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Original Article

Quantitative GC-MS and HPLC Profiling of n-hexane and Methanol Extracts from *Curcuma zedoaria* Leaves for Biomedical Applications

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Abstract

Curcuma zedoaria (Zingiberaceae), locally known as Temu putih or Kunyit putih, is widely used in Malaysia as a spice, flavouring agent, and postpartum remedy, often in combination with other medicinal plants. However, limited studies have explored the phytochemical composition of *C. zedoaria* leaves, particularly using extracts of varying polarities, despite the importance of this approach in capturing the full spectrum of bioactive compounds. HPLC analysis facilitated the detection of 18 polar constituents, notably flavonoids and coumarins, which are recognised for their therapeutic potential. In parallel, GC-MS profiling identified a broad spectrum of secondary metabolites, with the n-hexane extract yielding 37 compounds. In contrast, the methanol extract revealed 22 distinct peaks by GC-MS, reflecting a diverse range of phenolic derivatives and oxygenated diterpenes. Comparative results indicated distinct phytochemical patterns influenced by solvent polarity. These findings suggest that *C. zedoaria* leaves, often overlooked in favour of rhizomes, represent a substantial source of pharmacologically relevant compounds. This research contributes to expanding the phytochemical knowledge of the species and supports the valorisation of foliar biomass in pharmaceutical and industrial applications.

Keywords: *Curcuma zedoaria; GC-MS; HPLC*

Introduction

Herbal remedies have long played a central role in managing health and disease, forming the foundation of traditional and folk medicine across cultures (Allaq *et al*., 2025; Salim *et al*., 2023). Among these, medicinal spices function not only as dietary components but also as rich sources of phytochemicals with therapeutic properties, supporting their integration into holistic healthcare systems (Albaqami *et al*., 2022; Salim *et al*., 2022; Oreopoulou *et al*., 2021; Yuan *et al*., 2016). Although allopathic medicine became the dominant global healthcare model in the 20th century, recent scientific advances have renewed interest in traditional pharmacopoeias. This resurgence is driven by modern phytochemical techniques that enable systematic identification of bioactive natural compounds (Sidik *et al*., 2024). Integrating traditional knowledge with biomedical science has helped validate ethnomedicine and accelerate the discovery of sustainable therapeutic agents (Mukherjee *et al*., 2015; Sukor *et al*., 2022).

Given this newfound importance, the genus Curcuma (Zingiberaceae) has surfaced as a vital source of bioactive chemicals with considerable medicinal, nutritional, and industrial significance (Poudel *et*

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al., 2022). This genus, comprising over 93 species native to Asia, is globally cultivated and prized for its pharmacological properties, as shown in Figure 1.

Figure 1: The Distribution of *Curcuma zedoaria*

As shown in Figure 2*, C. zedoaria* is a herbaceous perennial that grows up to 0.4 meters, with green leaves marked by brownish-purple veins, highlighting its taxonomic significance. Its rhizomes are highly valued in traditional medicine for their anti-inflammatory, antimicrobial, and cytotoxic properties, making them integral to therapeutic practices (Ahmed Hamdi *et al*., 2014). In Malaysia, locally known as *Temu Putih* or *Kunyit Putih*, it is consumed as a spice, flavouring agent, and postpartum remedy, often combined with other plants (Malek *et al*., 2004). These diverse culinary and medicinal uses underscore its importance in both traditional and modern medical systems (Kumar *et al*., 2024).

Figure 2: *Curcuma zedoaria Leaves*

Emerging research suggests that *C. zedoaria* leaves also contain a diverse array of bioactive compounds, including curcuminoids, sesquiterpenoids, and essential oils, many of which exhibit strong antioxidant, antimicrobial, and anti-inflammatory effects (Dosoky & Setzer, 2018; Rahaman *et al*., 2020). These compounds are potentially useful in managing oxidative stress, inflammatory disorders, and antibiotic-resistant infections (Abdulsamad *et al*., 2024). Despite these indications, comprehensive chemical analysis of the leaves, particularly from South Asian ecotypes, remains limited. Most existing studies provide only preliminary or qualitative data, often without employing advanced analytical techniques (Yi *et al*., 2023).

To date, limited studies have comprehensively investigated the phytochemical composition of *C. zedoaria* leaves, and no detailed analysis has employed both high-performance liquid chromatography (HPLC) and gas chromatography-mass–mass spectrometry (GC-MS) on samples collected from Malaysia or comparable geographical regions (Can *et al*., 2024). Furthermore, comparative analysis of extracts using solvents of different polarities, such as methanol and n-hexane, is rare, despite its importance in capturing the full spectrum of phytochemicals (Babbar *et al*., 2014). The biomedical potential of these leaf-derived compounds, especially in addressing inflammation, oxidative stress, and microbial resistance, also remains underexplored (Ribeiro *et al*., 2025). This represents a significant gap in current phytochemical research and limits the translational value of this underutilised plant part (Odeku *et al*., 2024).

