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RESEARCH ARTICLE

Comprehensive phytochemical profiling of *Curcuma caesia* Roxb. rhizome using HRLC-MS and GC-MS

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Abstract

Curcuma caesia Roxb., also called black turmeric, belonging to the Zingiberaceae family, is a rhizomatous herb and is widely known for its medicinal importance, such as for its antibacterial, anti-inflammatory and antioxidant properties. Despite its medicinal properties, the characterisation of secondary metabolites remains scarce. Total phenolic and flavonoid content were evaluated to validate the therapeutic value of the plant. This research also focuses on the profiling of the methanolic extract of *C. caesia* rhizomes using high-resolution liquid chromatography-mass spectrometry (HR LC-MS) and gas chromatography-mass spectrometry (GC-MS). Through the metabolite profiling, the study revealed more than 50 phytochemical compounds, including phenols, flavonoids, terpenoids, alkaloids and fatty acids. Extracts also showed elevated peak intensities, like curcumene, valine, cinnamic acid and (+)-ar-turmerone. The medicinal importance of *C. caesia* is highlighted by its unique phytochemicals, found via GC-MS and LC-MS analysis, which are also present in other *Curcuma* species as reported in the literature. These results bridge the gap between previous research studies and contemporary analytical science, suggesting the inclusion of this plant in cosmetic, nutraceutical and pharmaceutical advancements for high-value product development.

Keywords: *Curcuma caesia*; GC-MS; LC-MS; phytochemical profiling; secondary metabolites

Introduction

Medicinal plants have long been known and used for therapeutic purposes. They represent a rich source of secondary metabolites for drug development and discovery (1). *Curcuma caesia* Roxb., also known as black turmeric, belonging to the family of Zingiberaceae, is widely known for its varied pharmacological activities (2). It originates from the Indian subcontinent and Southeast Asia and has been used for antioxidant, anti-inflammatory and antimicrobial activities (3, 4). Despite its great importance in the history of ethnomedicine, scientific research into its phytochemical constituents is relatively rare. Recent advances in analytical technology have revolutionised the field of natural product chemistry, helping in molecular fingerprinting to provide a view of in-depth identities, structures and functions of phytochemicals of herbs, which further validates the use of herbs while producing knowledge in the creation of suitable nutraceuticals and pharmaceutical formulations (5–7).

The rhizome of *Curcuma caesia* is rich in diverse classes of bioactive compounds, including curcuminoids, essential oils, phenolics, flavonoids and terpenoids (8, 9). These phytoconstituents contribute not only to the plants characteristic pigmentation and aroma but also to its reported antioxidant, anti-inflammatory, antimicrobial and anticancer properties (10, 11). Owing to these pharmacological activities, *C. caesia* has been widely used in Ayurveda and traditional medicine systems for managing inflammatory disorders, respiratory ailments and metabolic dysfunctions. Despite its ethnomedicinal importance, comprehensive scientific characterisation of its phytochemical composition remains limited (12). The chemical diversity and dynamic variability in metabolite concentration, affected by environmental, geographical and extraction conditions, pose significant challenges for accurate identification and reproducible quantification (13). While related species such as *Curcuma longa* have been extensively investigated (14, 15). *Curcuma caesia* remains comparatively underexplored, resulting in a knowledge gap that restricts its integration into evidence-based therapeutic frameworks (16). Advanced analytical techniques are essential to address these limitations, such as liquid chromatography-mass spectrometry (LC-MS), which combines high-resolution chromatographic separation with precise mass-based molecular identification, enabling sensitive detection of structurally diverse and low-abundance metabolites in complex plant matrices (17–19).

Although preliminary phytochemical investigations of *C. caesia* have reported the presence of major secondary metabolites, a systematic and comprehensive LC-MS-based profiling that elucidates its broad chemical composition and identifies potential bioactive markers remains insufficiently documented (20–22). The present study addresses this gap by conducting a comprehensive LC-MS analysis of *Curcuma caesia* rhizome extracts to establish a detailed phytochemical profile and

identify major bioactive constituents. By correlating chemical composition with reported pharmacological potential, this work aims to strengthen the scientific foundation underlying its traditional applications and facilitate future therapeutic, nutraceutical and standardisation efforts. This study contributes not only to natural product research but also to the integration of traditional medicinal knowledge with modern analytical science, promoting evidence-based and sustainable application of black turmeric in contemporary healthcare systems.

Materials and Methods

Reagents and instrumentation

For total phenolic content (TPC) and total flavonoid content (TFC), the reagents used were of analytical grade. For the extraction process, methanol (Merck, Mumbai, India) was used as the primary solvent. Folin-ciocalteu reagent and a saturated sodium carbonate solution (75 g L^{-1}) were utilised for the assay, purchased from HiMedia, Mumbai, India, with gallic acid (Sigma-Aldrich, Mumbai, India) serving as a standard for phenolics. For the flavonoid assay, the $NaNO_2$ (HiMedia, Mumbai) was prepared in distilled water. NaOH was procured from HiMedia (Mumbai). Standard used for flavonoid detection, Quercetin, was procured from Sigma-Aldrich, Mumbai, India. For the phase separation during flavonoid quantification, chloroform was used, which was procured from HiMedia, Mumbai. While methanol, acetonitrile (ACN) and formic acid, which were used for LC-MS analysis, were procured from Merck, India. Ultrapure water, deionised water and double-distilled water were obtained from a Milli-Q purification system. Liquid chromatography-mass spectrometry analysis was done through the thermo scientific vanquish UHPLC system coupled to a Q exactive plus orbitrap mass spectrometer (Thermo Fisher Scientific, USA).

Sample preparation

Fresh rhizomes of *Curcuma caesia* were collected, washed properly with distilled water and then kept for shade drying for 3 days at room temperature (25–30 °C) until constant weight was achieved. After 4 days, the samples were then ground into fine powder using a mechanical grinder and stored in airtight containers until extraction. Thereafter, 30 g of ground powder was used for extraction with 300 mL of analytical-grade methanol using Soxhlet. The extraction was carried out for 30 hr (approximately 3–4 cycles per hr) at the temperature of methanol heating at 20–30 °C until the solvent in the siphon tube became colourless, indicating exhaustive extraction. The methanolic extract was then filtered using Whatman quantitative filter paper Grade 41 (pore size: 20 µm, diameter: 12.5 cm) and concentrated under reduced pressure using a rotary evaporator at 40 °C to remove residual methanol. The samples were then stored at -20 °C for further phytochemical analysis (23).

