may inhibit bacterial growth. Studies on *C. fistula* leaves have reported antibacterial activity against both Gram-positive and Gram-negative bacteria. Panda et al. (2011) investigated different solvent extracts of the leaves and reported antibacterial activity against several bacterial strains. Bhalodia and Shukla (2011) also reported antibacterial activity of hydroalcoholic leaf extracts against medically important bacteria. These findings indicate the potential of *C. fistula* leaves as a source of antibacterial compounds.

The biological activity of plant extracts may vary with the plant part, extraction solvent, and experimental conditions. Therefore, further studies are needed to evaluate the phytochemical composition and biological properties of plant extracts. The present study was undertaken to investigate the phytochemical constituents of the aqueous leaf extract of *Cassia fistula*. The antioxidant activity was evaluated using the DPPH radical scavenging assay. The antibacterial activity was studied against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* using the agar well diffusion method. The study aims to provide preliminary information on the biological potential of *C. fistula* leaf extract.

2. Materials and methods

2.1 Plant material collection

Fresh, healthy, and disease-free leaves of *Cassia fistula* L. were collected from Delhi road, Amber, Jaipur, Rajasthan, India.

Following collection, the leaves were thoroughly washed with running tap water to remove adhering dust and surface contaminants. The leaves were subsequently immersed in sterile 0.9% (w/v) saline solution and rinsed repeatedly with deionized distilled water. Excess moisture was removed by blotting the leaves dry (Anuradha et al., 2015). The cleaned leaves were then shade-dried at room temperature for 8–10 days under well-ventilated conditions (Kamaraj et al., 2020).

To determine the dry weight, 5 g of freshly collected leaves were transferred to a laboratory cabinet dryer maintained at 40–50 °C and weighed at regular intervals until a constant weight was achieved (Anuradha et al., 2015). The completely dried leaves were ground into a fine powder using a laboratory grinder (Gupta et al., 2024) and subsequently sieved through a 100-mesh sieve to ensure uniform particle size and homogeneity (Suresh et al., 2015). The resulting powdered plant material was stored in airtight containers under appropriate conditions until further experimental use.

2.2 Preparation of aqueous leaf extract

An aqueous extract of *Cassia fistula* leaves was prepared from the previously dried and powdered plant material. For extraction, 10 g of leaf powder was accurately weighed and mixed with 100 mL of distilled water. The suspension was then subjected to ultrasonic treatment using a sonicator at 40°C for 10 min to facilitate the release of bioactive constituents into the aqueous medium (Shahzad et al., 2022). Following sonication, the mixture was cooled to ambient temperature and passed through Whatman No. 42 filter paper to separate the insoluble plant material (Ganaie et al., 2014). The resulting clear filtrate was collected and maintained at 4°C until required for subsequent experiments.

2.3 Phytochemical analysis of the leaf extract

Preliminary phytochemical screening of the selected plant extract was conducted to assess the presence or absence of phytochemicals, including proteins, carbohydrates, phenols, flavonoids, alkaloids, glycosides, saponins, and terpenoids, using standard methods with minor modifications.

2.4 Evaluation of antioxidant potential

The antioxidant potential of the extracted sample was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Briefly, 100 µL of the sample at different concentrations was added to 3.9 mL of freshly prepared 0.1 mM DPPH solution in methanol. The mixture was vortexed thoroughly to ensure uniform mixing, then incubated in the dark at room temperature for 30 min. Following incubation, absorbance was measured at 515 nm using a UV–Vis spectrophotometer, with methanol as the blank. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH Radical scavenging activity (\%)} = \left(\frac{\text{Ab}_{\text{control}} - \text{Ab}_{\text{sample}}}{\text{Ab}_{\text{control}}} \right) \times 100$$

Where $\text{Ab}_{\text{control}}$ is the absorbance of the DPPH solution without the sample, and $\text{Ab}_{\text{sample}}$ is the absorbance of the DPPH solution containing the test sample.

The half-maximal inhibitory concentration (IC_{50}) was determined using the linear regression equation obtained by plotting the percentage of DPPH radical scavenging activity (Y) against the corresponding sample concentration (X). The regression equation was expressed as:

$$Y = mx + c$$

where Y represents the percentage of DPPH radical scavenging activity, X represents the sample concentration, m is the slope, and c is the intercept of the regression line. The IC_{50} value, defined as the concentration of the extract required to achieve 50% inhibition of DPPH radicals, was calculated by substituting $Y = 50$ into the regression equation and solving for X . Thus, the antioxidant potential of the extracts was expressed in terms of their IC_{50} values. Lower IC_{50} values indicate stronger antioxidant activity, as a lower extract concentration is required to achieve 50% DPPH radical scavenging.

