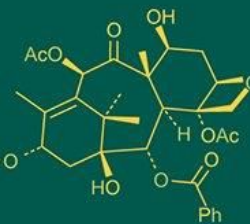
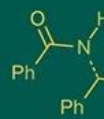


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(3): 605-610
www.biochemjournal.com
Received: 03-01-2026
Accepted: 05-02-2026

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Physicochemical profiling, phytochemical analysis, and antibacterial activity of the methanolic leaf extract of *Curcuma caesia* Roxb

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3Sh.7955>

Abstract

Curcuma caesia Roxb. (Black turmeric), a member of the family Zingiberaceae, is an important medicinal plant widely used in traditional medicine. While the rhizome of *Curcuma caesia* has been extensively investigated for its pharmacological properties, the leaves remain comparatively less explored despite their ethnomedicinal importance. Therefore, the present study was undertaken to evaluate the phytochemical composition and antibacterial potential of the methanolic leaf extract of *Curcuma caesia*. Fresh leaves were collected from the Hiranagar region of Kathua district, Jammu and Kashmir, India, and subjected to methanolic extraction. Preliminary organoleptic evaluation of the leaf powder and extract revealed characteristic green color, aromatic odor, and fine texture. Physicochemical analysis showed a percentage extractive yield of 18%, total ash content of 4%, and moisture content of 14%, indicating the basic quality parameters of the plant material. Qualitative phytochemical screening of the methanolic extract revealed the presence of several bioactive constituents, including alkaloids, saponins, glycosides, phenols, flavonoids, steroids, tannins, terpenoids, carbohydrates, proteins, and amino acids. The antibacterial activity of the extract was evaluated against six bacterial pathogens using the agar well diffusion method. The extract exhibited moderate antibacterial activity with zones of inhibition ranging from 6.15±0.10 mm to 9.00±0.55 mm, whereas the standard antibiotic streptomycin sulphate showed comparatively higher inhibitory activity. The results indicate that *Curcuma caesia* leaves contain diverse phytochemicals that may contribute to their antibacterial potential and support their possible use as a natural source of bioactive compounds.

Keywords: *Curcuma caesia*, black turmeric, methanolic leaf extract, phytochemical screening, antibacterial activity, medicinal plants

1. Introduction

Medicinal plants are recognized as valuable sources of therapeutic agents due to their rich content of bioactive secondary metabolites (Chatopadhyay *et al.*, 2004). *Curcuma caesia* Roxb., commonly known as black turmeric, belongs to the family Zingiberaceae. Its bluish-black rhizome is rich in bioactive compounds and has been traditionally employed to treat a wide range of ailments including piles, leprosy, asthma, wounds, fever and diarrhea (Prajeetha *et al.*, 2025) [13]. Previous studies have reported its antifungal (Banerjee and Nigam, 1976) [1], anti-ulcer (Das *et al.*, 2012) [5], antioxidant (Mangla *et al.*, 2010) [9] and antibacterial (Paliwal *et al.*, 2011) [12]. It has gained considerable scientific and ethnomedicinal interest due to its wide range of therapeutic applications. Native to India, *C. caesia* is primarily distributed across the north-eastern and central regions of the country. Although the rhizome of *C. caesia* is well-documented for its traditional applications, the leaves also possess notable medicinal value.

Ethnobotanical records indicate that the leaves are used externally as plasters to treat lymphangitis, furunculosis and localized swelling. In several indigenous communities of India, leaf preparations are applied for rheumatism, gout and arthritis, suggesting the presence of bioactive compounds with anti-inflammatory and analgesic properties. Despite its ethnomedicinal relevance, research on the phytochemical composition of *C. caesia* leaf is limited. Most phytochemical studies have focused on the rhizome, thereby overlooking the

potential of leaves as an alternative and sustainable source of bioactive compounds, which is especially important because *C. caesia* is an endangered medicinal species (Lenka *et al.*, 2025) [7]. Therefore, the present study was designed to investigate the qualitative composition of the methanolic leaf extract of *Curcuma caesia* Roxb.