Phytochemical screening

Preliminary qualitative phytochemical screening of *Curcuma caesia* rhizome extracts was performed following standard protocols to identify major classes of secondary metabolites before advanced instrumental analysis (24). Alkaloids were assessed using Mayer's and Wagner's reagents, where the formation of cream or reddish-brown precipitates indicated positive reactions. Phenolic compounds were detected using the ferric chloride test (green to bluish-black colouration) and gelatin precipitation test. Flavonoids were screened using the alkaline reagent test (yellow colouration reversible upon acidification) and lead acetate test (yellow precipitate formation). Fixed oils and fats were evaluated using the filter paper spot test, where persistent translucent staining indicated their presence. These preliminary assays provided an initial qualitative indication of phytochemical classes, which were subsequently characterised in detail using GC-MS and LC-MS analysis.

Total phenolic content

Total phenolic content (TPC) of *Curcuma caesia* rhizome extracts was determined using the Folin-ciocalteu (FC) colourimetric method, with slight modifications (25). Methanolic and aqueous extracts were prepared at a concentration of 1 mg mL^{-1}. Methanol was selected as an extraction solvent due to its high efficiency in extracting medium-to-polar phenolic compounds, while aqueous extracts were included to reflect traditional preparation practices and evaluate solvent-dependent variation in phenolic yield. For the assay, 0.75 mL of extract solution was mixed with 0.5 mL of FC reagent and incubated for 5 min at room temperature. Subsequently, 0.4 mL of 7.5 % (w/v) sodium carbonate solution was added and the reaction mixture was incubated in the dark for 2 hr at room temperature to allow complete colour development. Absorbance was measured at 760 nm using a UV-Visible spectrophotometer (CL-1320 Chemiline) against a reagent blank containing solvent instead of extract. Gallic acid (20–120 µg mL^{-1}) was used to construct a calibration curve ($R^2 \geq 0.99$). Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE g^{-1} DW). All extract samples were analysed in triplicate within the same experimental run to ensure statistical comparability. Differences among solvent extracts were analysed using one-way ANOVA, with $p < 0.05$ considered statistically significant.

Total flavonoid content

Total flavonoid content (TFC) was estimated using the aluminium chloride colourimetric method (26). Extracts were prepared at a 1 mg mL^{-1} concentration. Briefly, 0.1 mL of extract was mixed with 30 µL of 5 % sodium nitrite ($NaNO_2$) solution and incubated for 5 min at room temperature. Subsequently, 0.2 mL of 1 mM NaOH and 0.34 mL of distilled water were added to the mixture to develop the chromogenic complex. Absorbance was measured at 510 nm using the same spectrophotometer against a solvent blank. Quercetin (20–120 µg mL^{-1}) was used to generate the standard calibration curve ($R^2 \geq 0.99$). Results were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE g^{-1} DW). Similar to the TPC assay, all extracts were evaluated simultaneously in triplicate to allow direct statistical comparison among solvent systems using one-way ANOVA ($p < 0.05$).

GC-MS analysis (gas chromatography with tandem mass spectrometry)

Volatile constituents of *Curcuma caesia* rhizome extract were analysed using a Scion 436-GC (Bruker) system coupled with a triple quadrupole mass spectrometer operating in electron ionisation (EI) mode at 70 eV. Chromatographic separation was achieved using a fused silica capillary column BR-5MS (5 % diphenyl/95 % dimethyl polysiloxane; 30 m × 0.25 mm i.e., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. A 2 µL sample was injected in split mode (10:1) with an injector temperature of 250 °C. The oven temperature program was as

follows: initial temperature of 110 °C (held for 2 min), ramped at 10 °C min^{-1} to 200 °C, then at 5 °C min^{-1} to 280 °C, with a final isothermal hold at 280 °C for 9 min. The total run time was 46 min. The mass spectrometer operated in positive EI mode with a scan range of m/z 50–500 Da and a scan interval of 0.5 sec. The ion source temperature was maintained at 250 °C and the inlet temperature was 280 °C. A solvent delay of 3 min was applied. Compound identification was performed by comparing mass spectra with entries from the NIST library and ICAR-Medicinal and Aromatic Plants Research database. The relative abundance of each compound was calculated based on peak area normalisation. All compounds were putatively identified based on spectral database matching without confirmation using authentic reference standards and are therefore classified as metabolomics standards initiative (MSI) level 2 identifications. Compounds exhibiting pharmaceutical-like or synthetic structural signatures were carefully reviewed. Where spectral similarity scores were low or structurally implausible for plant matrices (e.g., synthetic anticholinergics or morphinan derivatives), such annotations were considered tentative and interpreted with caution, acknowledging the possibility of database misannotation or background contamination. GC-MS analysis was performed at the food quality testing laboratory, medicinal and aromatic plants research, Anand, Gujarat, India (27).

LC-MS analysis (liquid chromatography with tandem mass spectrometry)

The LC-MS analysis was performed at the SAIF (sophisticated analytical instrument facility) of the Indian Institute of Technology Bombay, India. The column used for the chromatographic separation was C_{18} reverse-phase (150 × 2.1 mm, 1.7 µm particle size) at 40 °C. The mobile phase consisted of two solvents, A- 0.1 % formic acid in water and B- methanol. The constant solvent flow rate was maintained at 0.3 mL min^{-1}. The gradient program was set at: 0–2 min: 5 % solvent B, followed by a linear gradient of 95 % solvent B for 2–20 min, 20–25 min: hold at 95 % Solvent B and re-equilibrium at 5 % solvent B between 26–30 min. 10 µL of the sample was injected into the system. The total run time of the system was 35 min. The mass spectrometric detection was determined by using a heated electrospray ionisation (HESI) source in positive ion mode. The operating parameters were set as follows: a scan range of 105–1500 m/z, resolution of 70000 for full MS and 17500 for MS/MS, AGC target of 1×10^6 for full MS and 1×10^5 for MS/MS, maximum injection time of 100 ms, dynamic exclusion of 10 s and stepped normalised collision energy (NCE) of 30 eV. Raw data were processed using thermo scientific xcalibur software. Metabolite annotation was performed by comparing accurate mass, retention time and MS/MS fragmentation patterns against public databases, including ChemSpider and PubChem. All detected metabolites were putatively annotated based on high-resolution mass spectral matching without validation using authentic standards. Therefore, compound assignments correspond to MSI level 2 (putatively annotated compounds) or MSI level 3 (tentatively characterised compound classes), where applicable. Relative abundance was estimated using peak intensity and peak area normalisation. Compounds with structural features inconsistent with known plant secondary metabolites (e.g., synthetic pharmaceuticals) were critically evaluated. Such annotations were flagged as tentative and interpreted cautiously, considering potential misannotation due to isobaric compounds, in -source fragmentation, or database limitations (28).