2.5 Evaluation of antimicrobial potential

The in vitro antibacterial activity of the test samples was evaluated using the standard agar well diffusion method. Nutrient agar was used as the culture medium for the antibacterial assay. The test samples were dissolved or diluted in dimethyl sulfoxide (DMSO), and four different concentrations of each sample were prepared. Sterile Petri plates containing the prepared nutrient agar medium were inoculated with the respective test microorganisms. The inoculum was uniformly distributed over the agar surface using a sterile spreader, and the inoculated plates were allowed to stand for 30 min.

Subsequently, 6-mm-diameter wells were aseptically prepared in the seeded agar plates. The

standard antibiotic, erythromycin, was used as the reference control and prepared at concentrations matching those of the test samples. A 30 μ L volume of each sample concentration and the standard antibiotic was carefully loaded into the respective wells. The plates were then incubated at 37°C for 24 h. Following incubation, antibacterial activity was assessed by measuring the diameter of the clear zone of inhibition (ZI) formed around each well. The antibacterial activity of the test samples was compared with that of the standard antibiotic using the inhibition zone diameters.

The Activity Index (AI) was calculated to determine the relative antibacterial activity of the test samples in comparison with the standard antibiotic using the following equation:

Activity Index (AI) = Inhibition zone diameter of sample / Inhibition zone diameter of standard antibiotic

3. Results

3.1 Phytochemical determination of the leaf extract

Flavonoids and phenols are important plant compounds because they are often associated with antioxidant activity. Saponins, glycosides, and terpenoids may also contribute to the biological properties of plant extracts. However, qualitative screening only confirms the presence or absence of these compounds. It does not measure their exact amounts or identify individual compounds.

The present findings are consistent with previous studies on *C. fistula*. Panda et al. (2011) reported flavonoids, carbohydrates, glycosides, proteins, saponins, and triterpenoids in leaf extracts. Bhalodia and Shukla (2011) also reported several phytochemicals, including flavonoids, saponins, glycosides, and triterpenoids, in hydroalcoholic leaf extracts. The differences in the detected compounds may be due to the extraction solvent and the methods used for phytochemical screening.

Table 1. Qualitative phytochemical profile of *Cassia fistula* leaf extract.

S.No	Test Name	Observation	Result
1	Carbohydrate Test (Fehling's Test)	Red Precipitate	Positive
2	Saponin Test (Foam Test)	Foam formed	Positive
3	Flavonoid Test (Lead Acetate Test)	Light Yellow Precipitate	Positive

4	Glycoside Test (NaOH Test)	Red Colour	Positive
5	Alkaloid Test (Dragendorff's Test)	No Precipitate	Negative
6	Terpenoid Test	Dark Brown Color	Positive
7	Phenol Test (Ferric Chloride Test)	Pale Green Color	Positive
8	Protein Test (Ninhydrin Test)	Bluish Purple Color	Positive

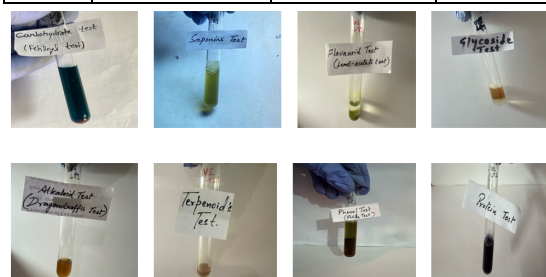


Figure 1: Qualitative phytochemical screening of *Cassia fistula* leaf extract.

3.2 Antioxidant assay

The antioxidant activity of the aqueous leaf extract of *Cassia fistula* was evaluated using the DPPH radical scavenging assay. The extract showed an increase in antioxidant activity with increasing concentration. The percentage of inhibition increased from $20.00 \pm 1.32\%$ at 20 mg/L to $37.57 \pm 0.61\%$ at 100 mg/L. The IC₅₀ value of the extract was 156.74 ± 2.75 mg/L.

The increase in antioxidant activity with concentration shows that the extract was more effective at higher concentrations under the tested conditions. The IC₅₀ value quantifies the extract's antioxidant activity. Ascorbic acid showed higher antioxidant activity than the plant extract at all tested concentrations. Its IC₅₀ value was 74.58 ± 0.59 mg/L. This indicates that ascorbic acid showed greater antioxidant activity than the extract in the present assay.

