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Probing the phytochemical profile of rhizomic and inflorescence extract of *Zingiber zerumbet* (L.) Smith by gas chromatography and mass spectroscopy

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Abstract

Zingiber zerumbet (L.) Smith, an underutilized species within the Zingiberaceae family, commonly known as shampoo ginger, is a perennial herb native to tropical and subtropical Asia, valued for its extensive medicinal potential and biological activities. Its rich phytochemical profile includes sesquiterpenoids, flavonoids and aromatic compounds, with zerumbone as the principal bioactive constituent, renowned for potent anti-inflammatory, anticancer, antimicrobial and antioxidant properties. Traditionally, *Z. zerumbet* has been used to treat a wide range of ailments, including inflammation, digestive disorders, skin diseases and infections, while its mucilaginous inflorescence gel is valued as a natural shampoo and conditioner. This study aimed to explore the phytochemical profile of *Z. zerumbet*, targeting to spotlight its therapeutic and pharmaceutical (in the field of cosmetic) significance and the need for further biotechnological research to harness its full potential. Fresh rhizomes and inflorescence gel were collected from plants grown under Eucalyptus trees in Horticultural Research Station, Pechiparai, Tamil Nadu and analyzed using GC-MS technique. The analysis revealed a complex spectrum of phytochemicals, identifying a total of 115 compounds in the rhizome and 56 in the inflorescence gel, with higher relative abundances in the latter. Key bioactive constituents in the rhizome included 4-allyl-1,2-diacetoxybenzene, (E)-β-farnesene and eucalyptol, comprising nearly 80% of the phytochemical content. The inflorescence gel was rich in 4-chromanol, β-sitosterol and caryophyllene, among others, known for anti-inflammatory, antimicrobial and antioxidant activities. These findings highlight the influence of plant part and environmental conditions on secondary metabolite synthesis. The diverse bioactive metabolites identified position *Z. zerumbet* as a promising candidate for pharmaceutical and natural cosmetic development. Further research on metabolite enhancement through biotechnology could unlock sustainable commercial applications from this valuable species.

1. Introduction

Plants serve as foundational biological resources across human sectors, including nutrition, agriculture, materials science and renewable energy production. Within this spectrum, their medicinal utility is significant, underpinning around 80% of primary healthcare worldwide through bioactive phytochemicals such as alkaloids, flavonoids and terpenoids. A wide array of plant species contributes to diverse pharmacological and ethnomedicinal applications, leveraging their rich phytochemical diversity to address multifarious therapeutic targets. These natural sources remain vital as preferences shift to plant-derived drugs for superior efficacy, biocompatibility and reduced toxicity over synthetics.

Among the millions of existing plant species, *Zingiber zerumbet* (L.) Smith emerges as a particularly versatile and pharmacologically promising perennial herb. Its taxonomic classification places it within the kingdom Plantae, division Angiosperms, class Monocots, order Zingiberales and family Zingiberaceae. This family, commonly known as the ginger family, comprises over 50 genera and more than 1,600 species of aromatic, rhizomatous herbs, including economically vital genera like Zingiber (ginger), Curcuma (turmeric), Alpinia (galangal) and Elettaria (cardamom) (Deng *et al.*, 2022). *Z. zerumbet* is commonly known as shampoo ginger, pinecone ginger or bitter ginger and is indigenous to tropical and subtropical Asia with a global distribution that has expanded through both natural dispersal and cultivation (Pradhan and Sarkar, 2023). The plant is now found widely across the Indian subcontinent, Southeast Asia (including Malaysia, Indonesia and Thailand), Southern China, the Pacific Islands (notably Hawaii) and parts of northern Australia and Central America, thriving in humid, shaded environments of lowland forests and along stream banks.

The Zingiberaceae family is renowned not only for its economic importance as a source of spices, flavorings and ornamentals but also for its exceptionally rich and diverse phytochemistry (Singh *et al.*, 2012). Characteristic bioactive compound classes within this family include volatile terpenoids (especially mono and sesquiter-

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penes), diarylheptanoids, phenylpropanoids, flavonoids and phenolic acids. These compounds are responsible for the distinctive aromas, flavors and a broad spectrum of pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial and anticancer effects, which underpin the family's extensive use in traditional medicine systems worldwide (Nag *et al.*, 2013).