Statistical analysis

All experiments were performed in triplicate (n = 3) and results are expressed as mean ± standard deviation (SD). For cell-based experiments (e.g., oleic acid-induced lipid accumulation assays), statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to compare treatment groups against the defined control group. In hepatoprotective assays, the oleic acid-treated group served as the disease control, while untreated cells served as the normal control. Extract-treated groups were statistically compared against the disease control to evaluate protective effects. For phytochemical quantification assays (TPC and TFC), one-way ANOVA was used to compare solvent extracts within the same experimental run. Where appropriate, Tukey's post hoc test was applied for pairwise comparison among extract groups. Differences were considered statistically significant at $p < 0.05$, with higher levels of significance indicated at $p < 0.01$. All statistical analyses and graphical representations were performed using GraphPad Prism software (version 8.4.2).

Results

Phytochemical screening

Qualitative phytochemical screening of *Curcuma caesia* rhizome extracts revealed the presence of multiple classes of secondary metabolites (Table 1). Alkaloids were detected through positive reactions in Mayers' and Wagners' tests, indicated by the formation of characteristic precipitates. Phenolic compounds were confirmed by a positive ferric chloride reaction and gelatin precipitation test. Flavonoids were detected through positive responses in both alkaline reagent and lead acetate tests. Fixed oils and fats were indicated by the formation of a persistent translucent spot in the filter paper test. Tannins were not detected under the experimental conditions employed.

Table 1. Preliminary investigation of phytochemical constituents in methanolic extract of the formulation

Phytochemical test	
Alkaloids	
Mayer test	+
Wagner test	+
Phenolic compounds	
$FeCl_3$ test	+
Gelatin test	+
Tannins	
Dilute iodine test	-
Flavonoids	
NaOH and acid test	+
Lead acetate test	+
Fixed oil and fats	
Spot test	+

Total phenolic content

Among all the extracts taken for testing, the methanolic extract [CC (MeOH)] showed significantly higher phenolic secretion when compared to other extracts (146.32 ± 2.14 mg GAE g^{-1}, ***$p < 0.001$) (Fig. 1). However, the aqueous [CC(AQ)] and hexane [CC(Hex)] extracts showed minimal levels of phenolic secretion at levels of 63.24 ± 1.86 and 67.45 ± 2.05 mg GAE g^{-1}, respectively. Additionally, the ethyl acetate [CC(Eth-Ac)] and chloroform [CC(Chl)] extracts showed the lowest phenolic secretion at the levels of 18.52 ± 0.92

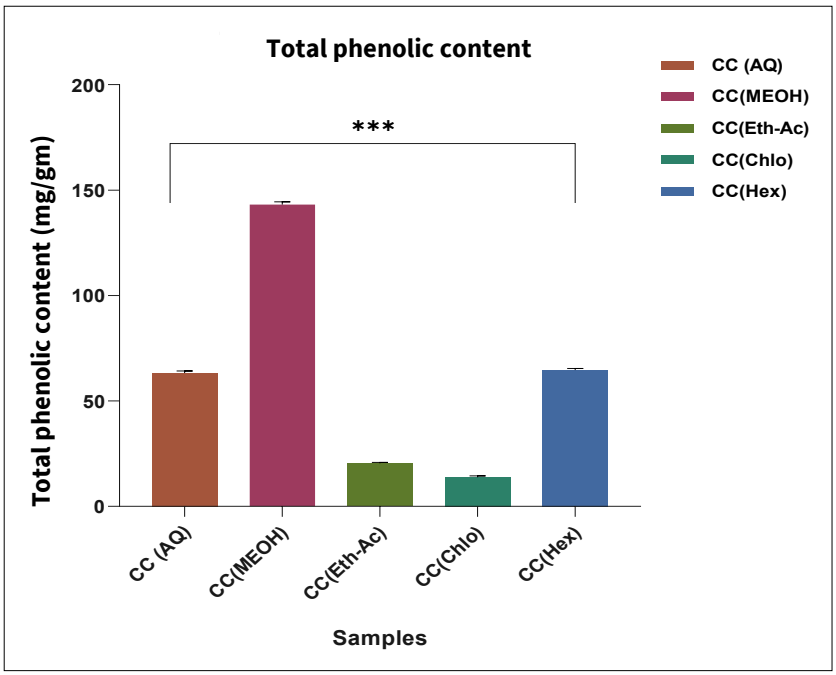

Fig. 1. Total phenolic content (TPC) of different solvent extracts of *Curcuma caesia* expressed as mg GAE g^{-1} dry weight. Among the extracts, the methanolic extract [CC(MeOH)] exhibited the highest phenolic content, followed by aqueous [CC(AQ)] and hexane [CC(Hex)] extracts, whereas ethyl acetate [CC(Eth-Ac)] and chloroform [CC(Chl)] extracts showed the lowest values. Data are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Dunnetts' test (**$p < 0.001$).

and 12.87 ± 0.75 mg GAE g^{-1}, respectively. The results showed that the polarity of the solvent had a significant impact on the metabolite extraction. Methanol demonstrated the highest efficiency in solubilising the phenolic compounds, because of its highest polarity compared to all other solvents. Statistical analysis using one-way ANOVA followed by appropriate post hoc testing confirmed significant differences among solvent extracts ($p < 0.05$).