- **Taxonomic classification**
- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Class:** Liliopsida
- **Order:** Zingiberales
- **Family:** Zingiberaceae
- **Genus:** *Curcuma*
- **Species:** *Curcuma caesia* Roxb.

2. Material and methods

2.1 Collection of sample

Fresh leaves of *Curcuma caesia* Roxb. were collected from the Hiranagar region of Kathua district, Jammu and Kashmir, UT, India, and transported to the laboratory of Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST-Jammu). The plant material was washed thoroughly with distilled water to remove adhering dust and impurities, cut into small pieces, and air-dried at room temperature until completely dried. The dried leaves were then ground into a fine powder using an electric grinder for further analysis.

2.2 Extraction

Approximately 10 g of powdered leaf sample was accurately weighed and extracted with 100 ml of methanol. The mixture was allowed to stand with occasional shaking to ensure complete extraction of phytochemicals. The solution was then filtered through Whatman No. 1 filter paper, and the filtrate was collected for further qualitative phytochemical analysis (Fig.1).

2.3 Macroscopic (Organoleptic) Evaluation

The macroscopic characteristics of both the leaf powder and methanolic extract were evaluated based on parameters such as color, odor, texture, appearance, and solubility using visual and organoleptic observations.

2.4 Physicochemical profiling

Dried powdered leaves of *Curcuma caesia* were subjected to physicochemical analysis to determine parameters such as percentage yield, total ash content and moisture content in order to evaluate the quality and purity of the plant material (Regupathi and Chitra 2015) [14].

2.4.1 Determination of Percentage Yield

The dried plant powder was extracted with methanol, and the solvent was evaporated to obtain the concentrated extract. The weight of the dried extract was recorded and used to calculate the percentage yield using the following formula:

$$\text{Percentage yield (\%)} = (\text{Weight of concentrated samples} / \text{Weight of powdered plant samples}) \times 100$$

Ash value represents the total inorganic content present in the plant material, which may include mineral salts, silica, and other inorganic impurities.

2.4.2 Determination of Total Ash

Approximately 2 g of dried leaf powder of *Curcuma caesia* was accurately weighed and placed in a pre-weighed silica crucible. The sample was then gradually heated and incinerated in a muffle furnace at 450°C until the material became completely white, indicating the absence of carbon. After incineration, the crucible was removed and allowed to cool in a desiccator, and the weight of the ash was recorded. The heating process was repeated until a constant weight of ash was obtained.

The percentage of total ash was calculated using the following formula:

$$\text{Total Ash (\%)} = (\text{Weight of ash} / \text{Weight of air-dried sample}) \times 100$$

2.4.3 Determination of Moisture Content (Loss on Drying)

About 2 g of powdered leaf sample was accurately weighed and placed in a previously weighed petri dish. The sample was dried in a hot air oven at 105-110°C for about 3 hours. After drying, the dish was cooled in a desiccator and weighed. The drying process was repeated until a constant weight was obtained. The percentage moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = (\text{Weight of wet sample} - \text{Weight of dry sample}) / \text{Weight of wet sample} \times 100$$

Moisture determination using the loss on drying method is commonly used to evaluate plant drug stability and prevent microbial deterioration.

2.5 Phytochemical Screening

The methanolic extracts were subjected to qualitative phytochemical screening to identify the presence of various bioactive compounds. The following tests were performed (Harborne, 1998) [6].

2.5.1 Alkaloid Test (Mayer's test)

Approximately 2ml of extract was acidified with a few drops of dilute hydrochloric acid and treated with Mayer's reagent. The presence of alkaloids was indicated by the formation of a yellow or cream coloured precipitate (Shaikh and Patil, 2020) [16].

