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RESEARCH ARTICLE

Polarity-driven extraction revealed potent bioactivities in rhizomes and leaves of *Curcuma caesia* Roxb.

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Abstract

The increasing demand for plant-based bioactive compounds has fueled interest in exploring natural antioxidants and antimicrobial agents. *Curcuma caesia* Roxb., commonly known as black turmeric, holds significant potential as a source of natural antioxidants and antimicrobial agents. This study investigated the antioxidant and antimicrobial potential of sequential extracts from *C. caesia* rhizomes and leaves, utilizing solvents of varying polarity (n-hexane, chloroform, ethyl acetate, methanol and water). The extraction yields varied between 1.69% and 6.34%, with n-hexane providing the highest yield of 6.34% for leaf extracts and 5.9% for rhizome extracts. Chloroform extracts were particularly rich in phenolics (total phenolic content: 95.17 ± 0.15 mg GAE/g for leaves and 84.16 ± 0.20 mg GAE/g for rhizome) and flavonoids (total flavonoids content: up to 75.98 ± 2.00 mg quercetin/g for leaves and 56.89 ± 0.15 mg quercetin/g for rhizomes). Antioxidant activity, determined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, showed the strongest results in chloroform extracts, with IC_{50} values as low as 0.75 ± 0.02 µg/mL for leaves. Additionally, nonpolar solvent extracts (n-hexane and chloroform) demonstrated significant antimicrobial activity against multidrug-resistant strains like *Klebsiella pneumoniae* and *Staphylococcus aureus*, with minimum inhibitory concentration (MIC) values as low as 3.12 µg/mL, comparable to standard antibiotics. These findings highlight *C. caesia* as a promising source of bioactive compounds for future phytopharmaceutical applications.

Keywords

antimicrobial activity; antioxidant activity; *C. caesia*; solvent extraction; total flavonoid content; total phenolic content

Introduction

The emergence of consumer interest in plant-derived phytochemicals has surged, driven by a growing emphasis on environmental sustainability over convenience (1). Herbs are rich in diverse active phytoconstituents with various pharmacological effects, each having distinct metabolic and binding properties (2). Secondary metabolites in plants, including phenols, terpenoids, flavonoids and alkaloids, play vital roles in plant defense and serve as valuable sources of microbicides, antioxidants and other pharmaceutical compounds (3).

Curcuma caesia Roxb., or black turmeric, is a lesser-known medicinal plant of the Zingiberaceae family. Its rhizomes are traditionally used to treat bronchitis, asthma, tuberculosis, menstrual disorders and cancer, among other ailments (4). The leaves are used for conditions such as furunculosis, leukoderma, piles, tumors, lymphangitis and adenitis (4). Rhizomes are

reported to possess diverse pharmacological properties, including antifungal, antioxidant, analgesic, antibacterial and anti-ulcer effects (5). Although *C. caesia* is endangered, its unexplored leaf extracts hold promise as sources of natural antioxidants and antimicrobial agents.

The extraction method of plant materials significantly influences their inhibitory potential against microbes (6). Sequential extraction has emerged as a preferred technique for isolating active components from natural sources. This method employs solvents of varying polarities, optimizing extraction efficiency and enhancing biological activity compared to direct solvent extraction (7). Solvents with different polarities significantly impact the total phenolic and flavonoid content, as well as the antimicrobial and antioxidant activities of plant extracts.

Free radicals, unstable molecules with unpaired electrons, contribute to degenerative diseases such as cancer, inflammation and neurodegenerative disorders. Antioxidants counteract by inhibiting or delaying oxidation, thus preventing damaging chain reactions (8). Concerns about the carcinogenic risks of synthetic antioxidants have increased interest in natural plant-based antioxidants, which are safer and exhibit low cytotoxicity, making them ideal for food preservation (9). These natural antioxidants not only protect plants but also offer nutritional and therapeutic benefits to humans by neutralizing free radicals and reactive oxygen species (ROS) (10). Epidemiological studies support the health benefits of consuming antioxidant-rich plant foods, which are associated with a reduced risk of oxidative stress-related diseases (11, 12).