Total flavonoid content

Among all the extracts for flavonoid testing, the chloroform extract [CC(Chlo)] showed the highest flavonoid content compared to all other extracts secreted at the level of 74.26 ± 1.54 mg QE g^{-1}. Similarly, the ethyl acetate extract [CC(Eth-Ac)] was also secreted in a good amount at the level of 63.14 ± 1.22 mg QE g^{-1}. However, the methanolic [CC(MeOH)] and aqueous [CC(AQ)] extracts showed mild secretion of flavonoids at the levels of 44.08 ± 1.05 and 35.42 ± 0.98 mg QE g^{-1}, respectively. Additionally, the hexane extract [CC(Hex)] demonstrated the least flavonoid secretion at the level of 10.63 ± 0.47 mg QE g^{-1}. The results highlighted that the semi-polar solvents have more efficiency in separating the flavonoid components compared to the polar solvents. Differences among solvent extracts were statistically significant ($p < 0.05$).

GC-MS analysis (gas chromatography with tandem mass spectrometry) analysis

The GC-MS analysis was generated within the time range between 0 and 46.5 min, as displayed in Fig. 2. The chromatogram showed various well-defined peaks in the early detection period between 1–5 min of retention period, corresponding to compounds such as leucine, isoleucine, valine and other amino acid derivatives, known

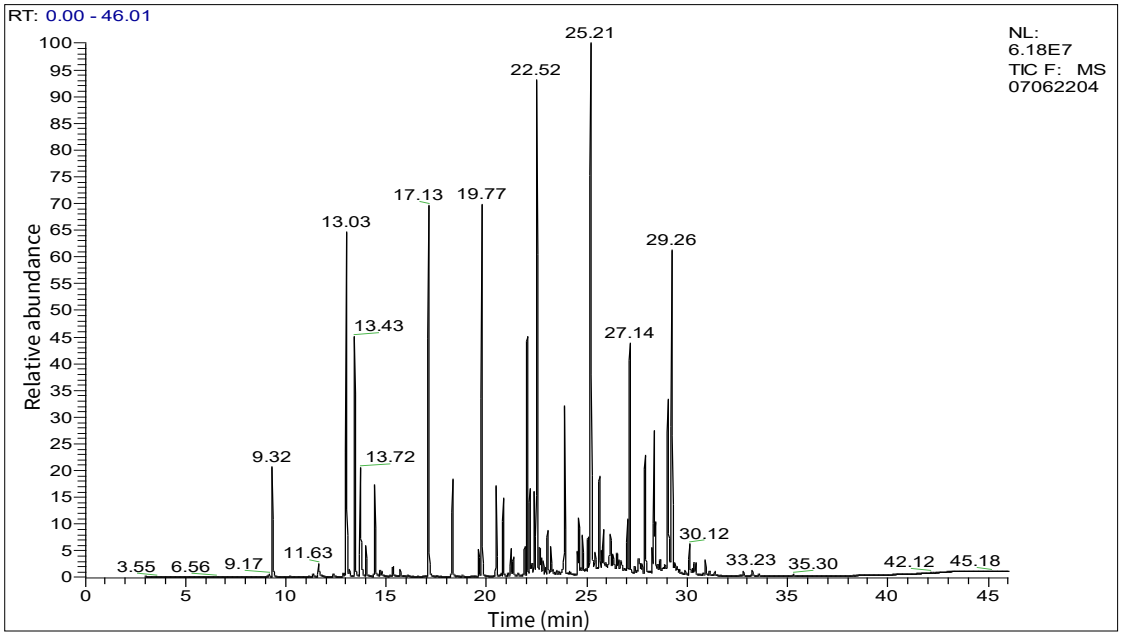

Fig. 2. Representative GC-MS chromatogram of the *Curcuma caesia* extract showing distinct peaks corresponding to different phytoconstituents identified across the retention time (RT) range of 0–46 min.

for their anticancer and antioxidant potential. Additionally, intermediate detection periods ranging between 10 and 20 min of retention periods also exhibited few peaks, representing few metabolites such as (+)-ar-turmerone, curcumene, guaiazulene and cinnamic acid, known for their antimicrobial, analgesic and hepatoprotective properties. The above-mentioned compounds were secreted in very high amounts, followed by other curcumene and turmerone derivatives, which were detected between the retention periods of 18–20 min. The last eluting periods, ranging between 25 and 46 min, showed low intensity compounds such as indole derivatives, naphthalene and other oxygenated hydrocarbons. These compounds were detected in min amounts; however, they too have important medicinal properties such as antiviral, neuroprotective and anti-diabetic properties. Overall, the chromatogram highlighted a wide spectrum of bioactive compounds ranging from phenolics and amino acids to flavonoids, alkaloids and terpenoids (Fig. 3).

Comparative results with the GC-MS chromatogram

Table 2 showed the list of compounds found in the GC-MS chromatogram, ranging between the retention times of 0–46.5 min. The major peak was seen at the retention times of 13.03, 19.77, 22.52, 25.21 and 29.26 min, which corresponded to camphor (10.29 %), caryophyllene (8.19 %), germacrone (11.79 %), curzerenone (13.26 %) and pyranophenanthrenol (6.52 %), respectively. The intermediate peaks corresponded to compounds such as norpinane (6.17 %), isoborneol (5.73 %) and isoledene (3.72 %). Himachalene, menthadienol and myrtenyl acetate were the compounds found in the min peak areas. The peak intensities in the chromatogram were mainly occupied by the compounds such as essential oils, where both monoterpenes, such as borneol, isoborneol, camphor and sesquiterpenes, such as curzerenone, caryophyllene, germacrone, were detected, suggesting their strong medicinal properties.