2.5.2 Saponins Test (Foam Test)

1 ml of the methanolic extract was mixed with 5 ml of distilled water and shaken vigorously for 5 minutes. The formation of stable foam indicated the presence of saponins (Mujeeb *et al.*, 2014) [11].

2.5.3 Glycoside Test (Alkaline reagent test)

A small quantity of the filtrate was mixed with 1 ml of distilled water followed by the addition of sodium hydroxide solution. The development of a yellow coloration confirmed the presence of glycosides (Roghini and Vijaylakshmi, 2018) [15].

2.5.4 Phenols Test (Ferric Chloride Test)

Two ml of distilled water was added to 1ml of extract followed by a few drops of 10% ferric chloride. The presence of phenols can be detected by the formation of blue or green colour (Lydia *et al.*, 2016) [18].

2.5.5 Flavonoids Test (Alkaline Reagent Test)

1 ml of sodium hydroxide was added to 2ml of extract. The presence of flavonoids can be detected by their changing colour to yellow (Lydia *et al.*, 2016) ^[8].

2.5.6 Test for steroids and Phytosteroids (Salkowski test)

One milliliter of chloroform followed by few drops of concentrated sulphuric acid was added to one milliliter of extracts. The presence of steroids and phytosteroids can be detected by the appearance of brown ring and bluish ring respectively (ushie *et al.*, 2022) ^[17].

2.5.7 Tannins Test (Braymer's Test)

Two ml of 5% ferric chloride was added to 1ml of leaf extract. The presence of tannins can be detected by their changing colour to greenish black or dark blue (Lydia *et al.*, 2016) ^[8].

2.5.8 Test for carbohydrates (Molisch's reagent)

Approximately, 1ml of the filtrate was treated with two drops of Molisch's reagent, followed by the careful addition of concentrated sulfuric acid along the sides of the test tube. The formation of a violet or reddish ring indicated the presence of carbohydrates (Lydia *et al.*, 2016) ^[8].

2.5.9 Test for Proteins (Millon's Test)

Two milliliters of the plant extract were treated with 2 ml of Millon's reagent and allowed to stand for two minutes. The formation of a white precipitate, which turned red upon gentle heating, confirmed the presence of proteins (Mishra *et al.*, 2024) ^[10].

2.5.10 Test for Amino Acids (Ninhydrin Test)

Two millilitres of the plant extract were treated with 2 ml of ninhydrin reagent and heated gently. The appearance of a violet coloration indicated the presence of amino acids (Bhati *et al.*, 2022) ^[2].

2.5.11 Terpenoids Test (Salkowski's Test)

One millilitre of the plant extract was mixed with 0.5 ml of chloroform, followed by the addition of 3-5 drops of concentrated sulfuric acid. The formation of a reddish-brown coloration confirmed the presence of terpenoids (Roghini and Vijaylakshmi, 2018) ^[15].

2.6 Antibacterial activity of plant extract

The antibacterial activity of crude plant extract was evaluated using the agar well diffusion method. Sterile Nutrient Agar medium was prepared and poured into Petri plates and allowed to solidify (Chanda *et al.*, 2011) ^[3]. The bacterial cultures were uniformly spread on the agar surface using spreader. Wells of 5mm diameter were made in the agar using sterile cork borer. The crude plant extract was prepared at concentration 80 mg/ml by dissolving the dried extract in 50% DMSO. About 50 µl of extract solution was introduced in each wells. Streptomycin sulphate was used as positive control. The plates were incubated at 37°C for 24hrs. After incubation, antibacterial activity was determined by measuring zone of inhibition (ZOI) in millimeters (mm) around the wells. All experiments were performed in triplicate and results were expressed as Mean±Standard deviation.