The ethnobotanical applications of *Z. zerumbet* demonstrate remarkable diversity, mirroring its morphological variations. This species is almost a multi-purpose medicinal herb, with various vegetative parts extensively utilized in traditional healing systems throughout Asia, India, China and the Arabic world. In Ayurveda and traditional Indian medicine, the rhizome is used as a carminative, digestive, and antiemetic agent. In Malay traditional medicine, it is applied to treat edema, wounds, and sores. In Chinese folk medicine, preparations are used for their antipyretic and analgesic properties. The rhizome has been the most extensively utilized in folk medicine to address an extensive range of ailments (Yob *et al.*, 2011). Traditional practitioners have used it to treat inflammation, diarrhea, stomach cramps, bacterial infections, fever, flatulence, allergies and even poisoning (Rawat *et al.*, 2023). In Hawaiian traditional medicine, the cooked and softened rhizome (Awapuhi) was applied directly to dental cavities to alleviate toothaches, while a preparation of ground and strained rhizome mixed with water was administered orally to relieve stomach ailments (Ghosh *et al.*, 2011). Additionally, the stems and rhizomes function as seasoning agents in culinary practices and the floral buds are consumed as vegetables, highlighting the plant's role as a functional food source (Raina and Misra, 2025).

A unique and defining characteristic of *Z. zerumbet*, which provides the basis for its common name, is the clear, fragrant, mucilaginous liquid contained within the mature inflorescence. This creamy substance, abundant in saponins, has been traditionally utilized as a natural shampoo and hair conditioner by communities in Hawaii and Southeast Asia (Rawat *et al.*, 2023). The gel not only effectively cleanses the scalp but also softens hair while imparting a pleasant fragrance-a practice that continues to the present day and has garnered significant interest from the modern cosmetic industry.

The volatile oil derived from the rhizome has been the subject of intensive scientific investigation. It is characterized by the presence of key sesquiterpenes, with zerumbone representing the most prominent and biologically significant constituent. Other notable components include humulene, camphene and limonene - the latter reportedly occurring exclusively in *Z. zerumbet* among closely related species, potentially serving as a chemotaxonomic marker (Koga *et al.*, 2016). Zerumbone, a unique monocyclic sesquiterpenoid first isolated from the rhizomes of this species, has been established as a potent anti-inflammatory agent, forming a fundamental basis for scientific interest in the plant (Matsuo and Takeyama, 2014). This compound further demonstrates pronounced anticancer activity by inhibiting tumor promotion and suppressing proliferation in colon, skin, liver, breast and leukemia cell lines-primarily through the induction of apoptosis (programmed cell death) (Yu *et al.*, 2008). Furthermore, its anti-HIV and antifungal activities have revealed promising avenues for developing new therapeutic agents and eco-friendly alternatives to synthetic fungicides (Kaur *et al.*, 2025).

Z. zerumbet significance is further enhanced with rising demand for natural, sustainable products, driven by concerns over commercial shampoos' harsh surfactants, parabens, silicones and fragrances causing scalp irritation, hair damage and pollution - which stimulated renewed interest in herbal and plant-based alternatives. Its traditional use and validated antimicrobial/antioxidant phytochemistry position it as a key "green" alternative for herbal shampoos, conditioners and skincare (Bhogal *et al.*, 2025).

Despite the considerable economic and therapeutic potential of *Z. zerumbet*, significant challenges and knowledge gaps persist. Wild Zingiberaceae species, including *Z.zerumbet,* have received comparatively limited attention from plant scientists and biotechnologists (Mohanty *et al.*, 2012). A comprehensive phytochemical profiling is a critical first step to bridge this gap, validate traditional uses, and identify novel bioactive compounds. Gas chromatography and mass spectrometry (GC-MS) analysis is an indispensable tool for this purpose. It allows for the precise separation, identification, and quantification of volatile and semi-volatile compounds within complex plant matrices. For *Z. zerumbet*, applying GC-MS to analyze both the rhizome oil and the inflorescence gel is particularly important. This dual analysis can provide a complete chemical fingerprint, revealing not only the known major constituents like zerumbone but also minor compounds that may contribute synergistically to its biological activities (Dash *et al.*, 2020). Furthermore, such profiling can clarify chemotaxonomic relationships, identify quality markers for standardization and pinpoint compounds responsible for its cosmetic and dermatological properties, thereby strengthening the scientific rationale for its development into commercial phytopharmaceuticals and cosmaceuticals. To thoroughly investigate the complete phyto-chemical profile of both the *Z. zerumbet* rhizome and its distinctive gel, this study aimed to perform GC-MS analysis on these two plant components, laying the groundwork for further research.

