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Insights into the composition of *Curcuma caesia Roxb.*

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Abstract

Black zedoary *(Curcuma caesia Roxb.)* is a distinctive medicinal plant recognized for its diverse therapeutic properties. The rhizome was subjected to proximate analysis, with results including moisture (63.9%), starch (42.4), carbohydrates (28.4), protein (3.19g), fat (2.39.g), fibre (1.94g), vitaminC (35.5mg), calcium(10.9mg), iron (21.37mg), phosphorus (27.7mg), potassium (9.45mg). Major chemical constituents in the methanolic extract of the rhizome were identified through cold maceration followed by High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) analysis. Using LC-MS, 20 non-volatile compounds were identified based on their retention times These findings suggest that the bioactive compounds present in the rhizome of black zedoary exhibit significant potential for further investigation as functional food ingredients, dietary supplements, or pharmaceutical agents due to their possible beneficial effects on human health.

Keywords: *Curcuma caesia*, black zedoary, rhizome, proximate composition, bioactive compounds, hr-lcms, functional food

Introduction

Black zedoary *(Curcuma caesia Roxb.)* is a perennial herbaceous plant distinguished by its rhizomatous nature and belongs to the *Zingiberaceae* family. It is an important medicinal species within the Curcuma genus, owing to its wide range of therapeutic properties and traditional uses. The rhizomes of black zedoary are modified underground stems that function as critical storage organs, accumulating essential nutrients as well as diverse secondary metabolites such as alkaloids, phenolics, and terpenoids. These bioactive compounds are mainly responsible for this plant's therapeutic potential such as anti-inflammatory, antimicrobial, and antioxidant activities. Due to these valuable properties, rhizomatous plants like black zedoary have been extensively employed in traditional medicinal systems such as Ayurveda, Siddha, and various indigenous folk medicines. These traditional systems have long relied on such plants for their healing potential, using them in remedies for a variety of ailments. Extensive biochemical profile of black zedoary rhizomes underscores their potential for further scientific research and development in pharmacology and natural product-based therapeutics (Kizhakkayil and Sasikumar, 2011) [18].

India is widely recognized as the major center of diversity for the *Zingiberaceae* family, which encompasses a vast array of genera and species that hold significant economic and medicinal value (Debnath and Vijayan, 2024) [9]. The state of Kerala, situated within the globally recognized Western Ghats biodiversity hotspot, is home to a rich variety of medicinal plants from the *Zingiberaceae* family, including several important species of the genus Curcuma. Among these species, *Curcuma caesia* commonly known as black turmeric or black zedoary it is particularly distinguished by its unique bluish black rhizome and its potent camphoraceous aroma, which contributes to its medicinal and aromatic significance. This species naturally occurs predominantly across the tropical and subtropical regions of India as well as Southeast Asia. It is especially prevalent in Indian states such as Madhya Pradesh, Chhattisgarh, and Odisha, in addition to the Western Ghats region. These populations represent crucial reservoirs of genetic and phytochemical diversity, underscoring the importance of conservation and sustainable utilization efforts for this valuable medicinal plant (Borah *et al.*, 2020) [6].

Rhizomatous plants have long been integral to traditional medicine systems like Ayurveda, Siddha, and local folk practices, especially in Kerala, a region rich in medicinal plants from the Zingiberaceae family such as Curcuma, Zingiber, and Alpinia (Soorya *et al.*, 2016) [34]. Among these, *Curcuma caesia Roxb*., known as black zedoary or black turmeric, is a lesser studied rhizome with notable therapeutic potential. Traditionally, its bluish black rhizome has been used across India to treat digestive issues, inflammation, respiratory problems, and skin conditions. Despite its traditional uses, black zedoary is less commercially developed and researched compared to other Curcuma species like *C. longa* (Victório, 2011) [38].

The therapeutic value of black zedoary is linked to its diverse chemical makeup, which includes essential oils rich in mono- and sesquiterpenes, as well as phenolics, flavonoids, and other secondary metabolites. These constituents vary based on geography, genotype, and environmental factors, influencing the rhizome's biological effects (Sabulal *et al.*, 2006) [28]. Ethnomedicinal knowledge supports its traditional use and guides scientific studies to validate these health claims, while also helping preserve Kerala's medicinal plant heritage (Gangwar, 2025) [11].