4. Result

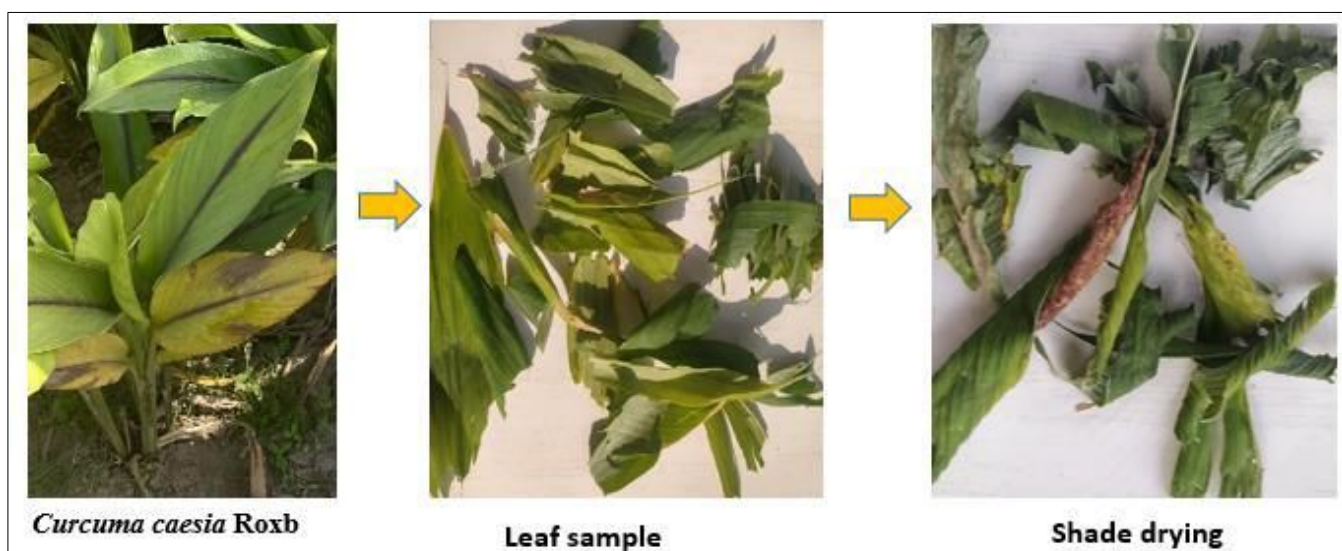
4.1 Macroscopic (Organoleptic) Characteristics

The macroscopic evaluation of the leaf powder and methanolic extract of *Curcuma caesia* revealed distinct organoleptic characteristics. The leaf powder was green in color with an aromatic, herbaceous odor and a fine, dry, homogeneous texture. In contrast, the methanolic extract appeared dark green with a strong aromatic odor and a semi-viscous to oily consistency. The extract was clear in appearance and showed complete solubility in methanol, indicating efficient extraction of phytoconstituents. The complete organoleptic characteristics of the leaf powder and methanolic extract are summarized in Table 1.

Table 1: Macroscopic characteristics of *Curcuma caesia* leaf powder and methanolic extract

S. No.	Parameter	Leaf powder	Methanolic extract
1.	color	Green	Dark green
2.	odor	Aromatic, herbaceous	Strong aromatic
3.	Texture	Fine powder	Semi-viscous to oily
4.	Appearance	Dry, homogeneous	Clear
5.	Solubility	Not applicable	Completely soluble in methanol