The antioxidant activity may be related to the phenols and flavonoids detected during phytochemical screening. These compounds are commonly associated with antioxidant properties. However, the exact compounds responsible for the activity cannot be confirmed from the present results.

Previous studies have also reported antioxidant activity in *C. fistula* leaves. Sharma et al. (2022) reported DPPH radical scavenging activity in an aqueous-ethanolic leaf extract, with an IC₅₀ value of 215.55 µg/mL. The difference between the previous and present results may be due to differences in the extraction solvent, plant material, and experimental conditions. The IC₅₀ values should therefore be compared carefully, as the units and assay conditions differ. Overall, the present study shows that the aqueous leaf extract of *C. fistula* has antioxidant potential and warrants further investigation.

Table 2: % Free radical scavenging potential of the extract.

Sample name	% Free radical scavenging activity at different concentrations (mg/L)					IC ₅₀ (mg/L)
	20	40	60	80	100	
Cassia extract	20.0	25.1	28.0	33.6	37.5	156.
	0±1.	9±0.	8±0.	9±0.	7±0.	74±2
	32	50	52	64	61	.75
Ascorbic acid	32.5	37.3	46.4	51.7	58.0	74.5
	2±1.	8±0.	3±0.	2±0.	7±0.	8±0.
	35	36	21	33	22	59

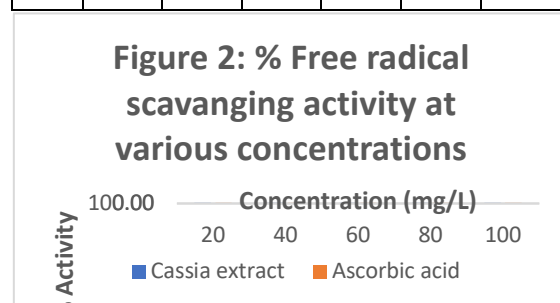


Figure 2. Antioxidant activity of *Cassia fistula* leaf extract determined by the DPPH assay.

3.3 Antimicrobial assay

The antibacterial activity of the aqueous leaf extract was evaluated against four bacterial strains using the agar well diffusion method. The extract showed inhibitory activity against all tested bacteria. The inhibition zones increased with increasing extract concentration in most cases. The results are presented in Table 3.

3.3.1 Antibacterial activity against *Escherichia coli*

The aqueous leaf extract of *Cassia fistula* showed antibacterial activity against *Escherichia coli*. The

inhibition zone increased from 7 mm at 6.25 mg/L to 21 mm at 100 mg/L. The activity index increased from 0.30 at 6.25 mg/L to 0.67 at 80 mg/L. At 100 mg/L, the activity index was 0.65. The highest inhibition zone was observed at 100 mg/L.

The increase in the inhibition zone with concentration indicates that the extract showed greater antibacterial activity at higher concentrations under the tested conditions. However, the activity index did not increase continuously at the highest concentration. This may be related to differences in diffusion of the extract compounds through the agar medium or variation in the assay.

Panda et al. (2011) reported antibacterial activity of *C. fistula* leaf extracts against *E. coli*. Their study also showed that antibacterial activity varied with the extraction solvent. The present findings support the antibacterial potential of *C. fistula* leaves. However, a direct comparison is difficult because the extraction methods and concentrations differed.

3.3.2 Antibacterial activity against *Staphylococcus aureus*

The aqueous leaf extract of *Cassia fistula* showed antibacterial activity against *Escherichia coli*. The inhibition zone increased from 7 mm at 6.25 mg/L to 21 mm at 100 mg/L. The activity index increased from 0.30 at 6.25 mg/L to 0.67 at 80 mg/L. At 100 mg/L, the activity index was 0.65. The highest inhibition zone was observed at 100 mg/L.

The increase in the inhibition zone with concentration indicates that the extract showed greater antibacterial activity at higher concentrations under the tested conditions. However, the activity index did not increase continuously at the highest concentration. This may be related to differences in diffusion of the extract compounds through the agar medium or variation in the assay.

Panda et al. (2011) reported antibacterial activity of *C. fistula* leaf extracts against *E. coli*. Their study also showed that antibacterial activity varied with the extraction solvent. The present findings support the antibacterial potential of *C. fistula* leaves. However, a direct comparison is difficult because the extraction methods and concentrations differed.

3.3.3 Antibacterial activity against *Bacillus subtilis*

The aqueous leaf extract of *Cassia fistula* showed antibacterial activity against *Bacillus subtilis*. The inhibition zone increased from 8 mm at 6.25 mg/L to 25 mm at 100 mg/L. The activity index increased from 0.53 to 1.08. The highest inhibition zone and activity index were recorded at 100 mg/L.