Phenolic compounds, in particular, are essential due to their electron-donating properties and their role in forming stable radicals. Phenolics not only neutralize free radicals in plant extracts but also offer protective benefits when consumed, aiding in the prevention of diseases such as cardiovascular issues and cancer (13). Furthermore, the global polyphenol market is projected to reach $1.82 billion by 2025, with a growth rate of 7.44% from 2020 to 2025, driven by increasing awareness of diet-health relationships and the role of antioxidants in slowing aging (14). Single-electron transfer-based methods, such as DPPH, are preferred for their accuracy in assessing the reduction potential of antioxidants, making them crucial in the study of plant-derived phenolics (15).

The rising prevalence of various diseases has led to an increased reliance on allopathic medicines; however, their associated adverse effects have sparked renewed interest in plant-derived bioactive compounds, which offer a natural alternative for health management (9). This shift is further underscored by the overuse of antibiotics, which has driven the emergence of multidrug-resistant pathogens, thereby reducing the effectiveness of conventional treatments (16). Considering these challenges, this study aims to assess the antioxidant and antibacterial activities, along with the total phenolic content (TPC) and total flavonoid contents (TFC) of sequential extracts from the rhizomes and leaves of *C. caesia* obtained through Soxhlet extraction.

Materials and Methods

Plant materials

C. caesia rhizomes and leaves were collected from Nongpoh, Meghalaya, located at 91.8337°E longitude and 25.8699°N latitude, with an elevation of 554 meters (Fig. 1). The area experiences a local climate with a maximum temperature of 22.16°C, a minimum of 14.65°C and an annual rainfall of 554 mm. A taxonomist confirmed the plant's identification and the voucher specimen (no. 2482/CBT) was deposited in the herbarium and maintained in the greenhouse at Centre for Biotechnology (CBT), Siksha O Anusandhan (SOA) University.

Preparation of extract

Shade-dried rhizomes and leaves of *C. caesia* (30 g each) were sequentially extracted using 250 mL of solvents in the following order: n-hexane, chloroform, ethyl acetate, methanol and water. After each extraction step, the plant material was air-dried at room temperature before proceeding to the next solvent. The extracts were then filtered using Whatman filter paper and concentrated to dryness using a rotary flash evaporator (17). The yield of each extract was recorded and the extracts were stored at 4°C until further analysis. Prior to bioactivity evaluation, the extracts were dissolved in methanol at a concentration of 1 mg/mL (17).

DPPH radical scavenging activity

The DPPH radical scavenging activity of various rhizome and leaf extracts was evaluated as described in the protocol (18). Each extract was dissolved in methanol at concentrations ranging from 1 to 20 µg/mL and 1 mL of a 0.1 mM DPPH solution was added to each sample. After incubating the mixtures in the dark at room temperature at 30 minutes, the absorbance was measured at 517 nm using a UV-visible spectrophotometer (Thermo Scientific™ Evolution™ 201). Quercetin, gallic acid and ascorbic acid served as positive controls. The scavenging activity was calculated as a percentage of inhibition using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Ac- As}}{\text{Ac}} \text{ X } 100$$

Where, As is the absorbance of sample; Ac is the absorbance of control.

Fig. 1. *Curcuma caesia* Roxb. (a) rhizome (b) leaf.

This method effectively highlights the antioxidant potential of the extracts by measuring their ability to neutralize DPPH radicals, demonstrating their potential role in mitigating oxidative stress.

Antimicrobial activity

Bacterial strains: Multidrug-resistant (MDR) bacterial strains were obtained from the Department of Microbiology at Institute of Medical Sciences and SUM Hospital, Siksha 'O' Anusandhan University, Bhubaneswar. The strains included Gram-negative bacteria - *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* as well as Gram-positive *Staphylococcus aureus*.