Table 2. Major compounds identified from GC-MS analysis and their relative abundance

Retention time (min)	Compound	Area (%)
9.32	Terpienol	1.76
11.63	Camphene	0.30
12.91	Myrtenyl acetate	0.10
13.03	Camphor	10.29
13.43	Isoborneol	5.73
13.72	Borneol	2.01
14.00	Terpienyl acetate	0.46
14.43	Carene	1.37
14.70	Myrtenol	0.11
15.34	Menthadienol	0.17
15.69	Cyclohexanol	0.15
17.13	Norpinane	6.17
18.31	Menthene	1.43
19.61	Humulene	0.39
19.77	Caryophyllene	8.19
20.48	Aristolene	1.28
20.83	Longifolene	1.13
21.22	Naphthalene	0.45
21.34	Seychellene	0.36
21.90	Cadinadiene	0.48
22.03	Isoledene	3.72
22.16	Valencene	1.42
22.38	Cyclopropanaphthalene	1.14
22.52	Germacrone	11.79
22.65	Guaiene	0.72
22.83	Himachalene	0.21
24.79	Guaiadiene	0.62
25.06	Elemene	0.57
25.21	Curzerenone	13.26
25.62	Cycloisolongifoline	1.88
25.83	Neoclovene	0.95
26.18	Himachaladiene	0.86
27.14	Longiverbenone	3.75
27.58	Cyclodecacycloteradecene	0.59
27.90	Verrucarol	2.01
28.36	Norethynodrel	3.27
29.04	Azulenofuranone	3.15
29.26	Pyranophenanthrenol	6.52

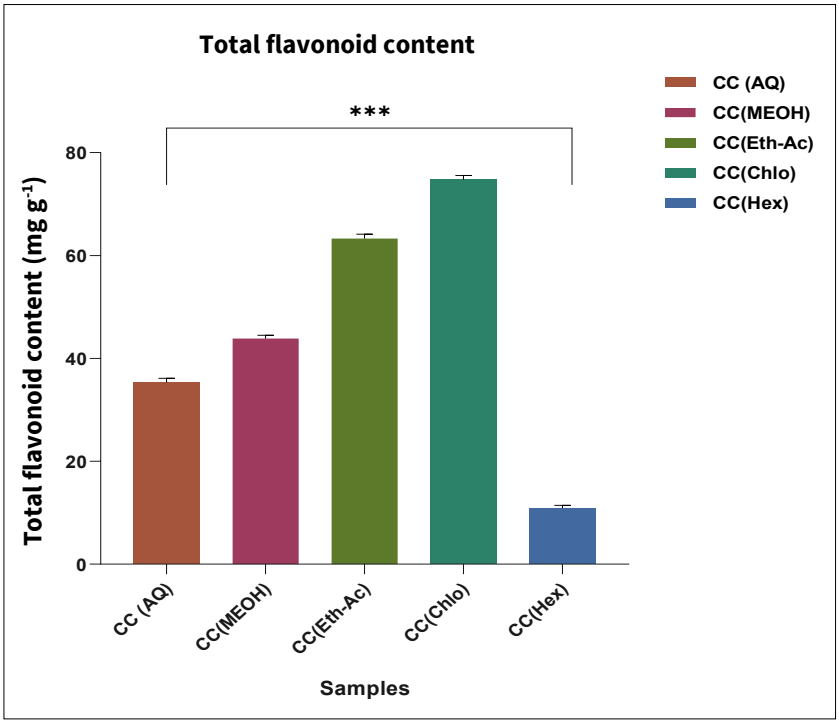

Fig. 3. Total flavonoid content (TFC) of different solvent extracts of *Curcuma caesia* expressed as mg QE g^{-1} dry weight. The chloroform extract [CC(Chlo)] exhibited the highest flavonoid content, followed by ethyl acetate [CC(Eth-Ac)], while the hexane extract [CC(Hex)] showed the lowest. Data are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Dunnett's test (**$p < 0.001$).

All compounds were putatively identified based on comparison of mass spectra with library databases (NIST and institutional libraries) without confirmation using authentic reference standards. Therefore, the identifications correspond to MSI level 2 annotations. Certain detected compounds exhibited structural similarity to synthetic or pharmaceutical molecules. Such annotations were carefully re-examined; however, given the absence of authentic standards and confirmatory fragmentation studies, these identifications remain tentative. Misannotation may arise due to- Isobaric compounds, similar fragmentation patterns, database matching limitations and background contamination. Accordingly, only compounds consistent with known plant-derived terpenoids and aromatic metabolites were emphasised in the primary analysis. No biological activity is experimentally validated in this study; therefore, any pharmacological relevance of detected compounds is discussed cautiously and based solely on previously reported literature.

LC-MS analysis (liquid chromatography with tandem mass spectrometry) analysis

The LC-MS chromatographic profile of the methanolic extract of *Curcuma caesia* is presented in Fig. 4. Multiple well-resolved peaks were detected in both positive and negative ionisation modes, indicating the presence of structurally diverse metabolites. In positive ionisation mode, prominent peaks were observed at retention times of 10.46, 11.70, 16.02, 16.33, 18.30 and 19.12 min, with the highest peak intensity recorded at 16.33 min. In negative ionisation mode, major peaks were detected at retention times of 10.17, 11.60, 15.63, 19.12, 22.92, 23.73, 24.34 and 29.24 min, with the most intense signals at 22.92 and 23.73 min. Putative metabolite annotation based on accurate mass, retention time and MS/MS fragmentation patterns suggested the presence of multiple chemical classes, including- amino acids and derivatives (e.g., valine, isoleucine, DL-stachydrine), phenolic acids and aromatic compounds (e.g., cinnamic acid derivatives, nicotinic acid), terpenoids and sesquiterpenoids (e.g., ar-turmerone, curcumene, guaiazulene), indole derivatives, sugars (e.g., maltose) and fatty acid derivatives (Table 3, 4). Several cyclic or peptide-like molecular features were also tentatively annotated; however, their structural identity requires further confirmation. The relative abundance of detected metabolites was estimated based on peak intensity and normalised peak area.