4.2 Physicochemical Analysis



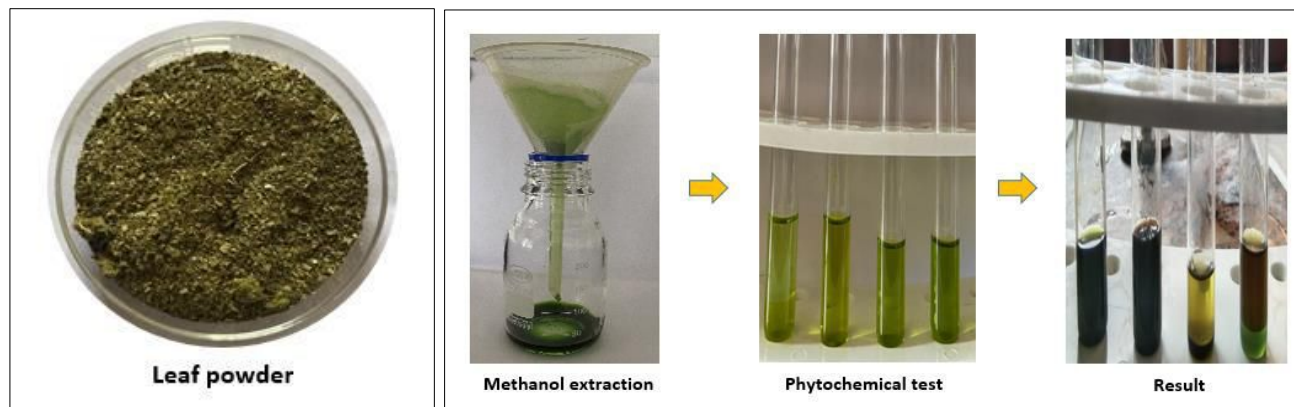


Fig 1: Flow chart of leaf extraction and phytochemical analysis

The physicochemical parameters of *Curcuma caesia* leaf powder were evaluated and the results are presented in Table 2 and Fig 2. The percentage yield of the methanolic leaf extract was found to be 18%. The total ash content was recorded as 4%, indicating the presence of inorganic mineral constituents in the plant material. The moisture content (loss on drying) was found to be 14%, which reflects the amount of water present in the dried leaf powder. These

physicochemical parameters are important for the quality evaluation and standardization of the plant material.

Table 2: Physicochemical analysis of *Curcuma caesia* leaf powder

S.No.	Parameters	%
1.	Yield	18%
2.	Total ash	4%
3.	Moisture content	14%

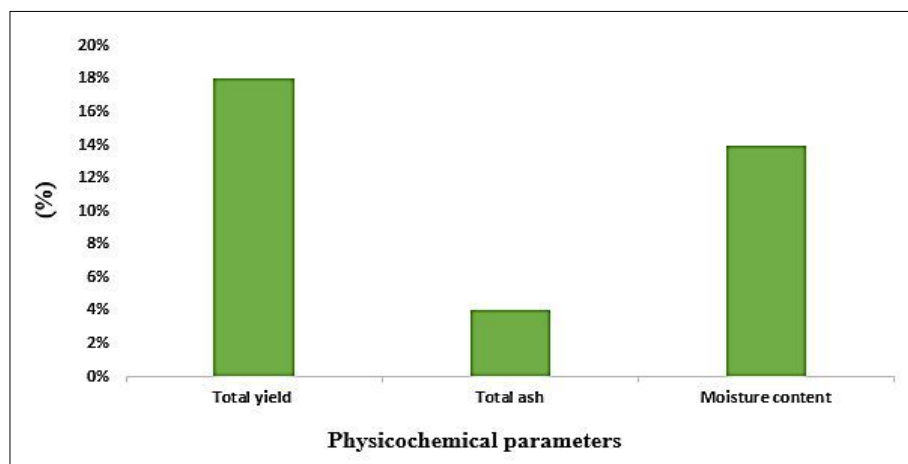


Fig 2: Graphical representation of physicochemical parameters of *Curcuma caesia* Roxb. leaf powder

4.3 Qualitative Phytochemical analysis of Plant Extract

Phytochemical analysis of the methanolic leaf extract of *Curcuma caesia* Roxb. was carried out to determine the presence of various bioactive constituents. The results revealed the presence of alkaloids, as confirmed by a positive Mayer's test. Saponins were also detected through the foam test, indicating the possible surfactant and biological properties of the extract. Glycosides were present, as evidenced by positive alkaline reagent test, suggesting potential therapeutic significance.