Although black zedoary has ethnomedicinal importance, data on its nutritional profile, chemical variation, mechanisms, and safety remain limited. Recent studies highlight its biological potential, but more systematic research is needed to confirm its nutritional and therapeutic roles and explore its use as a functional food or nutraceutical (Thomas *et al*., 2020). The rhizome contains carbohydrates (notably starch), proteins, fats, dietary fiber, and minerals like calcium, iron, phosphorus, and potassium, all contributing to its nutritional and functional properties. These components vary with genotype, maturity, location, cultivation, and processing (Sarangthem and Haokip, 2010) [32].

Antinutritional factors such as phytate, tannins, and oxalate can reduce mineral bioavailability but also possess biological activity, so their nutritional impacts should be balanced against functional benefits. Proper processing can minimize negative effects while preserving beneficial compounds (Sajitha and Sasikumar, 2015) [31]. The phytochemical profile of *C. caesia* is rich in phenolics, flavonoids, terpenoids, and essential oils, but levels vary widely among genotypes and regions, underscoring the need for chemical characterization and standardization (Tamang, 2022) [35].

Processing methods like drying, grinding, extraction, and storage also affect the rhizome's chemical and nutritional quality, making standardization crucial for consistent products (Thingujam *et al.*, 2026) [36].

Black zedoary has demonstrated various biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, neuroprotective, and cytoprotective effects, mostly in experimental models using extracts or isolated compounds. These findings support traditional uses but call for further mechanistic and clinical research (Kaur *et al.*, 2018) [17].

Antioxidant activity, widely studied, shows the plant's potential to scavenge free radicals and reduce oxidative damage, though *In Vitro* results alone don't confirm therapeutic benefit (Sutar *et al*., 2020). Anti-inflammatory effects align with traditional uses and suggest possible applications in chronic inflammatory conditions, but more research is needed to identify active compounds and mechanisms (Reenu *et al.*, 2015) [26].

Extracts and oils also show antimicrobial effects, varying by extract type, concentration, and microbial species, indicating potential as natural antimicrobial agents pending standardized studies (Bindal and Mohanty, 2024) [4]. Interest in antidiabetic and metabolic benefits arises from constituents that may influence glucose metabolism, oxidative stress, and inflammation, though active compounds and mechanisms require further study (Lobo *et al.*, 2009) [21].

Neuroprotection is an emerging field, with preliminary evidence suggesting benefits against oxidative stress and neuroinflammation—key factors in diseases like Alzheimer's. Computational studies aid in identifying potential molecular targets but need experimental validation (Sharma *et al.*, 2025) [33]. Traditional use for digestive ailments has prompted investigation into gastrointestinal benefits, especially in inflammatory bowel conditions. Early research shows promise, but detailed studies are necessary (Leela and Adheeba, 2024) [19].

Cytoprotective and antimutagenic effects have been observed, hinting at cellular protection from damage. However, differences in plant source, genotype, extraction, dosage, and models complicate comparisons, highlighting the need for standardized materials and methods to link chemistry with bioactivity reliably (Navarro *et al.*, 2002) [8].

Safety evaluation is vital for future development as a food or therapeutic resource. While traditionally used, systematic studies on dosage, toxicity, and long-term effects are essential to ensure safe applications (Haida *et al*., 2023) [12].

Curcuma caesia is a promising yet underutilized medicinal rhizome with valuable nutritional, phytochemical, and pharmacological features. Its diverse bioactivities and chemical composition justify further research focused on standardization, active compound identification, mechanism elucidation, safety, and clinical validation to support its use as a functional food, nutraceutical, or therapeutic source (Akter *et al.*, 2019) [1].

Materials and methods

Plant material

Fresh mature black zedoary *(Curcuma caesia Roxb*.) will be collected from the Research farm of Agronomy and Plantation, spices, medicinal and Aromatic crops, College of Agriculture Vellanikkara.