Among the four tested bacteria, *B. subtilis* showed the largest inhibition zone with the extract. The activity index of 1.08 indicates that the extract's inhibition zone was slightly greater than that of the standard antibiotic at 100 mg/L. This result shows strong antibacterial activity against *B. subtilis* under the present experimental conditions.

Panda et al. (2011) reported that *B. subtilis* was inhibited by different solvent extracts of *C. fistula* leaves. Their findings support the antibacterial activity observed in the present study. However, the present result does not prove that the extract is more effective than the antibiotic for clinical treatment.

3.3.4 Antibacterial activity against *Pseudomonas aeruginosa*

The aqueous leaf extract of *Cassia fistula* showed antibacterial activity against *Pseudomonas aeruginosa*. The inhibition zone increased from 8 mm at 6.25 mg/L to 22 mm at 100 mg/L. The activity index increased from 0.40 to 0.78. The highest inhibition zone was observed at 100 mg/L.

These results indicate that the extract inhibited the growth of *P. aeruginosa*. The increase in the inhibition zone suggests that higher extract concentrations produced greater antibacterial activity. The activity index remained below 1.0 at all tested concentrations. This shows that the inhibition zones were smaller than those of the standard antibiotic.

Bhalodia and Shukla (2011) reported antibacterial activity of hydroalcoholic leaf extracts of *C. fistula* against *P. aeruginosa*. Panda et al. (2011) also reported antibacterial activity of *C. fistula* leaf extracts against Gram-negative bacteria. The present findings are consistent with these earlier studies and support the antibacterial potential of the aqueous leaf extract.

3.4 Comparative antibacterial activity of the extract

The comparative results showed that the aqueous leaf extract inhibited all four tested bacteria. The highest inhibition zone was observed against *Bacillus subtilis* at 100 mg/L. It was followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. This shows that the extract had different levels of antibacterial activity against the tested bacteria.

The differences in activity may be related to bacterial cell structure and the diffusion of extract compounds through the agar medium. Gram-positive and Gram-negative bacteria have different cell envelope structures. These differences may affect their response to plant extracts. However, the present study did not examine the exact mechanism of antibacterial action.

The activity index was generally lower than that of the standard antibiotic. The only exception was *B. subtilis* at 100 mg/L, with an activity index of 1.08. This result indicates considerable inhibition against this organism under the tested conditions. Further studies using MIC, MBC, and purified compounds are needed to confirm the antibacterial activity.

Previous studies have reported antibacterial activity of *C. fistula* leaf extracts against both Gram-positive and Gram-negative bacteria. Panda et al. (2011) observed activity in different solvent extracts. Bhalodia and Shukla (2011) also reported activity

against medically important bacterial strains. These findings support the present results and suggest that *C. fistula* leaves may contain antibacterial compounds.

Table 3: Antibacterial potential of the *Cassia fistula* leaf extract.

Antibacterial potential of the plant extract at different concentrations (mg/L)										
	6.25		12.50		25		50		100	
	I	A	I	A	I	A	I	A	I	A
	Z	I	Z	I	Z	I	Z	I	Z	I
	(		(		(		(		(	
	m		m		m		m		m	
	m		m		m		m		m	
	)		)		)		)		)	
<i>E. coli</i>										
Sa	7	0	1	0	1	0	1	0	2	0
mp	.	4	.	6	.	9	.	1	.	6
le	3		5		6		6		6	
	0		6		1		7		5	
Sta	2		2		2		2		3	
nd	3		5		6		8		2	
ard										
<i>S. aureus</i>										
Sa	8	0	1	0	1	0	1	0	2	0
mp	.	2	.	5	.	8	.	4	.	7
le	5		7		7		7		2	
	7		5		8		2		2	
Sta	1		1		1		2		3	
nd	4		6		9		5		2	
ard										
<i>B. subtilis</i>										
Sa	8	0	1	0	1	0	1	0	2	1
mp	.	1	.	3	.	6	.	5	.	0
le	5		6		6		7		8	
	3		4		8		6		8	
Sta	1		1		1		2		2	
nd	5		7		9		1		3	
ard										
<i>P. aeruginosa</i>										

Sa	8	0	1	0	1	0	1	0	2	0
mp		.	0	.	4	.	7	.	2	.
le		4		4		5		6		7
		0		5		8		5		8
Sta	2		2		2		2		2	
nd	0		2		4		6		8	
ard										

