INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
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Vol. 27, Issue: 3, p. 102-106, 2025

RESEARCH PAPER OPEN ACCESS

Assessment of antimicrobial properties of *Curcuma caesia* Roxb. rhizome extracts against pathogenic microorganisms

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Key words: Zingiberaceae, Cucuma, Antimicrobial, Rhizome

DOI: https://dx.doi.org/10.12692/ijb/27.3.102-106 Published: September 13, 2025

ABSTRACT

Curcuma caesia Roxb is a rhizomatous herb of the Zingiberaceae family, which is traditionally used in ethnomedicine for treating infections, wounds, inflammation, and respiratory disorders. The present study assessed the antimicrobial activity of successive solvent extracts of *C. caesia* rhizomes using agar well and disc diffusion assays against *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Candida albicans,* and *Penicillium chrysogenum*. Among the extracts, ethyl acetate exhibited the strongest antimicrobial activity, with ZOI ranging from 4.6 ± 1.15 mm to 19.3 ± 0.57 mm in the well diffusion method and 8.6 ± 0.57 mm to 15.6 ± 0.57 mm in the disc diffusion method, followed by methanol, which showed moderate but consistent inhibition. Acetone and aqueous extracts displayed relatively weak and selective activity, being effective only against a few test organisms. Statistical analysis (ANOVA) confirmed significant differences among extracts ($p < 0.05$). These findings support the ethnomedicinal claims of *C. caesia* and highlight the ethyl acetate fraction as a promising candidate for the development of plant-based antimicrobial agents.

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INTRODUCTION

Curcuma caesia Roxb., commonly known as black turmeric, is a perennial rhizomatous herb of the family Zingiberaceae, widely distributed in North-East India and valued for its ethnomedicinal importance. Traditionally, the rhizomes have been employed by indigenous communities to treat ailments such as wounds, inflammation, skin infections, asthma, hemorrhoids, and snake bites, highlighting their significance in folk medicine (Kumar *et al.*, 2013; Lalhminghlua and Lalramnghinglova, 2011). This rich ethnobotanical knowledge underscores its potential as a reservoir of bioactive compounds.

Phytochemical investigations of *C. caesia* have revealed the presence of a diverse array of secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, tannins, and essential oils, which contribute to its broad spectrum of pharmacological activities (Nadkarni and Nadkarni, 2007; Singh *et al.*, 2011). The vital oils of the rhizome are reported to contain camphor, ar-turmerone, curcumene, 1,8-cineole, and borneol, compounds known for antimicrobial and antioxidant properties (Das *et al.*, 2012). Such phytochemical diversity provides a biochemical basis for its traditional therapeutic uses.

With the alarming rise of multidrug-resistant pathogens, the search for novel antimicrobial agents from plant sources has gained urgency (Dan, 2023). Crude plant extracts, being a complex mixture of bioactive molecules, often display synergistic effects that may provide broader efficacy than isolated compounds (Cowan, 1999). Therefore, assessing the antimicrobial potential of crude extracts from different ethnomedicinal plants like *C. caesia* is crucial to validate their ethnomedicinal claims and to explore new leads for drug discovery. Standardized antimicrobial assays, such as agar well diffusion provide scientific evidence of their efficacy against clinically significant pathogens (Parekh and Chanda, 2007).

MATERIALS AND METHODS

The fresh rhizomes of *C. caesia* were collected from different areas of the Upper Brahmaputra Valley Zone of Assam. They were carefully washed to remove adhering dirt, cut into small pieces, and shade-dried. The well-dried rhizomes were then ground into powder and stored separately in properly labeled airtight containers.

Successive solvent extraction of rhizome powder of *C. caesia* was carried out in a Soxhlet apparatus using solvents of increasing polarity, namely n-hexane and ethyl acetate (non-polar), acetone (dipolar), and ethanol and methanol (polar). A total of 200 g of powdered rhizome was extracted with each solvent at 50 °C for 5 h. After each extraction, the insoluble marc was dried before subjecting it to the next solvent of higher polarity. The extracts obtained were concentrated by evaporation, and the percentage yield was calculated for each solvent system. All extracts were stored at 4 °C until further use.