Physico-chemical qualities

Moisture

Moisture content of the fresh rhizome sample was determined according to the method described in Bhardwaj *et al.*,2023 [2]. A known quantity of the sample was weighed and dried in a hot air oven at 60–70°C. The dried sample was cooled in a desiccator and weighed. The process of heating, cooling and weighing was repeated until a constant weight was obtained. The loss in weight during drying was considered as the moisture content of the sample and was calculated accordingly.

Starch

Starch content was estimated by the colorimetric anthrone method described by Sadasivam and Manickam (1992) [30]. A 0.5 g portion of the rhizome sample was weighed and extracted with 80% ethanol to remove soluble sugars. The residue obtained was dried on a water bath, followed by the addition of 6.5 ml of 52% perchloric acid and 5 ml of distilled water. The mixture was extracted at 0°C for 20 minutes and the supernatant was collected and made up to 100 ml with water. An aliquot of 0.2 ml of the supernatant was diluted to 1 ml with water, followed by the addition of 4

ml of anthrone reagent. The mixture was heated for 8 minutes, cooled, and the absorbance was measured at 630 nm. A standard curve was prepared using serial dilutions of a standard glucose solution. The glucose concentration of the sample was obtained from the standard curve and multiplied by a conversion factor of 0.9 to determine the starch content, which was expressed as g per 100 g of sample.

Carbohydrate

The total carbohydrate content of the rhizome sample was determined using the anthrone method described by Sadasivam and Manickam (1997) [29]. About 100 mg of the dried sample was weighed and hydrolysed with 5 ml of 2.5 N hydrochloric acid (HCl) for 3 hours in a boiling water bath. The hydrolysate was cooled to room temperature and neutralized with sodium carbonate until effervescence ceased. The volume was then made up to 100 ml with distilled water and centrifuged. An aliquot of 0.2 ml of the clear supernatant was diluted to 1 ml with water, followed by the addition of 4 ml of anthrone reagent. The mixture was heated in a boiling water bath for 8 minutes, rapidly cooled, and the intensity of the green to dark green colour developed was measured at 630 nm. A standard calibration curve was prepared using serial dilutions of standard glucose solution. The total carbohydrate content of the sample was calculated from the standard curve and expressed as g per 100 g of sample.

Protein

The protein content of the rhizome sample was determined by Lowry's method as described by Sadasivam and Manickam (1997) [29]. Approximately 500 mg of the sample was extracted with 5–10 ml of Tris buffer (tris hydroxymethyl aminomethane) and centrifuged. From the resulting supernatant, 0.1 ml was transferred into a test tube and mixed thoroughly with 5 ml of alkaline copper reagent. The mixture was allowed to stand for 10 minutes, after which 0.5 ml of Folin–Ciocalteau reagent was added. The tubes were incubated at room temperature in the dark for 30 minutes, and the intensity of the blue colour developed was measured at 660 nm. A standard calibration curve was prepared using serial dilutions of the standard protein solution with alkaline copper and Folin–Ciocalteau reagents. The total protein content of the sample was calculated from the standard curve and expressed as g per 100 g of sample.

Fibre

The crude fibre content of the rhizome sample was determined according to the method described by Sadasivam and Manickam (1997) [29] [29]. About 2 g of the sample was boiled with 200 ml of 1.25% sulphuric acid for 30 minutes. The mixture was filtered and the residue was washed thoroughly with boiling water. The residue was subsequently boiled with 200 ml of 1.25% sodium hydroxide for 30 minutes and filtered through muslin cloth. It was then washed successively with 25 ml of boiling 1.25% sulphuric acid, three portions of 50 ml water and 25 ml alcohol. The resulting residue was transferred to a previously weighed ashing dish (W_1) and dried at 130°C for 2 hours. The dish was cooled in a desiccator and weighed (W_2). The dried residue was then incinerated in a muffle furnace at 600°C for 30 minutes, cooled in a desiccator and weighed again (W_3). The loss in weight on ignition was used for the calculation of crude fibre content.

Fat

The fat content of the fresh rhizome sample was determined by the Soxhlet extraction method described by Sadasivam and Manickam (1997) [29] . Approximately 5 g of the sample was placed in a Soxhlet thimble and plugged with cotton. The sample was continuously extracted with petroleum ether for 6 hours under gentle heating. Following extraction, the flask was cooled and the solvent was removed by heating. The weight of the extracted fat was recorded, and the fat content was calculated and expressed as g per 100 g of sample.