2. Materials and Methods

2.1 Plant material and authentication

The fresh experimental materials of *Z. zerumbet, i.e.*, rhizomes and inflorescence were collected from the plants grown amidst the Eucalyptus trees at Horticultural Research Station, Pechiparai, Kanyakumari District, Tamil Nadu.

Regarding the plant authentication, the specimen was botanically verified and authenticated by Dr. M. Johnson, Curator, Centre for Plant Biotechnology, St. Xavier's College, Palayamkottai, Tirunelveli, Tamil Nadu. Moreover, herbarium was deposited and preserved in Centre for Plant Biotechnology Herbarium (Indexed on 2022), St. Xavier's College, Palayamkottai, Tirunelveli with Voucher specimen number SXC-CPBH-5612 for future reference.

2.2 Cultivar description

The plant typically reaches heights of 1-1.5 m, occasionally extending to 2 m, featuring either sessile or petiolate leaves. It's inflorescence-a cone-shaped spike that emerges directly from the rhizome on a separate, shorter stalk. This inflorescence undergoes a notable color transformation from green in its youth to vibrant red upon maturation as shown in Figure 1, maintaining its appearance for several weeks (Chavanand Dey, 2023; Shinija *et al.*, 2009).

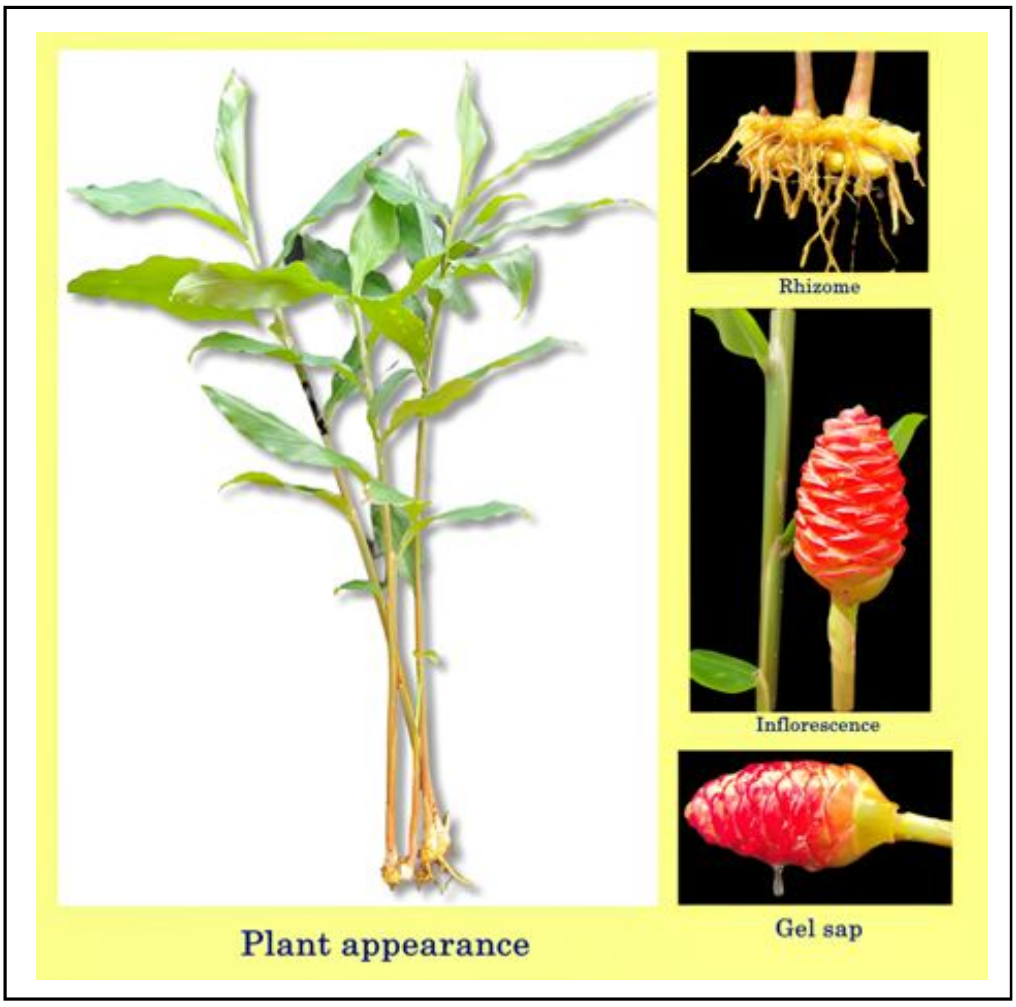

Figure 1: Morphological appearance of *Zingiber zerumbet* (L.).