Phenolic compounds were detected using the ferric chloride test, indicating the antioxidant potential of the extract. Flavonoids were confirmed by a positive alkaline reagent test, while steroids and terpenoids were identified through Salkowski's test, demonstrating the presence of important secondary metabolites with potential pharmacological

activities. Tannins were also present, as indicated by Braymer's test, which may contribute to antimicrobial and astringent properties.

In addition, carbohydrates, proteins, and amino acids were detected by positive Molisch's reagent, Millon's test and Ninhydrin test respectively, indicating the presence of primary metabolites along with secondary metabolites in the extract.

Overall, the phytochemical screening confirmed that the methanolic leaf extract of *Curcuma caesia* Roxb. contains a wide range of bioactive compounds, including alkaloids, saponins, glycosides, phenols, flavonoids, steroids, tannins, terpenoids, carbohydrates, proteins, and amino acids, which may collectively contribute to its potential medicinal and pharmacological properties. The detailed results of the qualitative phytochemical analysis are presented in Table 3.

Table 3: Qualitative phytochemical test of the methanol leaf extract of *Curcuma caesia* Roxb.

S.No	Phytochemical	Test name	Observation
1.	Alkaloids	Mayer's test	Positive
2.	Saponins	Foam test	Positive
3.	Glycosides	Alkaline reagent test	Positive

4.	Phenols	Ferric Chloride test	Positive
5.	Flavonoids	Alkaline Reagent Test	Positive
6.	Steroids	Salkowski's test	Positive
7.	Tannins	Braymer's test	Positive
8.	Carbohydrates	Molisch's reagent	Positive
9.	Protein	Millon's Test	Positive
10.	Amino acids	Ninhydrin test	Positive
11.	Terpenoids	Salkowski's test	Positive

4.4 Antibacterial Activity of extract

The antibacterial activity of *Curcuma caesia* leaf extract against six bacterial pathogens was evaluated using the agar well diffusion method. The results are presented in Table 4. The methanolic leaf extract (80 mg/ml) showed moderate antibacterial activity against all the tested bacterial strains. The highest zone of inhibition was observed against *Bacillus subtilis* and *Klebsiella pneumoniae* (9.00 ± 0.55 mm), followed by *Salmonella typhi* (7.50 ± 0.44 mm) and *Enterococcus faecalis* (7.00 ± 0.66 mm). Lower inhibitory activity was recorded against *Escherichia coli* (6.30 ± 0.22 mm) and *Pseudomonas aeruginosa* (6.15 ± 0.10 mm). In

comparison, the standard antibiotic streptomycin sulphate exhibited significantly higher antibacterial activity against all the tested pathogens with inhibition zones ranging from 15.0 ± 0.10 mm to 24.0 ± 1.52 mm. These results indicate that *Curcuma caesia* leaf extract possesses antibacterial potential against both Gram-positive and Gram-negative bacteria. The zone of inhibition observed against *Klebsiella pneumoniae* is shown in Fig. 3. and the comparative antibacterial activity of *Curcuma caesia* Roxb. leaf extract and streptomycin sulphate against the tested bacterial strains is illustrated in Fig 4.

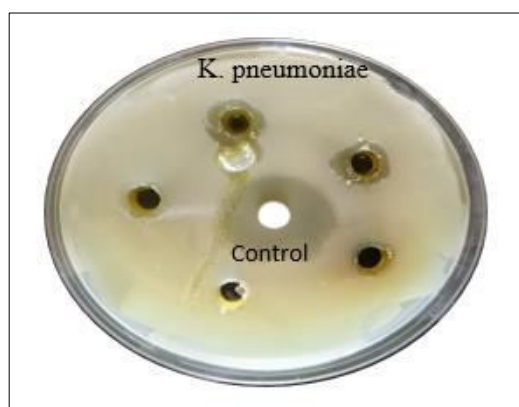


Fig 3: Antibacterial activity of *Curcuma caesia* leaf extract showing zone of inhibition against *Klebsiella pneumoniae* bacterial pathogen