Vitamin C

The vitamin C content of the rhizome sample was determined by the method described by Sadasivam and Manickam (1992) [30]. About 3 g of the sample was extracted using 4% oxalic acid, and the extract was made up to 100 ml with the same solution. The resulting supernatant was titrated against 2,6-dichlorophenol indophenol dye until a pink colour appeared and persisted for a few seconds, indicating the endpoint. The vitamin C content was calculated and expressed as mg per 100 g of sample.

Total Ash

The total ash content of the rhizome sample was determined according to the method prescribed by ISI (1980). Approximately 5 g of the sample was accurately weighed into a crucible and incinerated in a muffle furnace at 550–600°C for 5–6 hours. The crucible was then cooled to room temperature in a desiccator and weighed. The ash content was calculated and expressed as a percentage of the sample.

Calcium

Calcium content was estimated following the method recommended by Perkin–Elmer (1982). About 1 g of the rhizome sample was pre-digested using 10 ml of a diacid mixture containing nitric acid and perchloric acid in a 9:4 ratio. The resulting diacid digest was used for calcium estimation by Atomic Absorption Spectrophotometry. The calcium content was calculated and expressed as mg per 100 g of sample.

Iron

The iron content of the rhizome sample was determined using the method described by Perkin–Elmer (1982) [26]. One gram of the sample was pre-digested with 10 ml of a nitric acid and perchloric acid mixture in a 9:4 ratio. The resulting diacid extract was subjected to analysis using an Atomic Absorption Spectrophotometer. The iron content was expressed as mg per 100 g of sample.

Phosphorus

Phosphorus content was estimated colorimetrically according to the method of Jackson (1973) [15]. The method is based on the development of a yellow-coloured complex upon reaction with nitric acid–vanadate–molybdate reagent. Five millilitres of the pre-digested sample aliquot was

mixed with 5 ml of the reagent and the volume was made up to 50 ml using distilled water. After allowing the reaction to proceed for 10 minutes, the optical density was measured at 420 nm. A standard calibration curve was prepared using serial dilutions of a standard phosphorus solution. The phosphorus content was calculated from the standard curve and expressed as mg per 100 g of sample.

Preparation of Plant Extract

The plant extract was prepared using the cold maceration technique with hexane and methanol as extraction solvents. About 100 g of freshly sliced black zeodary rhizome was soaked in 250 ml of hexane for 24 hours and subsequently filtered. The extraction with hexane was repeated three times to ensure efficient removal of the hexane-soluble constituents. The remaining residue was then subjected to three successive extractions with methanol. The combined methanolic extract was concentrated using a rotary evaporator and diluted with methanol to obtain a 10ppm solution. The prepared extract was passed through a 0.22 µm PVDF syringe filter and transferred into a vial for further analysis.

High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LC-MS) Analysis

The methanolic extract of black zedoary rhizome was subjected to High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LC-MS) analysis for the characterization of its chemical constituents and generation of a chemical fingerprint. The analysis was carried out using an Agilent High-Resolution Liquid Chromatography–Mass Spectrometry system (Model G6550A) with a mass resolution of 0.01%. The mass acquisition range was set from 50 to 1000 m/z, with a scanning rate of one spectrum per second. The autosampler (Model G4226A) was operated at an auxiliary speed of 100 µl/min, an ejection speed of 100 µl/min and a flush-out volume of 5 µl, with an injection volume of 8 µl.

The total acquisition time was 30 minutes. During the initial 2 minutes, the solvent composition of mobile phases A and B was maintained at 95:5. The solvents used for the HR-LC-MS analysis were 100% water as solvent A and 100% acetonitrile as solvent B. The resulting chromatographic and mass spectral data were used to obtain the chemical fingerprint of the black zedoary rhizome extract and to identify its constituent compounds.