Minimum inhibitory concentration

To evaluate the effectiveness of sequential rhizome and leaf extracts against MDR bacterial strains, the MIC was determined. This procedure followed the clinical and laboratory standards institute (CLSI, 2013) guidelines and the methodology outlined in the protocol (19). The broth microdilution method was used with Mueller-Hinton Broth (MHB) to assess the antibacterial activity against four MDR strains. Extracts were prepared by dissolving them in dimethyl sulfoxide (DMSO) to a concentration of 100 µg/mL. The bacterial cultures were adjusted to 10^6 CFU/mL. Extracts were serially diluted two-fold in MHB, resulting in concentrations ranging from 25 to 0.048 µg/mL and added to a 96-well microtiter plate. Each well received a bacterial suspension at 10^6 CFU/mL. The plates were then incubated for 24 hours at 37°C. Ampicillin was used as a positive control. After incubation, 5 µL of 0.5 M 2,3,5-tetrazolium chloride (TTC) was added to each well and incubated for an additional 30 minutes at 37°C. The MIC was identified as the lowest concentration of extracts that did not produce a color change, indicating no bacterial growth. Each MDR strain was tested in triplicate to ensure accuracy and consistency of the results.

Minimum bactericidal concentration

This study outlines the procedure for determining the minimum bactericidal concentration (MBC), which is defined as the lowest extract concentration required to kill 99.9% of the tested bacterial strains (9). The same microdilution method used for MIC determination was applied. After a 24-hour incubation period of the bacterial cultures in the microtiter plates, a loopful of culture from each well was streaked onto MHA plates. These plates were then incubated for an additional 24 hours at 37°C. The MBC was determined by inspecting the agar plates for visible bacterial growth. The absence of bacterial growth at a specific concentration was used to identify the MBC.

Agar well diffusion method

The antibacterial activity of various rhizome and leaf extracts was evaluated using the agar well diffusion method, following the established protocol (8). Bacterial suspensions at concentrations of 10^6 and 10^8 CFU/mL were spread onto MHA plates. Wells on the plates were then filled with extracts at concentrations of 25, 50 and 75 µg/mL, followed by incubation. Ampicillin served as the positive control. After 24 hours of incubation at 37°C, the plates were examined for inhibition zones around the wells for each bacterial strain. The antibacterial effect was assessed by measuring the diameter of the inhibition zones (in mm). To ensure reliability and consistency, each assay was performed in triplicate.

Total phenolic and flavonoid content

The total phenolic content (TPC) of *C. caesia* leaf and rhizome extracts was determined using the Folin-Ciocalteu method, following the established protocol, with gallic acid as the standard reference (20). Extracts were diluted to ensure they fell within the standard curve range (0.0 to 500 µg gallic acid/mL). In the procedure, 250 µL of either the diluted extract or gallic acid solution was added to a test tube along with 1 mL of distilled water and 250 µL of Folin-Ciocalteu reagent. After a 5-minute incubation at room temperature, 2.5 mL of 7% sodium carbonate solution was added and the total volume was adjusted to 6 mL with distilled water. The absorbance of the resulting blue solution was measured at 760 nm after 90 minutes. TPC was calculated from the gallic acid standard curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. All measurements were performed in triplicate.

To measure the total flavonoid content (TFC) of *C. caesia* leaf and rhizome extracts, a modified method based on the formation of flavonoid-aluminum complexes was used, following the established protocol (20). One milliliter of a 2% aluminum chloride ethanol solution was mixed with 1 mL of the extract and the mixture was incubated at room temperature in the dark for one hour. The absorbance was recorded at 420 nm using a UV-VIS spectrophotometer. TFC was determined using a quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract. All experiments were performed in triplicate to ensure accuracy.