Table 4: Antibacterial activity of *Curcuma caesia* leaf extract against six bacterial pathogens

Plant extract	Zone of inhibition (mm) of plant extract (80 mg/ml)					
	<i>B. subtilis</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>
<i>C. caesia</i> leaf	9.00 ± 0.55	6.30 ± 0.22	7.00 ± 0.66	6.15 ± 0.10	9.00 ± 0.55	7.50 ± 0.44
Streptomycin sulphate	24.0 ± 1.52	15.30 ± 0.15	16.00 ± 0.55	21.80 ± 0.20	15.0 ± 0.10	22.00 ± 1.52

Data is Mean $\pm$ S.D. of three replicates

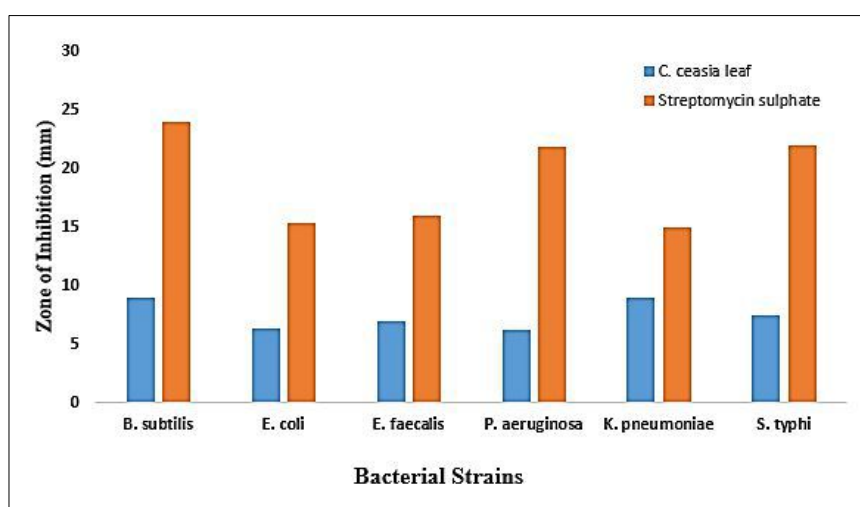


Fig 4: Graphical representation of antibacterial activity (zone of inhibition) of *Curcuma caesia* leaf extract and Streptomycin sulphate against six bacterial pathogens

5. Conclusions

The present study highlights the phytochemical composition and antibacterial potential of the methanolic leaf extract of *Curcuma caesia* Roxb. Physicochemical evaluation revealed an extractive yield of 18%, total ash content of 4%, and moisture content of 14%, indicating acceptable quality and standardization parameters of the plant material. Qualitative phytochemical screening confirmed the presence of diverse bioactive constituents including alkaloids, flavonoids, phenols, glycosides, saponins, tannins, steroids, terpenoids, carbohydrates, proteins, and amino acids, suggesting that the leaves of *Curcuma caesia* are a rich source of phytochemicals with potential biological activity.

The antibacterial assay demonstrated that the methanolic leaf extract exhibited moderate inhibitory activity against both Gram-positive and Gram-negative bacterial pathogens, with zones of inhibition ranging from 6.15 ± 0.10 mm to 9.00 ± 0.55 mm. Although the activity was lower than that of the standard antibiotic streptomycin sulphate, the observed antibacterial effect indicates the presence of naturally occurring antimicrobial compounds in the plant extract.

Overall, the findings of this study suggest that *Curcuma caesia* leaves possess significant phytochemical diversity and promising antibacterial properties, supporting their traditional medicinal use. The results also indicate that the leaves, which are relatively less explored compared to the rhizome, may serve as an alternative and sustainable source of bioactive compounds. Further studies involving quantitative phytochemical analysis, isolation and characterization of active constituents, and advanced pharmacological evaluations are required to fully explore the therapeutic potential of this plant.

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