Result and discussion

Table 1: Nutritional composition of Black zedoary rhizome

Nutrient	Amount
Moisture (%)	63.9
Starch(g/100)	42.4
Carbohydrates (g)	28.4
Protein (g)	3.19
Fat (g)	2.39
Fibre (g)	1.94
Viamin C (mg)	35.5
Total ash (%)	2.49
Calcium	10.9
Phosphorus	27.7
Potasium	9.45
Iron	21.37

The nutritional constituents of the fresh lotus rhizomes were estimated. The constituents like moisture, starch, carbohydrates, protein, fibre, vitamin C, total ash, calcium, iron, phosphorus, sodium, potassium content of the samples were analysed. The results pertaining to nutritional constituents of raw rhizome are presented in Table-1.

The fresh black zedoary rhizome contained a moisture level of 63.9 ± 3.12%, which is notably higher than the 9.6-9.9% moisture content reported by Lenka *et al.* (2025) [20] for fresh *Curcuma caesia* rhizomes from selected samples. Their measurements were made using the oven-drying method at 105 °C until the weight stabilized. The difference in moisture levels could be due to several factors, such as variations in the geographical location where the plants were grown, the specific plant accessions, their stage of maturity, harvesting techniques, and how the samples were handled post-harvest. Lenka and colleagues also emphasized that environmental and geographical influences can cause significant variation across different *C. caesia* accessions. The higher moisture content found in the fresh rhizomes here suggests that these samples have a larger water proportion, which highlights the need for careful drying and storage practices to avoid spoilage and maintain quality.

Starch content of *Curcuma caesia Roxb*. rhizome was measured at 42.4%, confirming starch as the primary carbohydrate stored in the rhizome. Sajitha and Sasikumar (2015) [31], compared starch levels across four Curcuma species, found starch contents between 45.24% and 48.48% on a dry-weight basis, slightly higher than the current findings. These differences in starch content could be due to variations in genotype, growing conditions, maturity, and environmental influences. Additionally, their research highlighted that *C. caesia* has unique starch properties, such as distinct swelling power, solubility, and water-holding capacity.

The fresh rhizome of black zedoary *(Curcuma caesia Roxb.)* was found to contain 28.4 ± 6.98% carbohydrates, highlighting carbohydrates as a significant nutritional component of the plant. This aligns with the findings of Bhardwaj *et al*. (2023) [2], who also reported carbohydrates as a major part of the proximate composition in *C. caesia* rhizomes. Supporting this, a recent metabolomic study showed that sugars made up about 29.93% of the primary metabolites detected in *C. caesia*, with sucrose and glucose being the predominant sugars. The carbohydrate content in the rhizome likely reflects the natural accumulation of starch and soluble sugars, as the rhizome serves as a storage organ for the plant. Variations in carbohydrate levels compared to other studies may be due to differences in plant varieties, growing locations, maturity stages, environmental conditions, moisture levels, and analytical techniques used. Overall, the carbohydrate content of around 28.4% underscores the nutritional potential of black zedoary rhizome as a valuable source of dietary carbohydrates.

The protein content of *Curcuma caesia Roxb*. rhizome in this study was found to be 3.19 g per 100 g. (Ramkumar,2023) [2] reported a crude protein value of 2.11%, while recorded an even lower protein content in their fresh rhizome analysis. This suggests that *C. caesia* rhizome offers a moderate amount of protein. Differences observed between studies may be due to variations in genotype,

geographical conditions, maturity at harvest, and the analytical methods used.

the fat content of *Curcuma caesia Roxb*. rhizome was measured at 2.39 g per 100 g. This is higher than the 0.9% crude fat content reported by (Lenka *et al.*,2025) [20]. Differences in fat levels may be influenced by factors such as genotype, geographical origin, environmental conditions, maturity, sample preparation, and analytical techniques. While *C. caesia i*s not a significant source of dietary fat, its rhizome does contain lipids and fatty acids that contribute to its overall phytochemical profile.

The vitamin C content was 35.5 mg/100 g, indicating that the rhizome contains an appreciable amount of ascorbic acid.

The fibre content of *Curcuma caesia Roxb*. rhizome was found to be 1.94 g/100 g in the present study. A recent study on different chemotypes of *C. caesia r*eported variation in nutritional composition among rhizome accessions, supporting the influence of genotype and growing conditions on the nutritional characteristics of the plant (Lenka *et al.*,2025) [20]. The variation in fibre content may be associated with differences in genotype, geographical location, maturity, cultivation conditions and analytical methodology. The presence of dietary fibre contributes to the nutritional quality of the rhizome and may support its utilization in value-added food products.