This study aimed to address existing gaps in the phytochemical understanding of *C. zedoaria* leaves by conducting a profiling and quantification of methanolic and n-hexane extracts using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). The analysis enabled the identification of key bioactive constituents, which were further assessed for their potential biomedical and pharmaceutical relevance. The findings not only strengthen the ethnopharmacological value of *C. zedoaria* but also offer promising insights into its applicability in the development of natural product-based therapeutics.

Materials and Methods

Collection samples

Fresh leaves of *C. zedoaria* were collected from Sungai Buloh farm, Selangor, Malaysia, in July 2024 and authenticated by Dr. Shamsul Khamis at the Department of Biological Sciences and Biotechnology, Universiti Kebangsaan Malaysia (UKM). Voucher specimens (ID118/2024) were deposited in the department's herbarium for documentation. The leaves were freeze-dried under shaded conditions to preserve bioactive constituents, ground into a fine powder, sieved through a 1400 µm mesh for uniformity and stored in polyethene bags under controlled dry conditions to ensure stability. This standardised preparation ensures sample integrity, supporting reliable phytochemical and pharmacological analyses.

Chemicals

n-Hexane (≥95% purity), methanol (≥95% purity), acetonitrile (MeCN) and the standards 4-hydroxycoumarin, quercetin, coumarin, and 6-methyl-coumarin were procured from Sigma-Aldrich (St. Louis, MO, USA) and ultra-pure water (UPW) was from Sartorius. All solvents, including those utilised for GC-MS analysis, were of HPLC grade to ensure high precision and accuracy.

Extraction Yield (%)

The extraction yield (%) was determined by calculating the ratio of the weight of the crude extract obtained after the extraction process to the initial weight of the plant powder used before the extract (Laleff *et al*., 2021). This ratio was then multiplied by 100 to express the extraction yield as a percentage, providing a quantitative measure of the efficiency of the extraction process.

$$\text{Yield Percentage (\%)} = (\text{Weight of Crude Extract} / \text{Weight of Plant Powder}) \times 100$$

Phytochemical Extraction

The extraction process employed successive and single-solvent methods to optimise the recovery of bioactive compounds from *C. zedoaria* leaves. In the successive method, 25 g of powdered leaves material was mixed with 125 mL of hexane in amber glass vials, followed by methanol, adhering to a polarity gradient for comprehensive compound recovery. Extractions were conducted at 30°C with stirring at 500 rpm for 24 hours, and the residue underwent three additional 30-minute extractions under identical conditions to maximise yield. The combined filtrates were concentrated via vacuum evaporation, yielding dried extracts reconstituted in methanol at 10 mg/mL for analysis. For the single-solvent method, 25 g of powdered material was extracted exclusively with methanol using the same protocol. Filtrates from both methods were pooled and evaporated to dryness for further investigation. This standardised approach ensured efficient recovery and reliable phytochemical and pharmacological properties analyses (Allaq *et al.*, 2021).

Metabolite Screening by HPLC

The 10 mg of the extracts were dissolved in acetonitrile and filtered using a 0.45 µm syringe filter. The filtered samples were subsequently injected into the HPLC system, along with the standards 4 4-hydroxycoumarin, quercetin, coumarin, and 6 6-methyl-coumarin. To quantify and identify the concentration of coumarin in A. galanga L. samples, a HPLC system (Dionex Ultimate 3000, Thermo Scientific Series, USA) was utilised. The system had a binary pump, a degasser, a thermostatic auto-sampler, and a diode array detector (DAD). The samples were separated on a Synergy 4u Hydro-RP 80A Phenomenex Column C-18 (250 mm x 4.6 mm, i.d 5µm) maintained at 25ºC. The HPLC parameters consist of a 1 ml/min flow and an injection volume of 20 µL. The mobile phase comprised UPW (A) and MeCN (B). A gradient elution program was used as follows: 5 –90 % B (0.00 – 15 min), 90% (15-20 min) and 5 % B (21 –23 min). After each injection, the needle was washed with 100 % MeCN. The detector wavelength was set to 210 nm to measure 4 4-hydroxycoumarin, quercetin, coumarin, and 6 6-methyl-coumarin concentration, and the analysis was performed over a runtime of 30 minutes. A measure 4 of 4-hydroxycoumarin, quercetin, coumarin, and 6 6-methyl-coumarin standard was prepared by dissolving in methanol. The resulting chromatogram was used for comparing the samples' retention time against the coumarin, 4- 4-hydroxycoumarin, 6- 6-methyl coumarin and quercetin standard qualitative (Geethanjali *et al.*, 2024). The phytochemicals within each sample based on known standards, were quantified using the following formula (Rana, Ganarajan & Kothiyal, 2015).