Statistical analysis

Statistical analyses were performed to determine the mean extract yield and bioactivity of various sequential extracts from rhizome and leaves. One-way ANOVA, followed by Tukey's HSD test ($p<0.05$) at a 95% confidence interval, was conducted using MINITAB 17 software to calculate the mean values and assess the statistical significance of differences in extract yield, bioactivity and correlation of IC_{50} values of different antioxidant assays with polyphenolic and flavonoid content among the sequentially obtained extracts. This approach allowed for the identification of significant variations in yield and bioactivity across the extracts.

Results and Discussion

Extract yield, total phenolic and flavonoid content

C. caesia rhizome and leaf extracts were obtained through sequential solvent extraction, yielding 1.69-5.90% for rhizomes and 2.3-6.34% for leaves (dry weight basis). Hexane extracts had the highest yield in both rhizomes (5.90%) and leaves (6.34%), while methanol yielded the least (1.69% and 2.3%, respectively), with significant differences ($p<0.05$) among solvents (Table 1). This variation reflects the diverse polarities of the extraction solvents and

suggests a higher proportion of nonpolar compounds in the plant. The extraction process is crucial for evaluating the biological and pharmacological properties of plants. Solvent selection is critical for maximizing the extraction of biologically active phytochemicals, which vary in structure and effects. The present study employed a polarity-based solvent sequence to extract different phytochemical classes. Differences in extraction yields and subsequent antioxidant and antimicrobial activities emphasize the importance of solvent choice in determining biological and pharmacological outcomes. These findings align with previous studies demonstrating varying biological activities in extracts obtained with different solvents (21).

The TPC and TFC of *C. caesia* extracts were analyzed using five solvent fractions: *n*-hexane, chloroform, ethyl acetate, methanol and water. TPC for rhizomes ranged from 32.14 ± 0.15 mg GAE/g (water) to 84.16 ± 0.20 mg GAE/g (chloroform), while for leaves, it ranged from 42.15 ± 0.03 (water) mg GAE/g to 95.17 ± 0.15 mg GAE/g (chloroform). Chloroform extracts exhibited the highest phenolic content ($p<0.05$), followed by ethyl acetate and *n*-hexane extracts. The TFC ranged from 31.28 ± 0.20 (water) to 56.89 ± 0.15 (chloroform) mg quercetin/g for rhizomes and 39.16 ± 0.20 mg quercetin/g (water) to 75.98 ± 2.00 (chloroform) mg quercetin/g for leaves, with chloroform extracts showing significantly higher flavonoid content ($p<0.05$) (Table 2). The observed variation in TPC and TFC reflects the role of solvent polarity, with polar solvents more effective at extracting phenolic and flavonoid compounds. The efficiency of Soxhlet extraction, particularly when using solvents at their boiling points, enhances the recovery of these compounds. Phenolic compounds are efficient free radical scavengers, characterized by hydroxyl groups linked to aromatic rings (22).

Antioxidant activity

In this study, the *in vitro* antioxidant potential of sequential extracts from dried rhizomes and leaves of *C. caesia*, extracted using solvents of varying polarity, was assessed using DPPH radical scavenging assay. The antioxidant capacity of both rhizome and leaf extracts from *C. caesia* was evaluated using the DPPH radical scavenging assay, revealing high scavenging activities across different solvent extracts. Chloroform and ethyl acetate extracts exhibited stronger antioxidant activity (lower IC_{50} values) compared to methanol and aqueous extracts as depicted in the Table 3. Standard antioxidants, including quercetin, gallic acid and ascorbic acid, showed better scavenging potential with the lowest IC_{50} values (Table 3). The antioxidant potential, based on IC_{50} values, followed the decreasing order: chloroform > n -hexane > ethyl acetate > methanol > aqueous (Table 3). Particularly, the moderately polar solvent chloroform extracts exhibited the strongest antioxidant activity, with IC_{50} values of 1.02 ± 0.02 µg/mL for the rhizome and 0.75 ± 0.20 µg/mL for the leaf. This was followed by the nonpolar solvent *n*-hexane extracts, which also showed potent activity, with IC_{50} values of 1.47 ± 0.02 µg/mL for the rhizome and 1.26 ± 0.02 µg/mL for the leaf. These findings suggest that nonpolar solvents efficiently extract lipophilic antioxidant compounds, particularly from leaves, which showed superior performance in some cases compared to the rhizomes. In comparison, extracts using more polar solvents such as ethyl acetate, methanol and water showed progressively lower scavenging activities (Table 3). These observations highlight the influence of solvent polarity on the extraction efficiency of bioactive compounds. Furthermore, the antioxidant potential was benchmarked against standard compounds such as gallic acid (IC_{50}: 0.67 ± 0.10 µg/mL), quercetin (IC_{50}: 0.6 ± 0.20 µg/mL) and ascorbic acid (IC_{50}:4.21 ± 0.10 µg/mL). The chloroform and *n*-hexane extracts of leaves, in particular, displayed greater activity than ascorbic acid, while others were less effective than the pure compounds gallic acid and quercetin. This disparity may be due to the complex nature of the extracts, which