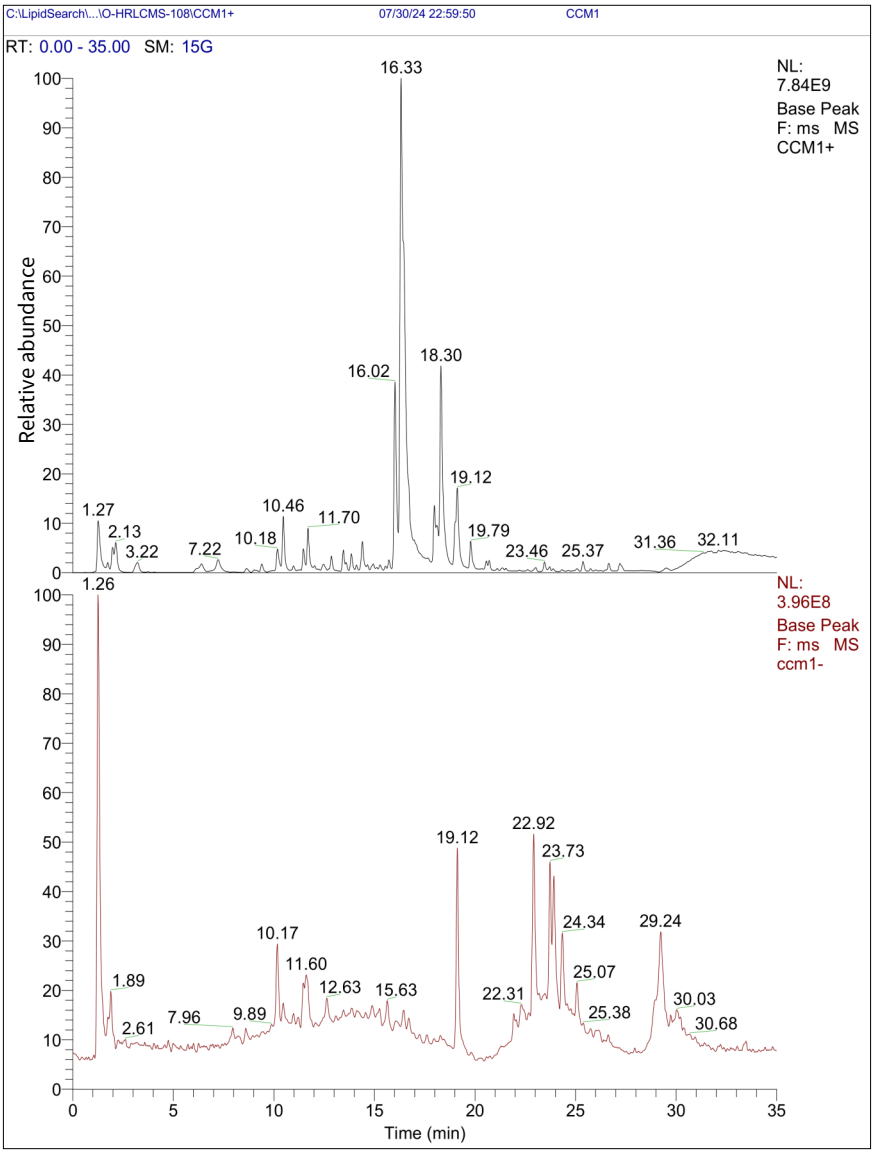

Fig. 4. LC-MS chromatograms of the methanolic extract of *Curcuma caesia* recorded in positive ionisation mode (top) and negative ionisation mode (bottom). Multiple peaks at different retention times indicate the presence of diverse phytochemicals, with major peaks detected at 16.33 min (positive mode) and 22.92–23.73 min (negative mode). The chromatograms highlight the abundance and complexity of metabolites extracted in methanol.

Table 3. LC-MS/MS-based identification of phytochemicals in the methanolic extract of *Curcuma caesia*

Compound	RT	MW	Peak area	DB diff (ppm)	Hits (DB)
Cyclo (isoleucylleucylisoleucylleucylleucyl)	19.113	565.41961	27149811178.2884	-1.25	1
NP-018534	10.46	266.15143	9803185093.45076	-1.44	170
(+)-ar-Turmerone	31.655	216.15108	9724174018.1603	-1.56	262
Valine	1.258	117.07896	9257000238.17272	-0.14	186
(+)-ar-Turmerone	18.149	216.15108	6308545236.38065	-1.55	262
Cinnamic acid	18.299	148.05226	5415353758.90884	-1.12	158
L-Norleucine	2.139	131.09451	5205400672.0705	-0.88	254
UV6260496	27.993	123.07968	4394880298.47278	0.29	161
Jasmonal	18.308	202.1354	4377869581.7755	-1.82	309
DL-Tryptophan	6.399	204.08963	4183082517.62851	-1.19	482
UV6260496	25.527	123.07968	4109954900.64749	0.29	161
5,6,7,8-Tetrahydro-1,4,5-naphthalenetriamine	7.218	177.12644	3693079239.57286	-0.91	110
(3R,3aR,11aR,13bR)-3a,11,11,13b-Tetramethyl-3-{(1S)-1-[(2S)-5-methyl-6-oxo-3,6-dihydro-2H-pyran-2-yl]ethyl}-2,3,3a,4,5,11,11a,13b-octahydroindeno[5',4':4,5] cyclohepta[1,2-c]oxepin-9(1H)-one	20.701	462.27667	3685463008.87085	-0.73	8
Isoleucine	1.977	131.09451	3110948012.04319	-0.88	254
(+)-ar-Turmerone	16.972	216.15108	3011665675.45725	-1.55	262
4-Phenylcyclohexanone	16.311	174.10419	2899966847.11381	-1.59	391
benactyzine	12.854	327.18303	2721629027.70689	-1.26	98
Isoleucine	1.415	131.09451	2629229862.61273	-0.87	254
1-Ethyl-3-pyrrolidinyl cyclopentyl(hydroxy)phenylacetate	16.118	317.1985	2437365156.6987	-1.87	75
D-(+)-Maltose	1.251	342.11539	2359991983.24549	-2.39	58
Curcumene	19.007	202.17196	2349757363.08845	-0.92	78
4,5,12-Trimethoxy-9-azatetracyclo[7.5.2.0~1,10~.0~2,7~]hexadeca-2,4,6-trien-3-ol	13.84	319.17779	2254741142.95905	-1.77	96
Curcumene	19.333	202.17197	2217463625.32045	-0.9	78
guaiazulene	16.321	198.14061	2191895750.91191	-1.21	78
2-Methyl-1-Tetralone	16.311	160.08854	2176388085.23718	-1.71	339
Cinnamic acid	3.214	148.05225	2026688545.6372	-1.21	158
Resorcinol diglycidyl ether	9.399	222.08903	1985057132.20206	-0.78	563
Naphthalene	16.466	128.06231	1777094476.70754	-2.26	27
3,4-Dihydroxybenzaldehyde	16.702	138.03147	1752136864.87524	-1.64	62
Valine	1.476	117.07896	1686548725.41796	-0.15	186
Cucurbitacin I	13.591	514.29277	1676169674.93126	-0.55	11
Indoline	3.219	119.07353	1655934752.27688	0.29	59
Valine	1.74	117.07897	1652100320.64349	-0.11	186
Methyl 3,10-dihydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydro-2-picenecarboxylate	18.075	480.28724	1625544482.21774	-0.69	9
trans-3-Indoleacrylic acid	6.237	187.0631	1599356509.17549	-1.24	354
3-Methoxy-17-methylmorphinan-4,14-diol	16.398	303.18291	1584661278.25822	-1.76	127
Gly-Arg	31.213	231.13344	1548393435.90479	1.3	9
guaiazulene	32.223	198.14075	1491804267.16268	-0.51	78
Cumene	16.324	120.09387	1439657932.576	-0.22	122
Ethyl 6-[2-(4-morpholinylmethyl)phenyl]-6-oxohexanoate	15.543	333.19353	1318895904.21432	-1.43	70
5,6,7,8-Tetrahydro-1,4,5-naphthalenetriamine	6.99	177.12644	1293461848.88836	-0.91	110
(3R,3aR,11aR,13bR)-3a,11,11,13b-Tetramethyl-3-{(1S)-1-[(2S)-5-methyl-6-oxo-3,6-dihydro-2H-pyran-2-yl]ethyl}-2,3,3a,4,5,11,11a,13b-octahydroindeno[5',4':4,5] cyclohepta[1,2-c]oxepin-9(1H)-one	20.566	462.2765	1290826075.2326	-1.1	6
3-Ethyl-o-xylene	16.325	134.10935	1272292707.38669	-1.52	179
(2R)-4-Methyl-2-({[(3S,4R,5R)-2,3,4,5-tetrahydroxytetrahydro-2H-pyran-2-yl] methyl}amino)pentanoic acid (non-preferred name)	2.007	293.14692	1264424850.31403	-1.81	3
Indane	16.396	118.07827	1263044539.44165	0.14	47
NP-018534	10.951	266.15141	1250660347.48057	-1.48	170
Tetralin	16.465	132.09378	1154238065.49427	-0.89	108
DL-Stachydrine	1.303	143.09436	1143261875.14271	-1.87	423
Indole	16.321	117.05786	1114352360.66984	0.12	45
Nicotinic acid	1.737	123.03199	1100130309.21659	-0.3	39