Antibacterial activity was tested against *B. subtilis*, *S. aureus*, *E. faecalis* (Gram-positive), and *E. coli* (Gram-negative), while antifungal activity was evaluated against *C. albicans* and *P. chrysogenum*. Extracts were assessed using agar well diffusion and disc diffusion methods. In agar well diffusion, 15 mg/ml of each extract was prepared in DMSO, inoculated on nutrient agar/PDA plates, and zones of inhibition were recorded after 24 h incubation at 37 °C, with ciprofloxacin and amphotericin as positive controls. In disc diffusion, extracts (1000 µg/disc) were applied on inoculated nutrient agar/PDA plates, with streptomycin and fluconazole serving as standards. Antimicrobial efficacy was determined by measuring inhibition zones, with all assays performed in triplicate and mean values considered for analysis.

RESULTS AND DISCUSSION

The antimicrobial activity of *Curcuma caesia* rhizome extracts varied significantly depending on the extraction solvent (Tables 1–4; Figs. 1–4).

In the agar well diffusion assay, the ethyl acetate extract exhibited the broadest and most potent antimicrobial spectrum, with inhibition zones ranging from 4.6 ± 1.15 mm to 19.3 ± 0.57 mm against all tested organisms (*B. subtilis, S. aureus, E. faecalis, E. coli, C. albicans,* and *P. chrysogenum*) (Fig. 1).

Table 1. Statistical comparison of mean antimicrobial activity of different extracts of rhizome of *C. caesia* (Agar well diffusion assay)

Extract	Mean	Std. Deviation	Std. Error	95% confidence interval for mean	
				Lower bound	Upper bound
Ethyl acetate	90.66666667	4.16333	2.4037	80.3244	101.009
Methanol	75.33333333	2.51661	1.45297	69.0817	81.5849
Water	28.66666667	0.57735	0.33333	27.2324	30.1009
Acetone	15	1	0.57735	12.5159	17.4841

Table 2. Statistical comparison (ANOVA) of antimicrobial activity of different extracts of rhizome of *C. caesia* (Agar well diffusion assay)

	Sum of Squares	df	Mean Square	F	Sig
Between groups	11856.9	3	3952.31	632.369	0.000
Within groups	50	8	6.25		
Total	11906.9	11			

Table 3. Statistical comparison of mean antimicrobial activity of different extracts of rhizome of *C. caesia* (Disc diffusion assay)

Extract	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
				Lower bound	Upper bound
Ethyl acetate	77.3333	1.52753	0.88192	73.5388	81.1279
Methanolic	54	4.58258	2.64575	42.6163	65.3837
Water	27.3333	0.57735	0.33333	25.8991	28.7676
Acetone	10	1	0.57735	7.51586	12.4841

Table 4. Statistical comparison (ANOVA) of antimicrobial activity of different extracts of rhizome of *C. caesia* (Disc diffusion assay)

	Sum of Squares	df	Mean Square	F	Sig
Between groups	7894.33	3	2631.44	426.721	0.000
Within groups	49.3333	8	6.16667		
Total	7943.67	11			

Methanolic extract also showed strong and broad-spectrum activity, with ZOI values between 4.3 ± 0.57 mm and 16.3 ± 0.57 mm, though slightly lower than ethyl acetate. By contrast, acetone and aqueous extracts were weaker, with acetone active only against *B. subtilis, E. faecalis,* and *E. coli,* while aqueous extract showed small but measurable inhibition against most organisms except *C. albicans*. Statistical comparison confirmed significant differences in activity among solvents (Table 1; Table 2, $p < 0.001$).

The disc diffusion assay results mirrored these findings (Fig. 3). Ethyl acetate extract again showed the strongest inhibition across all pathogens (ZOI 8.6 ± 0.57 to 15.6 ± 0.57 mm), followed by methanolic extract, which was active against five of six microorganisms but lacked activity against *P. chrysogenum*. Acetone displayed limited activity, while aqueous extract produced only weak inhibition. ANOVA confirmed significant solvent-dependent differences in antimicrobial potential (Table 3; Table 4, $p < 0.001$).