$$The\ percentage\ of\ compound\ = \{\ (Peak\ area\ of\ sample\ /\text{Peak area of standard})\ \times\ (\text{Concentration of standard}\ /\text{Concentration of sample})\ \}\ \times \text{ purity of standard}$$

GCMS conditions

Phytochemical screening of the aggregated *C. zedoaria* leaves samples were performed using a GC-MS system (Agilent 7890A) equipped with an auto-injector (Agilent 7693 Series) and a mass detector (Agilent Technologies 5975 Inert XL EI/CI Mass Selective Detector with Triple-Axis Detector). The crude n-hexane and methanolic extracts were separated on an HP-5 MS capillary column (Agilent; 0.25 mm × 30 m, 0.25 µm) utilising an auto-sampler to ensure precision. The temperature program was meticulously optimised to achieve efficient separation of phytochemicals. The analysis commenced at an initial temperature of 100°C, which was maintained for 2 minutes, followed by an increase to 120°C at a rate of 10°C/min, held for an additional 2 minutes. Subsequently, the temperature was raised to 280°C at a rate of 5°C/min and held for 10 minutes. The final step involved maintaining the column at 280°C for 2 minutes, resulting in a total run time of 66 minutes per sample. Helium was the carrier gas at a 3.0 mL/min flow rate to ensure optimal separation efficiency. The detector and injector temperatures were consistently maintained at 200°C, with the analysis conducted in split mode at a ratio of 10:1 for precise sample injection. The MS results were interpreted using the NIST database, where the spectra of unknown compounds were compared against known entries in the library, facilitating accurate compound identification (Allaq *et al.*, 2021).

Results

Extractive Yield

The extractive yields of *C. zedoaria* varied significantly with the solvent type (Table 1). Due to its high polarity and broad solubility, methanol achieved the highest yield (15.5%), while the non-polar n-hexane produced the lowest (3.5%). These results highlight the importance of solvent polarity in optimising the recovery of bioactive compounds, essential for effective phytochemical extraction and analysis.

Table 1: Percentage Yield of Different Extracts of *Curcuma zedoaria*

No	Solvent used	Weight of extract (g)	% Yield
1	Methanol	0.1550 g	15.5 %
2	n-hexane	0.0357 g	3.5 %

Qualitative Analysis of C. zedoaria

Samples were conducted using HPLC, with four commercial standards: 4-hydroxycoumarin, quercetin, coumarin, and 6-methyl coumarin standard employed to quantify their respective concentrations. The separation process was performed on a C18 reverse-phase column, utilising high-performance liquid chromatography with a runtime of 30 minutes to ensure efficient compound resolution. The chromatograms displayed sharp, symmetrical peaks corresponding to 4-hydroxycoumarin, quercetin, coumarin, and 6-methyl coumarin, which were identified at retention times of 7.167, 9.660, 9.86 and 10.927 minutes, respectively, as shown in Figure 3. The concentrations of these compounds in *C. zedoaria* were quantified as 7.45 mg/kg DW for 4-hydroxycoumarin, 6.79 mg/kg DW for quercetin, 14.41 mg/kg DW for coumarin, and 0.088 mg/kg DW for 6-methylcoumarin. These results highlight the presence of bioactive phytochemicals in the plant and provide a foundationn for further exploration of its pharmacological potential.