The total ash content of *Curcuma caesia Roxb. r*hizome was measured at 2.49%, reflecting its mineral content. Other studies have reported a range of ash values, such as 9.00% on an air-dried basis, 5.75% w/w by Pandey *et al*. (2025) [22], and a lower value around 1.08% (10.76 mg/g). These variations can be attributed to factors like geographical origin, soil mineral composition, maturity of the rhizome, cultivation methods, moisture content, and how samples were prepared. Because ash represents the inorganic material remaining after burning, differences in mineral absorption and environmental conditions naturally cause fluctuations in ash content.

The calcium content in *Curcuma caesia Roxb*. rhizome was measured at 10.9 mg per 100 g in this study. Calcium is recognized as an important mineral in C. caesia rhizomes, alongside other minerals like potassium, magnesium, phosphorus, sodium, and iron, as confirmed by Paudel *et al.* (2022). The presence of calcium highlights the rhizome's contribution to the plant's overall mineral profile. Variations in calcium levels may be influenced by factors such as geographic location, soil mineral availability, genotype, cultivation conditions, rhizome maturity, and analytical methods.

Potassium content was found to be 9.45 mg per 100 g. It is one of the key minerals present in *C. caesia* rhizomes. (Van hung, 2017) [37] also detected potassium along with several other minerals, but their reported potassium levels were significantly higher than those found in this study, indicating notable variation among samples. Factors like soil composition, geographic origin, genotype, and testing methods should be considered when comparing these results.

Iron was present at a relatively high concentration of 21.37 mg per 100 g, making it one of the more abundant minerals in the sample. Bhardwaj *et al.* (2023) [2] similarly confirmed iron in *C. caesia* rhizomes through elemental analysis. However, since mineral content can vary widely depending on soil and growing conditions, this high iron value should ideally be verified with repeated tests and detailed reporting of analytical techniques.

Phosphorus content was measured at 27.7 mg per 100 g, aligning well with previous findings. Bhardwaj *et al.* (2023) [2] identified phosphorus as a key mineral in *C. caesia*, and earlier work by Bhardwaj, Hait, and Kashyap reported phosphorus levels around 0.03% (approximately 30 mg per 100 g), which is close to the current study's result. This consistency supports the reliability of the phosphorus measurement in the rhizome.

Table 2: Bioactive compounds in methanol extract of Black zedoary

Compounds	Retention time	Mass	Chemical formula
3-(4-Hydroxyphenyl) propionic acid	5.721	166.0630	$C_9H_{10}O_3$
Suspensolide F	5.805	478.2050	$C_{21}H_{34}O_{12}$
1,6-Dihydroxy-3,7-dimethoxy-3-(3-methyl-2-butenyl)-8-(2-oxo-3-methyl-3-butenyl)-xanthone	5.863	438.1679	$C_{25}H_{28}O_7$
Methyl7-epi-12-hydroxyjasmonate glucoside	6.498	402.1890	$C_{19}H_{30}O_9$
Methyl7-epi-12-hydroxyjasmonate glucoside	6.498	402.1890	$C_{19}H_{30}O_9$
Kanokoside A	6.717	476.1894	$C_{21}H_{32}O_{12}$
Chrysin 7-[(rhamnosyl-(1→4)-glucoside)]	9.139	562.1686	$C_{27}H_{30}O_{13}$
Amaralin	10.537	264.1362	$C_{15}H_{20}O_4$
Erythronolide B	11.249	402.2613	$C_{21}H_{38}O_7$
Phloroionolic acid	11.813	332.2563	$C_{18}H_{36}O_5$
Apotrichodiol	11.873	252.1725	$C_{15}H_{24}O_3$
Corchorifattly acid F	12.133	328.2250	$C_{18}H_{32}O_5$
9,10-Dihydroxy-12,13-epoxyoctadecanoate	12.375	330.2406	$C_{18}H_{34}O_5$
Oryzalic acid A	14.179	350.2093	$C_{20}H_{30}O_5$
Lathyrol	15.945	334.2144	$C_{20}H_{30}O_4$
Callicarpone	16.101	332.1988	$C_{20}H_{28}O_4$
Valdiate	18.487	310.1780	$C_{17}H_{26}O_5$
α-Linolenic acid	20.599	278.2246	$C_{18}H_{30}O_2$
16-Hydroxyhexadecanoic acid	20.769	272351	$C_{16}H_{32}O_3$
Palmitic acid	23.315	256.2402	$C_{16}H_{32}O_2$