Compounds were identified based on database matching, retention time (RT), molecular weight (MW), peak area and mass accuracy (Δ ppm). The results indicate the presence of amino acids, phenolic acids, terpenoids, alkaloids and cyclic peptides.

Table 4. Medicinal activities of bioactive compounds identified through GC-MS analysis, along with their retention time (RT), molecular weight (MW)

Medicinal activity	Compound(s)	RT (min)	MW	Reference
Antioxidant	Cyclo(isoleucylleucylleucylleucylleucyl), Cucurbitacin I, Curcumene, Guaiazulene, UV6260496, 5,6,7,8-Tetrahydro-1,4,5-naphthalenetriamine	19.11, 13.59, 19.00–19.33, 16.32–32.22, 25.52–27.99, 6.99	565.42, 514.29, 202.17, 198.14, 123.08, 177.12	(1, 4)
Anti-inflammatory	NP-018534, Indoline, 5,6,7,8-Tetrahydro-1,4,5-naphthalenetriamine, Complex terpenoids	7.21, 12.85, 16.39–20.70	266.15, 119.07, 177.12, 462.27	(19)
Antimicrobial	(+)-ar-Turmerone, Curcumene, 3-Ethyl-o-xylene, (3R,3aR,11aR… triterpenoid)	16.97–31.65, 19.33, 16.32, 20.70	216.15, 202.17, 134.10, 462.27	(4)
Anticancer	Valine, Isoleucine, Guaiazulene, Methyl tetradecahydro -2-picenecarboxylate, (2R)-4-Methylpentanoic acid derivative	1.25–1.97, 16.32, 18.07, 2.00	117.08, 131.09, 198.14, 480.28, 293.14	(21)
Hepatoprotective	Cinnamic acid, 4-Phenylcyclohexanone, 3-Methoxy-17-methylmorphinan-4,14-diol, NP-018534	3.21, 16.31, 16.39, 10.95	148.05, 174.10, 303.18, 266.15	(1)
Cardioprotective	DL-Tryptophan, D-(+)-Maltose, Ethyl 6-oxohexanoate derivative, Nicotinic acid, Valine	1.25–6.39, 15.54, 1.73, 1.47	204.08, 342.11, 333.19, 123.03, 117.08	(9)
Neuroprotective	Jasmonal, Indole, Cumene, 3,4-Dihydroxybenzaldehyde, 1-Ethyl-3-pyrrolidinyl cyclopentyl acetate	6.23–18.30, 16.32, 16.32, 16.70	202.13, 117.05, 120.09, 138.03, 317.19	(21)
Analgesic	(+)-ar-Turmerone, Trans-3-Indoleacrylic acid, 2-Methyl-1-tetralone, Indane	6.23, 16.31–19.00	216.15, 187.06, 160.08, 118.07	(9)
Anti-diabetic	UV6260496, Isoleucine, Guaiazulene, Naphthalene, DL-Stachydrine	1.30–27.99, 16.32, 16.46, 1.97	123.08, 131.09, 198.14, 128.06, 143.09	(4)
Antiviral	Gly-Arg, Benactyzine, Tetralin, Resorcinol diglycidyl ether	9.39–31.21, 12.85, 16.46	231.13, 327.18, 132.09, 222.08	(19)

All metabolites were putatively identified based on high-resolution mass matching and database comparison without confirmation using authentic reference standards. Therefore, compound identifications correspond to MSI level 2 (putatively annotated compounds) or level 3 (tentatively characterised compound classes). Certain detected molecular features displayed similarity to cyclic peptides, synthetic molecules, or pharmaceutical -like structures. Such annotations should be interpreted cautiously, as misannotation may occur due to isobaric mass overlap, in-source fragmentation, similar MS/MS spectral patterns, database overfitting and background contamination. Additional structural validation using authentic standards, NMR spectroscopy, or targeted MS/MS fragmentation studies would be required to confirm these assignments. No direct biological activity was experimentally validated in this study; therefore, pharmacological implications of detected compounds are not inferred solely from their presence in the extract.