Table 1. Extract yield of rhizome and leaves of *C. caesia* Roxb.

Extract	Yield%	
	Rhizome	Leaf
n-hexane	5.9±0.02^{a}	6.34±0.02^{a}
Chloroform	5.1±0.01^{b}	5.86±0.02^{b}
Ethyl acetate	3.7±0.01^{c}	4.2±0.01^{c}
Methanol	1.69±0.01^{e}	2.3±0.15^{e}
Aqueous	2.5±0.15^{d}	3.2±0.15^{d}

Data are shown as mean ± standard deviation (SD) where *n*=3. Mean with different letter in a column were significantly different according to Tukey's HSD test at $p<0.05$.

Table 3. IC_{50} values of rhizome and leaf extracts of *C. caesia* Roxb. by DPPH radical scavenging assay

Extracts / positive controls		IC_{50} value (µg/mL)
n-hexane	Rhizome extract	1.47±0.02^{e}
	Leaf extract	1.26±0.02^{e}
Chloroform	Rhizome extract	1.02±0.02^{f}
	Leaf extract	0.75±0.20^{f}
Ethyl acetate	Rhizome extract	3.02±0.02^{d}
	Leaf extract	2.12±0.15^{d}
Methanol	Rhizome extract	6.58±0.02^{b}
	Leaf extract	4.33±0.20^{b}
Aqueous	Rhizome extract	12.68±0.10^{a}
	Leaf extract	10.26±0.15^{a}
Quercetin		0.6±0.20^{g}
Gallic acid		0.67±0.10^{g}
Ascorbic acid		4.21±0.10^{c}

Data are shown as mean ± standard deviation (SD) where *n*=3. Mean with different letter in a column were significantly different according to Tukey's HSD test at $p<0.05$. IC_{50}- half-maximal inhibitory concentration, µg/mL- microgram per millilitre.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of sequentially obtained *C. caesia* Roxb.

Extract	Rhizome		Leaf	
Extract	TPC (mg GAE/g)	TFC (mg QE/g)	TPC (mg GAE/g)	TFC (mg QE/g)
n-hexane	69.19±0.15^{c}	40.59±0.10^{c}	75.13±0.15^{c}	60.84±0.03^{c}
Chloroform	84.16±0.20^{a}	56.89±0.15^{a}	95.17 ± 0.15^{a}	75.98 ± 2.00^{a}
Ethyl acetate	78.35±0.15^{b}	46.65±0.15^{b}	88.15± 0.02^{b}	68.26±0.03^{b}
Methanol	53.74±0.02^{d}	37.19±0.10^{d}	58.12±0.02^{d}	49.23±0.05^{d}
Aqueous	32.14±0.15^{e}	31.28±0.20^{e}	42.15 ± 0.03^{e}	39.16± 0.20^{e}

Data are shown as mean ± standard deviation (SD) where *n*=3. Mean with different letter in a column were significantly different according to Tukey's HSD test at $p<0.05$. Total phenolic content (TPC) and total flavonoid content (TFC), mg GAE/g- milligrams of gallic acid equivalents per gram, mg QE/g- milligrams of quercetin equivalent per gram.

contain a mixture of compounds, whereas gallic acid and quercetin are pure compounds with well-defined antioxidant properties (23, 24).