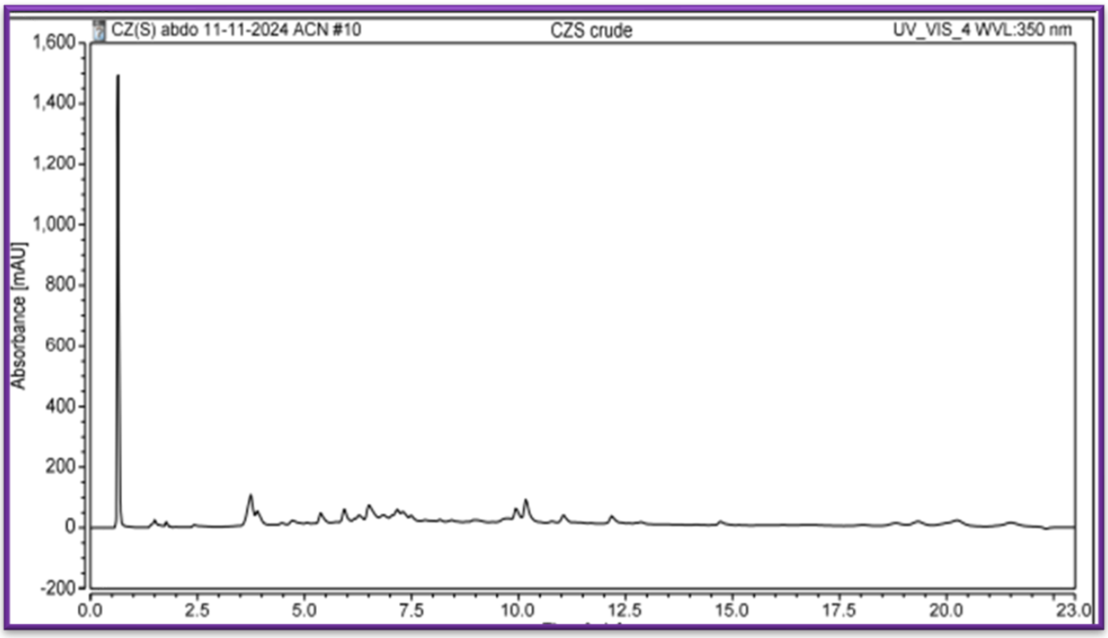

Figure 3: HPLC Quercetin Chromatogram Pattern of Different Varieties of 4-Hydroxycoumarin, Quercetin, Coumarin, And 6-Methyl-Coumarin, Standard

Advanced chromatographic techniques have been extensively utilised to identify and quantify coumarins and related phytochemicals across diverse plant matrices. Applied molecular networking and DDA-MS/MS to analyse Curcuma wenyujin in vivo, successfully detecting 4-hydroxycoumarin at a retention time of 3.05 minutes (Wang *et al*., 2020). Similarly, Phyllanthus acidus leaves and fruits were investigated using HPLC-MS/MS, identifying 4-hydroxy coumarins with intensities of 63.34 (CHI

2.8) and 36.47 (CHI 7.0), along with coumarin intensities of 57.51 (CHI 2.8) and 61.35 (CHI 7.0), highlighting their bioactive significance (Ilijaš *et al*., 2013).

Further advancements in HPLC methodologies have enhanced the accuracy of coumarin detection in complex matrices. HPLC-DAD was employed to quantify 4-hydroxycoumarin and its derivatives in propolis, identifying retention times of 20.790 and 23.230 minutes based on UV spectral data (190–400 nm) (Kulyal *et al*., 2021). Subsequently, the detection method was refined to 280 nm, achieving shorter retention times of 2.11 and 2.34 minutes, demonstrating improved sensitivity (Machyňáková & Hroboňová, 2017). The HPLC-ESI-QqQLIT-MS/MS method was further optimised for cinnamon samples, identifying coumarin at 8.38 minutes with a 320–340 nm wavelength range, confirming the precision of the method (Hroboňová *et al*., 2013). In turmeric (Curcuma longa L.), characterised by methyl-7-methoxy coumarin, the molecular formula ($C_{11}H_{10}O_3$), molecular weight (190.1953), and retention time (67927) were defined, further elucidating its bioactive role (Kulyal *et al*., 2021). Cinnamon biscuit extracts using HPLC-PDA detected a coumarin peak at 12.3 minutes, although chamomile infusions interfered at the same retention time, emphasising the necessity of standard verification (Scotter, Roberts & Rees, 2011).

Beyond coumarins, quercetin detection has been refined through various chromatographic approaches. RP-HPLC was employed to analyse *Curcuma angustifolia* Roxb. rhizomes, identifying quercetin at 10.01 minutes with a 266 nm detection wavelength (Allabaksh & Senthilraj, 2024). An optimised RP-HPLC method was developed for curcumin (99%) and quercetin, detecting the latter at 8.78 minutes, showcasing the technique’s reliability (Patil & Mahajan, 2022). These findings were further validated, quantifying quercetin in polyherbal formulations, reporting concentrations of 9.5 mg (HPLC) and 9.7 mg (HPTLC), with minor variations across methods (Baghel *et al.,* 2017). A Shimadzu-LC system was utilised for quercetin separation, achieving a retention time of 32.195 minutes (Ang *et al*., 2014), while *Phyllanthus acidus* was analysed using UHPLC-MS, identifying a target compound at 2.83 minutes, further illustrating the robustness of modern chromatographic systems (Abd Ghafar *et al.,* 2018).