Discussion

Chemical Diversity of *Curcuma caesia*

The comprehensive GC-MS and LC-MS profiling of *Curcuma caesia* rhizome extract revealed a chemically diverse metabolomic composition comprising terpenoids, phenolic compounds, amino acids, alkaloid-like features, sugars and fatty acid derivatives (29–31). The predominance of monoterpenes and sesquiterpenes in the GC-MS profile, alongside phenolic acids and nitrogen-containing metabolites detected via LC-MS, reflects the complex secondary metabolism characteristic of the Zingiberaceae family. Among the putatively annotated metabolites, sesquiterpenes such as (+)-ar-turmerone, curcumene, germacrone and curzerenone were detected with relatively higher peak intensities (32). These compounds are frequently reported in *Curcuma* species and are considered characteristic constituents of the genus. The detection of cinnamic acid derivatives and other aromatic compounds further supports the presence of phenylpropanoid pathway metabolites within the extract. In addition, the identification of amino acids such as valine and isoleucine reflects primary metabolic components that coexist with secondary metabolites in plant matrices. The presence of these metabolites likely contributes to the overall chemical fingerprint of the rhizome rather than indicating specific therapeutic roles (33). Importantly, all metabolite identifications are putative and based on high-resolution mass spectral matching without confirmation using authentic standards. Therefore, the results should be interpreted as a metabolomic overview rather than definitive structural confirmation (34).

Comparative analysis within the *Curcuma* genus

The phytochemical profile of *C. caesia* demonstrates both shared and distinctive features when compared to other members of the genus, particularly *Curcuma longa*. While both species contain terpenoid-rich essential oil fractions and phenolic constituents, variations in relative abundance and specific metabolite patterns suggest species-level metabolic specialisation (35). The prominence of certain sesquiterpenes in *C. caesia* may serve as chemotaxonomic markers differentiating it from other *Curcuma* species. However, quantitative comparison with standardised reference extracts would be necessary to establish definitive interspecies variation (36). The observed diversity in metabolite classes highlights the potential value of *C. caesia* as a chemically distinct species within the genus. Nevertheless, comparative functional studies are required before attributing biological uniqueness.

Methodological considerations and analytical insights

The use of dual-mode LC-MS (positive and negative ionisation) enabled broader metabolite coverage, capturing both basic nitrogen-containing compounds and acidic phenolic constituents. GC-MS further complemented this analysis by profiling volatile and semi-volatile components. The metabolomic approach adopted in this study provides a holistic chemical fingerprint of *C. caesia*, moving beyond traditional single-marker analysis (37). Such untargeted profiling can serve as a foundation for- standardisation of extracts, chemotaxonomic comparison and hypothesis generation for future bioassay-guided studies. However, certain annotations, including cyclic peptide-like structures and synthetic-

appearing molecules, require cautious interpretation. These may represent misannotations due to database overlap or isobaric mass similarities. Confirmatory techniques such as NMR spectroscopy and targeted MS/MS validation would be necessary for structural confirmation (38).

Conservation and sustainable utilisation

Comprehensive phytochemical profiling contributes to establishing chemical reference standards for *C. caesia*, which may aid in quality control of herbal preparations. Given the increasing commercial interest in medicinal plants, standardised chemical characterisation becomes essential for ensuring batch-to-batch consistency. Moreover, documentation of metabolomic diversity supports conservation efforts by providing baseline chemical data that can be correlated with geographical and environmental variations in future studies (39).

Future research directions and clinical implications

The present metabolomic profile provides a foundation for future research. Subsequent studies should focus on- bioassay-guided fractionation, isolation and structural confirmation of key metabolites, targeted quantification of marker compounds, evaluation of seasonal and geographical variation and mechanistic biological validation. Such investigations would clarify which specific constituents contribute to reported traditional uses and enable evidence-based development of standardised formulations (40).

Limitations and considerations

The present study was quite thorough. Several limitations must be acknowledged, like all metabolite identifications are putative (MSI level 2/3) and lack confirmation with authentic standards. The biological activities of detected compounds were not experimentally validated in this study (41). Seasonal and geographical variability of phytochemical composition was not assessed. Relative abundance is based on peak area normalisation and does not represent absolute quantification. Therefore, the findings should be interpreted as chemical characterisation rather than confirmation of pharmacological efficacy (42).

Conclusion

The LC-MS-based phytochemical profiling of *Curcuma caesia* Roxb. rhizome revealed a chemically diverse metabolomic composition comprising terpenoids, phenolic compounds, amino acid derivatives and other secondary metabolites. The detection of more than 50 putatively annotated metabolites highlights the chemical complexity of this species and supports its recognition as a rich source of structurally diverse natural products. While several identified metabolites have been previously reported in the literature for biological relevance, the present study does not establish direct therapeutic efficacy. Rather, these findings provide a chemical foundation for future bioactivity-guided investigations, targeted isolation and mechanistic studies aimed at validating specific pharmacological properties. The comprehensive metabolomic profile generated in this work contributes to chemotaxonomic understanding, quality control standardisation and sustainable utilisation strategies for *C. caesia*. By integrating traditional knowledge with advanced analytical methodologies, this study offers a reference framework for subsequent experimental validation and natural product research, rather than direct therapeutic application.

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Authors' contributions

RP contributed to the methodology, investigation, data curation and formal analysis of the study and prepared the original draft of the manuscript. ER was responsible for visualisation and contributed to the review and editing of the manuscript. DB conceptualised the study and assisted in reviewing and editing the manuscript. AVR contributed to the review and editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, artificial intelligence tools were used solely to improve the grammar, clarity and organisation of the text. The authors take full responsibility for the scientific content, data interpretation and conclusions presented in the manuscript.

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