2.3 Sample preparation and metabolite extraction

2.3.1 Crude methanolic extract from rhizome

Dried and powdered rhizomes of *Z. zerumbet* (2 g) were mixed with methanol (1:10 w/v) and subjected to extraction by reflux in a Soxhlet apparatus at 65°C for 35 min. The methanolic extract was filtered and the solvent was removed under reduced pressure using a rotary evaporator at 40°C to obtain the crude extract. The recovery yield was determined using the formula given below. Extractions were conducted in triplicate.

Rhizome extraction yield (%) =

$$\frac{\text{Mass of crude extract}}{\text{Mass of initial rhizome powder}} \times 100 \text{ (w/w)}$$

2.3.2 Crude lipophilic extract from inflorescence

Mature inflorescences of *Z. zerumbet* were harvested, washed and squeezed manually to obtain semi-liquid pulp (200 g fresh weight). The pulp was macerated with distilled water (1:3 w/v) by hand-kneading for 20 min to extract the mucilaginous, soap-like gel, followed by squeezing through four layers of muslin cloth to collect the aqueous gel filtrate. The filtrate was centrifuged at 4000 rpm for 10 min at 4°C to obtain the supernatant (100 ml), which was transferred to a separatory funnel. The supernatant was partitioned with n-hexane (1:1 v/v) in three successive extractions. The combined upper organic layers were dried over anhydrous sodium sulfate (3 g) for 30 min, filtered and concentrated under reduced pressure using a rotary evaporator at 40°C to yield the crude lipophilic extract. All extractions were performed in triplicate.The lipophilic fraction yield was computed as follows:

Inflorescence extract yield (%) =

$$\frac{\text{Mass of crude extract}}{\text{Mass of initial pulp obtained}} \times 100 \text{ (w/w)}$$

Both extracts were stored at 4°C in airtight vials prior to GC-MS analysis.

2.4 GC-MS analysis

Instrument specification: Perkin Elmer TurboMatrix 150 thermal desorber (USA) integrated with the system, operated at a 10:1 split ratio and helium carrier gas flow of 20 psi. The oven temperature was programmed to rise from 50°C to 250°C at 10°C/min. Mass

spectral detection employed positive ion electron impact ionization at 70 eV on a DB-5 capillary column (30 m × 0.25 mm, 0.25 m film thickness).

Analysis protocol: The solvent-free rhizome and inflorescence gel extracts were reconstituted in HPLC-grade methanol prior to analysis. An Agilent 7890A GC coupled to a 5975C MS system was used, featuring an initial oven temperature of 60°C held for 1.36 min, followed by a ramp to 325°C maintained for 23 min. The injector operated at 280°C with a 100:1 split ratio and 1 µl injection volume; helium served as carrier gas at 1 ml/min, yielding a total run time of 23 min, with mass spectra acquired across m/z 50-350.

2.5 Data interpretation

Compounds were identified by comparing the generated mass spectra against reference entries in the NIST (National Institute of Standards and Technology) mass spectral library. Relative quantification of major compounds detected in both rhizome and inflorescence gel extracts was achieved *via* GC-MS, based on peak areas from the corresponding total ion chromatograms.

3. Results

3.1 Recovery yield

The methanolic and lipophilic extract obtained from corresponding rhizome and inflorescence parts of *Z. zerumbet* is depicted in Figure 2. Results revealed that rhizomic extract had an higher average crude yield of 8.6%, whereas inflorescence gel extract recorded comparatively lower average lipophilic fraction of 2.36%.

3.2 GC-MS profiling

GC-MS analysis of both rhizome and inflorescence gel samples of *Z. zerumbet* displayed a wide spectrum of bioactive compounds and phytochemicals. The identification and confirmation of these phytoconstituents were carried out through the observation of peak areas and the distinctive properties of the screened secondary metabolites. The GC-MS screening of *Z. zerumbet* identified a total of 100 phytochemicals in the rhizome extract and 43 bioactive compounds in the inflorescence extract. However, the peak areas of corresponding secondary metabolites were higher in the inflorescence gel, indicating a greater relative abundance of certain compounds compared to the rhizome extract.