The results from this study emphasize the significant antioxidant potential of *C. caesia* extracts, especially those obtained using chloroform as a solvent and support their potential use as natural alternatives to synthetic antioxidants. Further in-depth research, including *in vivo* studies, is warranted to fully explore the therapeutic applications of these extracts in combating oxidative stress-related conditions.

Antimicrobial activity

The antimicrobial efficacy of different solvent extracts of *C. caesia* rhizome and leaf was investigated against four MDR strains: three gram-negative bacteria (*A. baumannii*, *E. coli*, *K. pneumoniae*) and one gram-positive bacterium (*S. aureus*). MIC, MBC and inhibition zone assays were performed, with comparisons to the commercially available antibiotic, ampicillin.

The rhizome extracts demonstrated significant inhibitory activity against *A. baumannii*, with MIC and MBC values ranging from 3.12-25 µg/mL and 6.25-25 µg/mL, respectively. Leaf extracts exhibited superior antimicrobial activity against *K. pneumoniae* and *S. aureus*, with MIC values ranging from 3.12 to 25 µg/mL and MBC values from 6.25 to 25 µg/mL. In comparison, the antibiotic ampicillin showed MIC and MBC values of 32 µg/mL for *K. pneumoniae* and 8 µg/mL for *S. aureus*. These results indicate that the susceptibility of these bacterial strains to *C. caesia* leaf extracts is comparable to that of the conventional antibiotic ampicillin, particularly in the case of *K. pneumoniae*.

Non-polar solvent extracts, such as *n*-hexane and chloroform, from both the rhizomes and leaves showed significant antimicrobial activity across all tested bacterial strains. In contrast, the sequential aqueous extracts displayed some observable inhibitory effects, indicating that water-based extractions had a moderate impact on isolating antimicrobial compounds from *C. caesia*. This suggests that while the polar nature of the aqueous solvent extracted certain components, it may not have fully captured the bioactive compounds responsible for stronger antimicrobial activity.

No previous reports have assessed the antimicrobial potential of sequentially extracted *C. caesia* against the four MDR strains examined in this study. These results suggest that *C. caesia* leaf extracts, particularly those extracted using non-polar solvents, hold promise as natural antimicrobial agents, especially against *K. pneumoniae* and *S. aureus*, which are known for their resistance to conventional antibiotics like ampicillin.

The IZD results further indicated that the inhibitory effects of the extracts were concentration dependent. *n*-hexane and chloroform extracts of both rhizome and leaf of *C. caesia* exhibited antimicrobial activity even at 25 µg/mL against all test bacteria, highlighting their strong antibacterial properties (Table 4). Conversely, the aqueous extracts demonstrated limited antimicrobial activity, even at the highest tested concentration (75 µg/mL) (Table 4).

The methanolic extract showed moderately lower antimicrobial activity compared to other solvents. Previous studies demonstrated significant activity of *C. caesia* crude methanolic extract against *E. coli* (16, 17). The reduced activity in this study may be attributed to the sequential extraction process, where chloroform, with a lower polarity (0.259), likely dissolved most medium-polarity phytochemicals, leaving fewer active compounds for methanolic extraction. Additionally, the sequential extraction may have resulted in the loss of sterols, key terpenes with pharmacological activity, potentially contributing to the reduced efficacy of the methanolic extract. Furthermore, the variation in antimicrobial activity is likely influenced by multiple factors, including environmental conditions affecting plant growth, the type of solvent used and the sequential extraction method employed (25). The use of sequential extraction with solvents of increasing polarity is particularly advantageous, as it allows for the isolation of active compounds according to their solubility profiles while minimizing potential antagonistic effects between compounds in the extract (25).