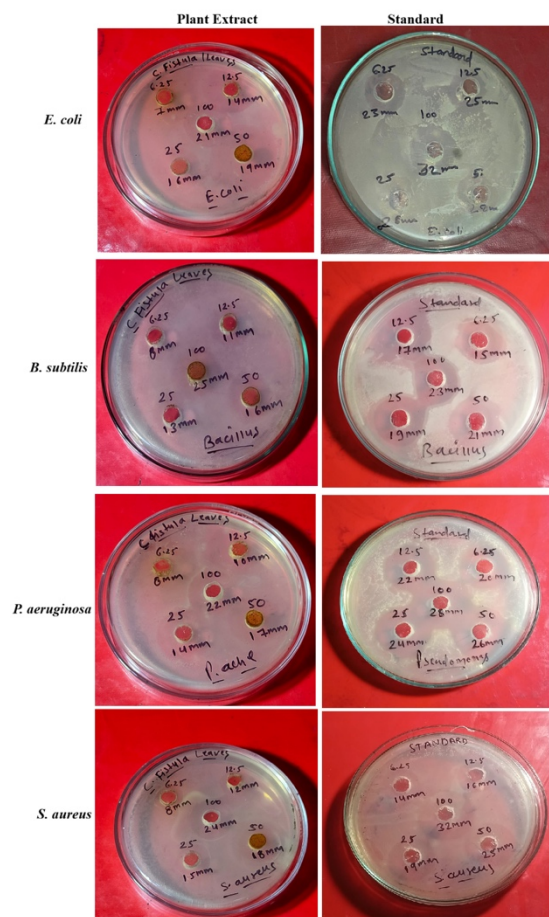


Figure 3: Representative petri plates showing the antibacterial activity of *Cassia fistula* leaf extract. The present study showed that the aqueous leaf extract of *Cassia fistula* contains several phytochemical groups. It also showed antioxidant and antibacterial activities. The DPPH assay showed that antioxidant activity increased with extract concentration. The antibacterial assay showed inhibition of all four tested bacterial strains. The highest inhibition zone was recorded against *Bacillus subtilis*. The observed biological activities may be related to the phytochemicals present in the extract. Phenols and flavonoids are commonly associated with antioxidant activity. Other secondary metabolites may also contribute to antibacterial activity. However, the present study did not establish a direct relationship between any specific phytochemical and the observed biological activity.

Previous studies have reported similar activities in *C. fistula* leaf extracts. Panda et al. (2011) and Bhalodia and Shukla (2011) reported antibacterial activity of *C. fistula* leaf extracts. Sharma et al. (2022), Arulpandi et al. (2012), and Ganesan et al. (2024) reported antioxidant activity in aqueous-ethanolic leaf extracts. The present study adds information on the antioxidant and antibacterial potential of an aqueous leaf extract collected from Jaipur, Rajasthan.

The study provides preliminary evidence of the biological potential of *C. fistula* leaves. Further studies should include quantitative phytochemical analysis, MIC and MBC determination, and identification of individual bioactive compounds. These studies may help identify the compounds responsible for the observed antioxidant and antibacterial activities.

4. Conclusion

The present study investigated the phytochemical composition, antioxidant activity, and antibacterial potential of the aqueous leaf extract of *Cassia fistula*. The extract contained carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, and proteins. Alkaloids were not detected. The extract exhibited concentration-dependent DPPH radical-scavenging activity, with an IC₅₀ of 156.74 ± 2.75 mg/L.

The extract also showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The highest inhibition zone was 25 mm against *B. subtilis* at 100 mg/L. These findings indicate that *C. fistula* leaves contain bioactive compounds with antioxidant and antibacterial potential.

Further studies are required to identify the active compounds and understand their biological mechanisms. Quantitative phytochemical analysis, MIC and MBC studies, and compound identification may provide more information. The present findings provide preliminary evidence for future research on the medicinal properties of *C. fistula* leaves.

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Author contributions

Syed Burhan Geelani: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, and Writing – original draft.

Arindam Saraf: Investigation, Data curation, Formal analysis, and Resources.

Anuradha Jayaraman: Supervision, Validation, and Writing – review and editing.

Indu Sharma: Supervision, Validation, and Writing – review and editing.

Sanjeevi Ramakrishnan: Supervision, Validation, and Writing – review and editing.

Conflict of interest

The authors have no conflict of interest to declare concerning the research, authorship, and publication of this article.

Data Availability Statement

All data generated and analyzed during this study are included in this published article and its supplementary information.

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