GC-MS analysis of *C. zedoaria*

The GC-MS chromatographic analysis of n-hexane leaves extracts of *C. zedoaria* identified 37 peaks corresponding to various bioactive compounds.

These compounds were characterised by correlating their retention time, peak area (%), and mass spectral fragmentation patterns with the reference data from the National Institute of Standards and Technology (NIST) library, as shown in Figure 4.

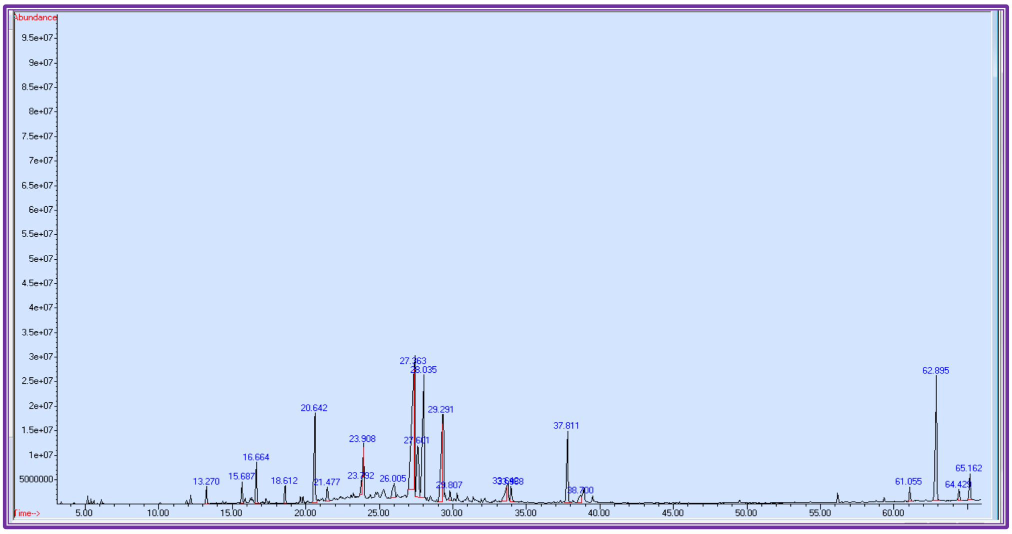

Figure 4: GC-MS Chromatogram of the n-hexane Extract of *C. zedoaria* Leaves

Among the 36 compounds identified in the n-hexane leaf extracts of **C. zedoaria** using GC-MS, 11 compounds were highlighted for their high peak areas (Table 2). Epicurzerenone (2.51%), curcumenol (4.63%), (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho\[2,3-b] furan-4(6H)-one (4.50%), isofuranodienone (11.89%), and vitamin E (2.99%) were among the key bioactive compounds identified. These compounds represent the dominant phytochemicals, with isofuranodienone showing the highest area percentage (11.89%). These results confirm the significance of *C. zedoaria* as a valuable source of bioactive compounds with potential pharmacological applications.

Table 2: Chemical Constituents Identified in n-hexane Leaves Extracts of *C. zedoaria*

NO	RT (min)	Molecular Mass (g/mol)	Area (%)	Compound Name	Chemical Formula	Qual (%)	Class
1	5.206	152.23	0.24	(+)-2-Bornanone	C10H16O	98	Terpenoid
2	5.442	144.12	1.21	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	58	Pyrones
3	16.631	204.34	0.71	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	C15H24	91	Terpenes
4	20.544	248.31	2.51	Epicurzerenone	C15H20O3	99	Terpenoids
5	21.462	220.34	1.01	Isospathulenol	C15H24O	96	Terpenoids
6	23.832	218.33	2.54	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (Z,E)	C15H22O	99	Terpenoids
7	24.145	125.21	0.79	2-(1-Cyclohexenyl) ethylamine	C8H15N	43	Amines
8	24.54	220.34	0.73	cis-Z,alpha Bisabolene epoxide	C15H24O	46	Bisabolane
9	24.722	164.2	0.39	Tricyclo[3.2.2.0]nonane-2-carboxylic acid	C10H12O2	50	Carboxylic Acid
10	24.857	220.34	0.75	Longipinocarveol	C15H24O	45	Alcohols
11	25.163	234.33	4.63	Curcumenol	C15H22O2	99	Terpenoids
12	25.856	96.17	2.18	3-Heptyne	C7H12	35	Alkyne
13	25.954	164.25	3.44	Tetrydamine	C10H16N2	25	Indazoles
14	26.819	162.26	0.54	3,4-Nonadien-6-yne, 5-ethyl-3-methyl	C12H18	45	Alkene
15	27.046	110.15	23.22	Bicyclo[3.1.0] hexan-3-one	C7H10O	52	Alkanes
16	27.417	232.31	4.5	Furanodienone	C15H20O2	95	Terpenes
17	27.716	232.31	11.89	Isofuranodienone	C15H20O2	99	Furanodiene Derivative
18	29.035	196.28	13.4	Fragranyl acetate	C12H20O2	50	Ester
19	29.704	234.37	1.13	4-Cycloocten-1-one, 8-(4-octen-4-yl)	C16H26O	48	Ketone
20	29.822	184.23	0.71	3-Hydroxy-3-(4-methylcyclohex-3-enyl) propionic acid	C10H16O3	53	Carboxylic Acid
21	31.927	270.44	0.39	Hexadecanoic acid	C17H34O2	97	Fatty Acid
22	32.133	232.31	0.88	(3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-b] furan-2(3H)-one	C15H20O2	91	Terpenoids
23	32.39	290.39	0.74	Benzenepropanoic acid	C18H26O3	99	Carboxylic Acid
24	33.34	256.42	0.75	Palmitic Acid	C16H32O2	96	Fatty Acid
25	33.606	216.12	1.04	1,3-Cyclohexadiene, 1,2,5,5,6,6-hexafluoro-3,4-dimethyl	C8H6F6	38	Fluorinated Compound