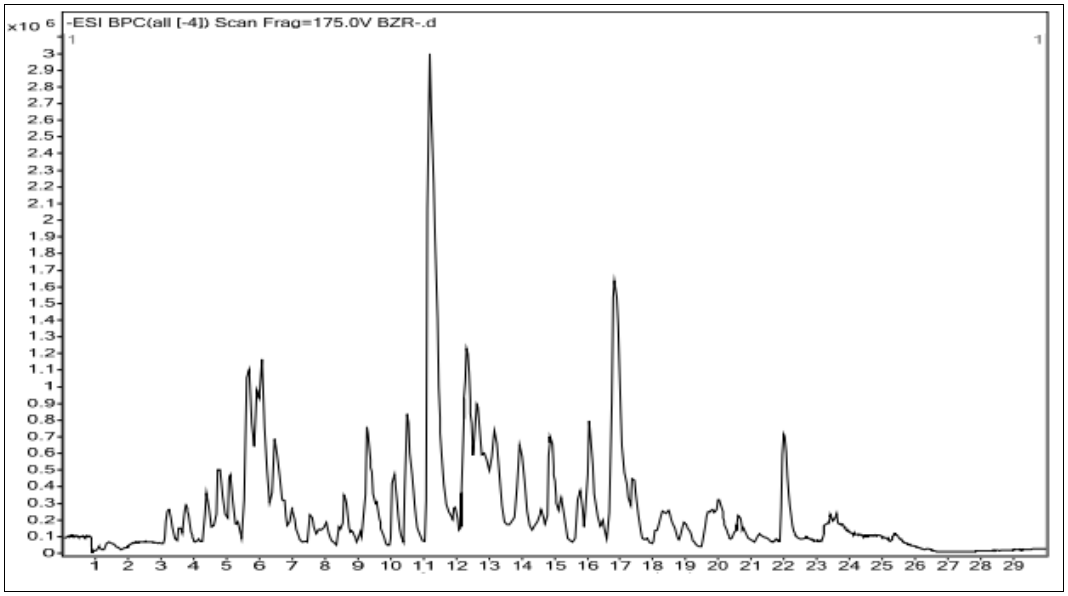

Fig 1: Chromatogram of methanolic Extract of Black zedoary rhizome

HR-LCMS analysis of the methanolic extract of *Curcuma caesia* rhizome identified several compounds with retention times ranging from 5.721 to 23.315 minutes. The compounds detected at shorter retention times included 3-(4-hydroxyphenyl) propionic acid (5.721 min), suspensolide F (5.805 min), the identified xanthone derivative (5.863 min), methyl 7-epi-12-hydroxyjasmonate glucoside (6.498 min), and Kanokoside A (6.717 min). The presence of these compounds indicates the chemical diversity of the rhizome extract. 3-(4-Hydroxyphenyl) propionic acid is a phenolic acid derivative and may contribute to the antioxidant potential of the extract. Phenolic compounds are important plant metabolites that are commonly associated with antioxidant and anti-inflammatory properties. Previous studies have also reported different phenolic compounds in *C. caesia* rhizomes, supporting the presence of phenolic constituents in the plant (Joseph *et al.*, 2023) [16].

Chrysin 7- [rhamnosyl-(1→4)-glucoside] was detected at a retention time of 9.139 min, while amaralin was detected at 10.537 min. The detection of the chrysin glycoside indicates the presence of flavonoid compounds in the rhizome. Flavonoids are known for their antioxidant and anti-inflammatory potential and may contribute to the biological properties of *C. caesia*. Joseph *et al.* (2023) [16] also reported several polyphenolic compounds in *C. caesia* rhizome extracts using LC-MS/MS analysis. Amaralin represents another specialised plant metabolite and may contribute to the overall phytochemical diversity of the extract. The presence of flavonoids and other secondary metabolites is consistent with the medicinal importance of *C. caesia* reported in previous studies (Ibrahim *et al.*, 2023) [13].