In conclusion, the results underscore the potential pharmaceutical value of *C. caesia* extracts, particularly those obtained using non-polar solvents, in the development of novel antimicrobial agents. Further studies are warranted to isolate and characterize the specific bioactive compounds responsible for the observed antimicrobial effects.

Correlation between antioxidant activities and phenol/ flavonoid content

Statistical analysis was conducted to explore the correlation between the antioxidant potential and phenol/flavonoid content of various sequentially obtained extract fractions. The correlation analysis between TPC (mg GAE/g) and the antioxidant assay revealed strong negative relationships, indicating that higher TPC levels are associated with lower IC_{50} values and consequently, stronger antioxidant activity. Specifically, the correlation coefficients for TPC with the DPPH assay of rhizome and leaf are -0.956 and -0.950, respectively with *p*-value below 0.05, confirming statistical significance. These results suggest that TPC plays a crucial role in enhancing antioxidant capacity, as increased phenolic content consistently correlates with improved free radical scavenging assay (Table 5). The correlation analysis between TFC (mg QE/g) and the DPPH antioxidant assay revealed a moderate negative relationship across the board. Specifically, TFC showed a correlation coefficient of -0.810 and -0.797 with the DPPH assay of rhizome and leaf. This value suggests that as TFC increases, the IC_{50} values for these assays decrease, implying improved antioxidant activity. The results demonstrate that phenolic compounds (TPC and TFC) are strongly correlated with enhanced antioxidant activity. However, further research is necessary to fully establish the specific contribution and potential of TPC/TFC in antioxidant mechanisms.

Table 4. MIC (µg/mL), MBC (µg/mL) and inhibition zone diameter (mm) of sequentially obtained rhizome and leaf extracts of *C. caesia* Roxb.

Rhizome extract	Microorganisms								IZD (mm)											
	A. baumannii		*S. aureus*		*K. pneumoniae*		*E. coli*		*A. baumannii* (µg/mL)			*S. aureus* (µg/mL)			*K. pneumoniae* (µg/mL)			*E. coli* (µg/mL)		
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	25	50	75	25	50	75	25	50	75	25	50	75
n-hexane	3.12	6.25	6.25	6.25	12.5	25	6.25	6.25	18±0.15	20±0.03	23±0.04	15±0.03	17±0.03	20±0.02	18±0.02	19±0.02	21±0.02	17±0.05	19±0.03	22±0.02
Chloroform	3.12	6.25	3.12	6.25	12.5	12.5	12.5	12.5	20±0.15	23±0.02	27±0.02	19±0.05	24±0.02	27±0.02	22±0.15	25±0.03	28±0.01	21±0.05	25±0.04	28±0.01
Ethyl acetate	12.5	25	6.25	12.5	12.5	12.5	3.12	3.12	17±0.02	19±0.12	21±0.15	13±0.02	15±0.02	17±0.02	15±0.15	17±0.02	20±0.02	15±0.02	17±0.02	19±0.02
Methanol	12.5	12.5	6.25	6.25	12.5	25	12.5	12.5	14±0.15	16±0.20	18±0.02	9±0.02	11±0.02	12±0.15	11±0.15	13±0.02	16±0.02	11±0.02	13±0.05	15±0.02
Aqueous	25	25	25	25	25	25	25	25	12±0.02	14±0.20	15±0.01	7±0.15	8±0.02	9±0.03	10±0.15	11±0.02	12±0.02	8±0.05	9±0.03	11±0.02
Ampicillin	0.5	0.5	8	8	32	32	0.5	0.5	24±2.0	26±1.5	30±1.5	23±1.5	29±1.5	32±1.0	27±0.02	30±0.02	35±1.5	27±1.0	33±1.5	36±1.5