26	33.922	250.33	1.18	Zederone	$C_{15}H_{22}O_{3}$	46	Terpenes
27	34.042	252.47	1.3	1-Octadecene	$C_{18}H_{36}$	98	Alkene
28	34.616	192.33	3.24	1,8-Nonadiene, 2,7-dimethyl-5-(1-methylethenyl)	$C_{14}H_{24}$	43	Terpenoids
29	36.133	200.31	4.26	Dodecanoic acid	$C_{12}H_{24}O_{2}$	51	fatty acid
30	36.869	141.55	2.53	6-Chloronicotinic aldehyde	$C_{6}H_{4}ClNO$	35	Aldehyde
31	37.303	84.16	1.17	Cyclohexane	$C_{6}H_{12}$	49	Cycloalkanes
32	37.717	296.52	2.52	Phytol	$C_{20}H_{40}O$	91	Terpenes
33	38.791	141.55	1.15	6-Chloronicotinic aldehyde	$C_{6}H_{4}ClNO$	43	Pyridines
34	43.09	228.28	2.99	Triphenylene	$C_{18}H_{12}$	68	Chrysenes
35	62.761	430.69	2.99	Vitamin E	$C_{29}H_{50}O_{2}$	98	Tocopherol
36	64.405	356.69	1.11	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(3-fluoroanilino) propionate	$C_{13}H_{13}ClF_{4}N_{2}O_{3}$	50	Amino Acid

GC-MS analysis of methanol extracts from *C. zedoaria* identified esters, volatile compounds, long-chain alcohols, and acids Figure 5.