Compounds detected at intermediate retention times included erythronolide B (11.249 min), phloionolic acid (11.813 min), apotrichodiol (11.873 min), corchorifatty acid F (12.133 min), 9,10-dihydroxy-12,13-epoxyoctadecanoate (12.375 min), oryzalic acid A (14.179 min), lathyrol (15.945 min), callicarpone (16.101 min), and valdiate (18.487 min). These compounds further demonstrate the presence of different oxygenated and lipid-related metabolites in the rhizome. Corchorifatty acid F is of particular interest because corchorifatty acids have been reported to possess anti-inflammatory potential. (Masayuki *et al.*,1998) reported inhibitory effects of corchorifatty acids on nitric oxide production in macrophages. However, the biological activity of corchorifatty acid F in *C. caesia* requires further confirmation.

Lathyrol, detected at 15.945 min, is another compound of potential therapeutic interest. Previous studies have reported anti-inflammatory activity associated with lathyrol-related compounds (Wang *et al.*, 2023). Chen *et al.* (2023) [7, 39] also reported that lathyrol showed anticancer activity by inhibiting cancer cell proliferation and promoting apoptosis in lung cancer cells. Therefore, the detection of lathyrol in the present *C. caesia* extract suggests possible biological significance. However, its actual contribution to the therapeutic activity of the whole rhizome extract cannot be confirmed from LC-MS identification alone.

Among the compounds detected at longer retention times, α-linolenic acid was identified at 20.599 min with a molecular formula of $C_{18}H_{30}O_2$. α-Linolenic acid is an omega-3 fatty acid with well-documented anti-inflammatory properties. (Ren and Chung 2007) [27] reported that α-linolenic acid reduced nitric oxide production and inhibited inflammatory pathways involving NF-κB and MAPK. Zhu *et al.* 2020) [40] also reported anti-inflammatory and antioxidant effects of α-linolenic acid in an experimental model of acute lung injury. Therefore, the presence of α-linolenic acid in the *C. caesia* rhizome extract may contribute to its reported anti-inflammatory potential.

16-Hydroxyhexadecanoic acid and palmitic acid were detected at retention times of 20.769 and 23.315 min, respectively. Their presence indicates the occurrence of long-chain fatty acids in the methanolic extract. These compounds contribute to the lipid composition of the rhizome, although their specific therapeutic contribution to *C. caesia* cannot be established from the present findings. Palmitic acid, in particular, should not be directly considered a beneficial therapeutic compound based only on its detection.

Overall, the LC-MS analysis showed that *C. caesia* rhizome contains a wide range of phenolic, flavonoid, terpenoid, oxygenated and fatty-acid-related compounds. Compounds such as 3-(4-hydroxyphenyl) propionic acid, chrysin glycoside, lathyrol and α-linolenic acid are particularly

interesting because of their reported biological activities. The diverse chemical profile observed in the present study agrees with previous metabolomic studies of *C. caesia* (El-Akad *et al.*, 2025) [10]. Recent research has also supported the antioxidant and anti-inflammatory potential of *C. caesia* rhizome extracts (Biswas *et al.*, 2026) [5]. However, the compounds identified by HR-LCMS should be considered putative until confirmed using MS/MS fragmentation and authentic standards. Further studies are required to determine their concentration, individual biological activities and contribution to the therapeutic potential of black zedoary.

Conclusion

The rhizome of black zedoary *(Curcuma caesia Roxb.)* is nutritionally valuable, with starch as its primary component, accompanied by protein, fiber, vitamin C, and important minerals. High resolution LCMS analysis has uncovered a wide variety of bioactive compounds, such as phenolic acids, flavonoid derivatives, glycosides, terpenoids, and fatty acids, highlighting its rich phytochemical profile. These substances likely play a role in the rhizome's known antioxidant and anti-inflammatory effects. Still, several compounds detected through HR-LCMS need further confirmation with MS/MS methods and comparison against authentic standards. To fully realize black zedoary's potential in functional foods, nutraceuticals, and pharmaceuticals, more research is necessary focusing on precise quantification, isolating individual compounds, validating their biological activities, and assessing safety.

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