Leaf extract	Microorganisms								IZD (mm)											
	A. baumannii		*S. aureus*		*K. pneumoniae*		*E. coli*		*A. baumannii* (µg/mL)			*S. aureus* (µg/mL)			*K. pneumoniae* (µg/mL)			*E. coli* (µg/mL)		
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	25	50	75	25	50	75	25	50	75	25	50	75
n-hexane	6.25	6.25	3.12	6.25	3.12	6.25	6.25	6.25	22±1.0	23±1.5	24±1.0	24±1.0	25±1.0	27±1.0	23±1.5	27±1.0	31±1.0	21±1.0	22±1.0	25±1.0
Chloroform	3.12	3.12	3.12	3.12	6.25	12.5	6.25	12.5	24±1.0	26±1.0	28±2.0	26±1.0	29±1.0	31±1.0	25±1.5	28±2.0	32±1.0	23±1.0	25±1.5	28±1.0
Ethyl acetate	12.5	12.5	12.5	12.5	12.5	12.5	6.25	6.25	20±1.5	21±1.0	22±1.0	20±2.0	21±1.0	23±1.5	20±1.0	22±1.0	23±1.0	20±1.5	21±2.0	23±1.5
Methanol	12.5	12.5	6.25	6.25	12.5	25	12.5	12.5	15±2.0	16±1.5	17±1.0	17±1.5	19±1.0	20±1.0	18±1.5	20±1.0	21±1.0	18±1.0	19±1.0	21±2.0
Aqueous	25	25	25	25	25	25	25	25	11±2.0	12±1.0	14±1.0	10±1.0	12±1.0	13±1.5	14±1.0	16±2.0	18±1.5	11±1.5	13±1.0	14±1.5
Ampicillin	0.5	0.5	8	8	32	32	0.5	0.5	29±1.0	30±1.0	32±1.5	30±1.5	32±1.5	34±1.0	29±1.0	33±1.0	35±1.0	27±1.5	32±1.5	38±1.5

IZD-inhibition zone diameter, µg/mL– microgram per millilitre, MIC-minimum inhibitory concentration, MBC-minimum bactericidal concentration, mm-millimetre.

Table 5. Correlations between the IC_{50} values of antioxidant activity and phenolic and flavonoid content of *C. caesia* Roxb.

Assay (µg/mL)	TPC (mg GAE/g) Correlation Coefficient	TFC (mg QE/g) Correlation Coefficient
DPPH assay (rhizome)	-0.956 (p = 0.011)*	-0.810 (p = 0.096)
DPPH assay (leaf)	-0.950 (p = 0.013)*	-0.797 (p = 0.106)

Each value in the table is represented as Mean ± SD (n = 3). *indicates significance at p<0.05.

Total phenolic content (TPC) and total flavonoid content (TFC), mg GAE/g- milligrams of gallic acid equivalents per gram, mg QE/g- milligrams of quercetin equivalent per gram, DPPH - 2,2-Diphenyl-1-picrylhydrazyl.

Conclusion

With increasing technological advancement, reliance on commercial products persists, yet a growing need for greater awareness of nature's therapeutic potential remains. Medicinal plants, such as *C. caesia*, are invaluable reservoirs of bioactive compounds with notable medicinal and aromatic properties. This study reveals the potent antioxidant and antimicrobial activities of *C. caesia* rhizome and leaf extracts, particularly when extracted sequentially using solvents with varying polarities. Chloroform and *n*-hexane extracts displayed the highest antioxidant activity, outperforming synthetic antioxidants like ascorbic acid in some assays. Moreover, these extracts demonstrated substantial antimicrobial efficacy against multidrug-resistant strains, particularly *K. pneumoniae* and *S. aureus*, suggesting their promise as natural alternatives to synthetic drugs. The variations in bioactivity based on the solvent used confirm the importance of sequential extraction for maximizing the recovery of bioactive phytochemicals. Further research is required to isolate and identify the specific active compounds responsible for these effects and to explore their therapeutic potential through *in vivo* studies.

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Authors' contributions

JL contributed to writing the original draft, formal analysis, investigation, methodology and validation. BK and SS contributed to conceptualization, supervision, visualization, review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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