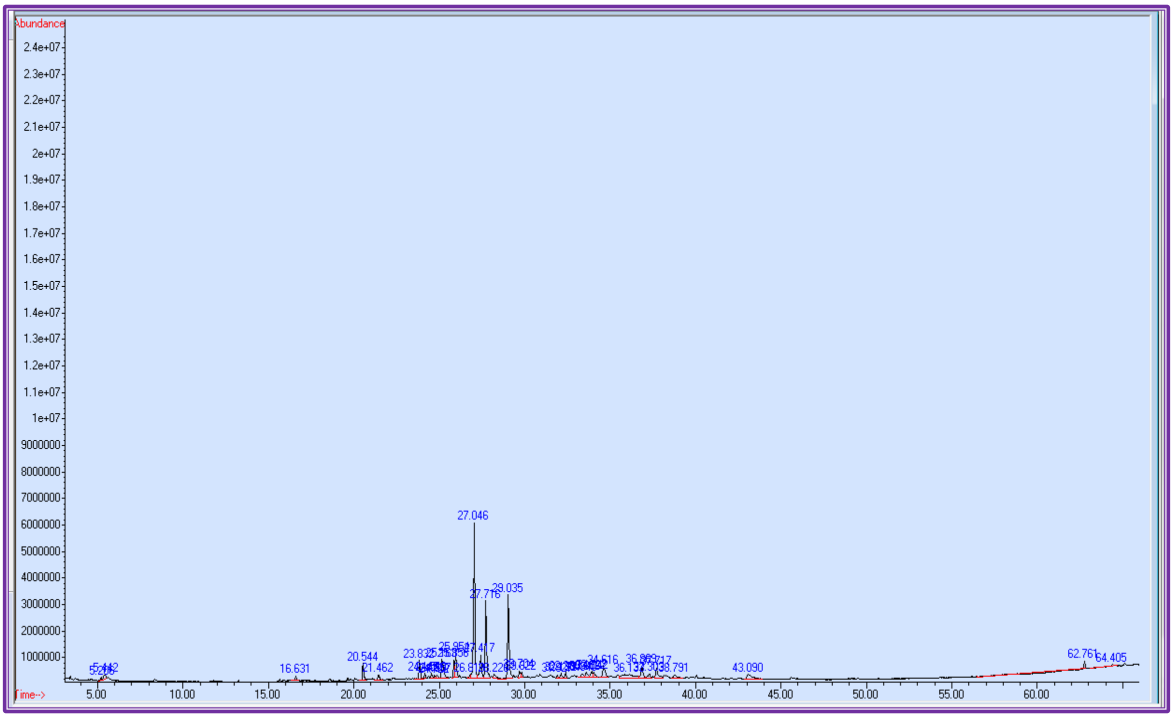

Figure 5: GC-MS Chromatogram of the Methanolic Extract of *C. zedoaria* Leaves

Table 3 presents the chemical constituents identified in the methanolic leaf extract of *C. zedoaria*. The major compounds included 5-hexenal, 4-methylene (21.3%), isofuranodienone (15.2%), vitamin E (12.0%), cis, cis-2,7-nonadiene (10.3%), epicurzerenone (7.8%), furanodienone (6.9%), and phytol (5.7%). Other notable constituents were 9,12-octadecadienoic acid (*Z, Z*) (1.1%), caryophyllene (1.0%), germacrene D (1.2%), and n-hexadecanoic acid (1.9%). In total, twenty-two distinct peaks were detected, reflecting the rich phytochemical diversity present in the methanol extract of *C. zedoaria* leaves.

Table 3 : Chemical Constituents Identified in Methanol Leaves Extracts of *Curcuma zedoaria*

NO	RT (min)	Molecular Mass (g/mol)	Area (%)	Compound Name	Chemical Formula	Qual (%)	Class
1	13.27	204.34	1.03	Caryophyllene	$C_{15}H_{24}$	99	Terpenes
2	15.687	204.34	1.28	Germacrene D	$C_{15}H_{24}$	99	Terpenes
3	16.664	204.34	2.78	ethenyl-1-methyl-2,4-bis(1-methylethenyl)	$C_{15}H_{24}$	93	Terpenes
4	18.612	204.34	1.05	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethylidene)-, (E,E)	$C_{15}H_{24}$	99	Terpene
5	20.642	232.31	7.88	Epicurzerenone	$C_{15}H_{20}O_2$	99	Terpenoids
6	21.477	220.34	1.17	Isospathulenol	$C_{15}H_{24}O$	99	Terpenoids
7	23.792	232.31	1.03	(4aS,8aR)-3,8a-Dimethyl-5-methylene-4,4a,5,6,7,8,8a,9-octahydronaphtho[2,3-b] furan	$C_{15}H_{20}O$	70	Terpenes
8	23.908	218.33	1.16	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (Z,E)	$C_{15}H_{22}O$	99	Terpenoids
9	26.005	196.12	2.04	4H-1-Benzopyran-3-carboxaldehyde, 6-fluoro-4-oxo	$C_{10}H_5FO_3$	35	benzopyran
10	27.363	110.15	21.36	5-Hexenal-4-methylene	$C_7H_{10}O$	49	Fatty acids
11	27.601	230.31	6.93	Furanodienone	$C_{15}H_{18}O_2$	95	Terpenes
12	28.035	230.31	15.21	Isofuranodienone	$C_{15}H_{18}O_2$	99	Terpenes
13	29.291	124.24	10.32	cis-2,7-Nonadiene	C_9H_{16}	38	alkenes
14	29.807	192.31	0.64	4-(6,6-Dimethyl-2-methylene cyclo hex-3-enylidene) pentane-2-ol	$C_{13}H_{20}O$	41	Terpene alcohol
15	33.642	256.42	1.92	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	99	Fatty acid
16	33.968	246.31	1.22	Zederone	$C_{15}H_{18}O_3$	99	Terpenes
17	37.811	296.52	5.79	Phytol	$C_{20}H_{40}O$	98	Terpenes
18	38.7	280.45	1.15	Linoleic acid	$C_{18}H_{32}O_2$	99	Fatty acid
19	61.055	400.68	1.04	Gamma-Tocopherol	$C_{28}H_{48}O_2$	99	Tocopherol
20	62.895	430.69	12.07	Vitamin E	$C_{29}H_{50}O_2$	99	Tocopherol
21	64.429	400.68	0.91	Campesterol	$C_{28}H_{48}O$	95	Steroids
22	65.162	414.72	2.02	Stigmasterol	$C_{29}H_{48}O$	99	Steroids