The consistently superior performance of the ethyl acetate extract (Figs. 2 and 4) is likely due to its semi-polar nature, enabling efficient extraction of bioactive compounds such as sesquiterpenes, curcuminoids, and flavonoids that possess strong antimicrobial properties. This trend is consistent with reports on other Curcuma species. For example, *Curcuma longa* extracts prepared with ethyl acetate showed greater antibacterial potency compared to methanol and water extracts (Kim *et al.*, 2005). Similarly, *Curcuma xanthorrhiza* demonstrated the highest antimicrobial efficacy in its ethyl acetate fraction, compared to methanol and n-hexane fractions (Haroen *et al.*, 2025).

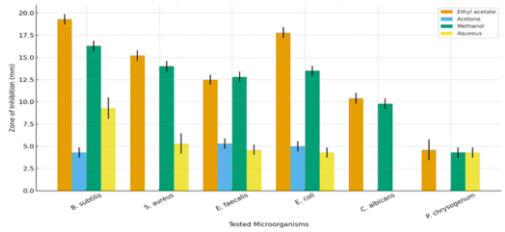

Fig. 1. Antimicrobial activity of *C. caesia* rhizome extracts (Well diffusion assay)

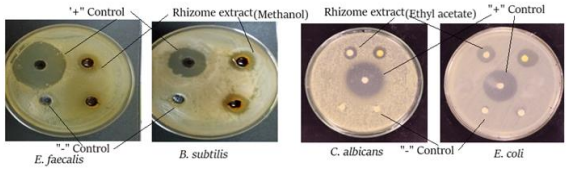

Fig. 2. Antimicrobial activity of different crude extracts of the rhizome of *C. caesia*

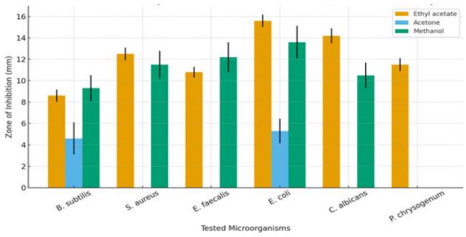

Fig. 3. Antimicrobial activity of *C. caesia* rhizome extracts (Disc diffusion assay)

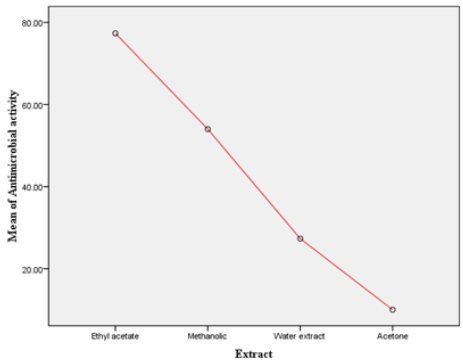

Fig. 4. Mean antimicrobial activity of different extracts of *C. caesia*

The relatively low activity of acetone and aqueous extracts may be attributed to the poor solubility of lipophilic antimicrobial compounds in these solvents or the inefficient extraction of key phytoconstituents. These results underscore the importance of solvent selection in phytochemical extraction for antimicrobial testing.

Overall, the findings confirm that ethyl acetate extract of *C. caesia* rhizome possesses the most effective and broad-spectrum antimicrobial activity, followed by methanolic extract, while acetone and aqueous extracts are markedly less active. These results highlight the therapeutic potential of *C. caesia* in managing pathogenic infections, particularly due to the strong inhibition observed against both Gram-positive and Gram-negative bacteria, as well as fungi.

CONCLUSION

The present investigation clearly demonstrates that the antimicrobial efficacy of *C. caesia* rhizome is strongly influenced by the choice of extraction solvent. Among the tested extracts, the ethyl acetate fraction consistently exhibited the strongest and broadest spectrum of activity against both bacterial and fungal pathogens in agar well and disc diffusion assays.

This superior effect can be attributed to the solvent's ability to extract semi-polar bioactive constituents such as sesquiterpenes and flavonoids, compounds widely reported for their antimicrobial potency. Methanolic extracts also showed substantial inhibitory effects against most of the test organisms, though their activity was comparatively lower than ethyl acetate.

In contrast, acetone and aqueous extracts were less effective, with antimicrobial activity restricted to a few strains. The observed differences were statistically significant, underscoring the non-homogeneity of antimicrobial responses across solvents. The results validate the traditional use of *C. caesia* in folk medicine and highlight the potential of its ethyl acetate and methanol extracts as a promising source of natural antimicrobial agents.

Further work focusing on the isolation and characterization of active compounds will be essential to develop new therapeutic leads against drug-resistant pathogens.

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