Discussion

The variation in compounds extracted using methanol and n-hexane from *_C. zedoaria_* leaves is largely influenced by the polarity of the solvents (Zhang *et al*., 2018). Methanol, being polar, extracted flavonoids and other hydrophilic compounds, while n-hexane, a non-polar solvent, selectively extracted terpenoids, fatty acids, and other lipophilic constituents. Several bioactive compounds appeared in both extracts, showing similar structural features with slight variations in retention times (Setyani *et al*., 2020). These solvent-dependent differences impact the pharmacological profiles of the extracts and highlight the importance of solvent selection in targeting specific bioactive groups. The presence of unique compounds in each extract may indicate underexplored or novel metabolites worthy of further investigation (Lee *et al*., 2024).

For example, 3,7-cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene) showed retention times of 23.908 minutes in hexane and 23.832 minutes in methanol. Similarly, cyclohexane, 1-ethenyl-1-

methyl-2,4-bis (1-methylphenyl), appeared at 16.664 and 16.631 minutes, respectively. Compounds such as epicurzerenone, furanodienone, isofuranodienone, isospathulenol, n-hexadecanoic acid, phytol, vitamin E, and zederone also exhibited minor shifts in retention time, underscoring the influence of solvent polarity on extraction efficiency and compound behaviour.

Recent studies support the pharmacological potential of these compounds. For instance, 3,7-cyclodecadien-1-one, a major bioactive from Rhizoma *Curcuma*, has demonstrated anticancer, antioxidant, and antiviral activities (Wang & Kannan, 2019). β-Elemene, isolated from *Curcuma wenyujin*, has been shown to inhibit tumour growth, induce apoptosis, and exert minimal toxicity, marking it as a promising chemotherapeutic agent (Bai *et al.*, 2021; Chen *et al.*, 2023). Epicurzerenone exhibits both antimicrobial and antioxidant effects (Liang *et al.*, 2020; Syahbirin, Aditianingrum & Mohamad, 2024).

Furanodienone, extracted from *Lindera pulcherrima*, possesses antioxidant, anti-inflammatory, and antibacterial properties, and shows cytotoxic effects against lung cancer cells (Aggarwal *et al.*, 2013; Al Saqr *et al.*, 2022). Isofuranodienone also demonstrates cytotoxic, antimicrobial, and anti-inflammatory potential (Sura & Cheng, 2024). Isospathulenol has been highlighted for its potent antioxidant activity, while n-hexadecanoic acid is associated with enzyme regulation, antimicrobial properties, and traditional medicinal use (Alves dos Santos *et al.*, 2025; Arya *et al.*, 2024; Zahara *et al.*, 2024).

Phytol has been extensively studied for its antitumoral, antioxidant, and cosmeceutical applications (Alencar *et al.*, 2018; Rosa *et al.*, 2024). Vitamin E contributes to cellular membrane stabilisation, enhances bioavailability, and plays a crucial role in redox regulation and cognitive health (Farina *et al.*, 2017; Schmölz *et al.*, 2016). Finally, zederone, a compound native to **C. zedoaria**, exhibits cytotoxic, antimicrobial, and neuroprotective properties, supporting its potential in cancer treatment and cosmetic formulations (Hamdi *et al.*, 2015; H. Zhang *et al.*, 2020).

Conclusion

This study demonstrates that *Curcuma zedoaria* (*C. zedoaria*) leaves are a valuable source of phytochemicals exhibiting notable biological activity. The chemical profiles of the n-hexane and methanol extracts differ significantly, though some constituents overlap, emphasising the importance of solvent selection in isolating bioactive compounds. The results uncover the largely untapped therapeutic and industrial value of *C. zedoaria* leaves, with promising applications in pharmaceuticals, agrochemicals, and biotechnology. By shifting attention to non-rhizome plant parts, this research challenges the conventional focus on rhizomes and promotes a more comprehensive exploration of the plant's phytochemistry. Future investigations should focus on isolating and characterising individual compounds, alongside assessing their specific biological activities, to unlock their full potential. The detection of antioxidant, anti-inflammatory, and antimicrobial agents, correlated with solvent polarity, further underscores the need for strategic extraction methods in phytopharmacological research. To validate these findings, subsequent studies should incorporate bioactivity-guided isolation, mechanistic analysis, and toxicity assessments. Clinical trials and formulation development are also recommended to support the advancement of *C. zedoaria* leaf-derived compounds in therapeutic applications.

Conflict of Interest

The authors declare that they have no competing interests.

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