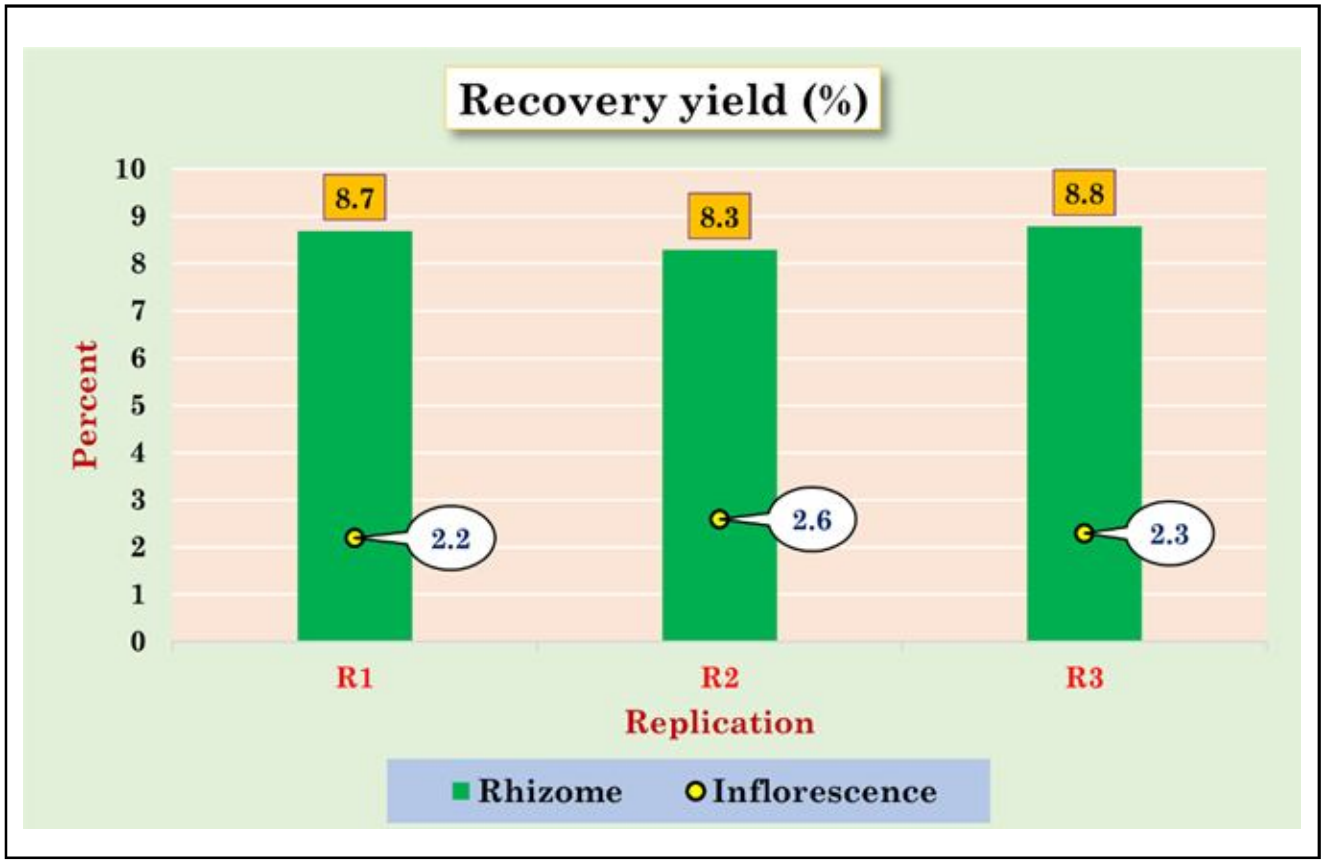

Figure 2: Recovery yield of rhizome and inflorescence extract of *Z. zerumbet*.

3.2.1 Rhizome

Chromatogram displayed in Figure 3 highlights peaks of multiple compounds found in the GC-MS analysis of *Z. zerumbet* rhizome extract along with the spectra and chemical structure of the chief phytochemical, *i.e.*, 4-allyl-1,2-diacetoxybenzene.

Among the 100 compounds identified, primary phytochemicals include 4-allyl-1,2-diacetoxybenzene, (E)-β-famesene, eucalyptol, humulene, 1-chlorohexadecane, chavibetol (m-eugenol) and farnesol, acetate with peak areas of 63.79%, 7.28%, 4.64%, 2.38%, 2.15%, 1.24% and 1.15%, respectively. These bioactive compounds collectively occupy nearly 80% of the overall phytochemical makeup. Table 1 presents details on key secondary metabolites screened, along with their peak areas (%) and retention times.

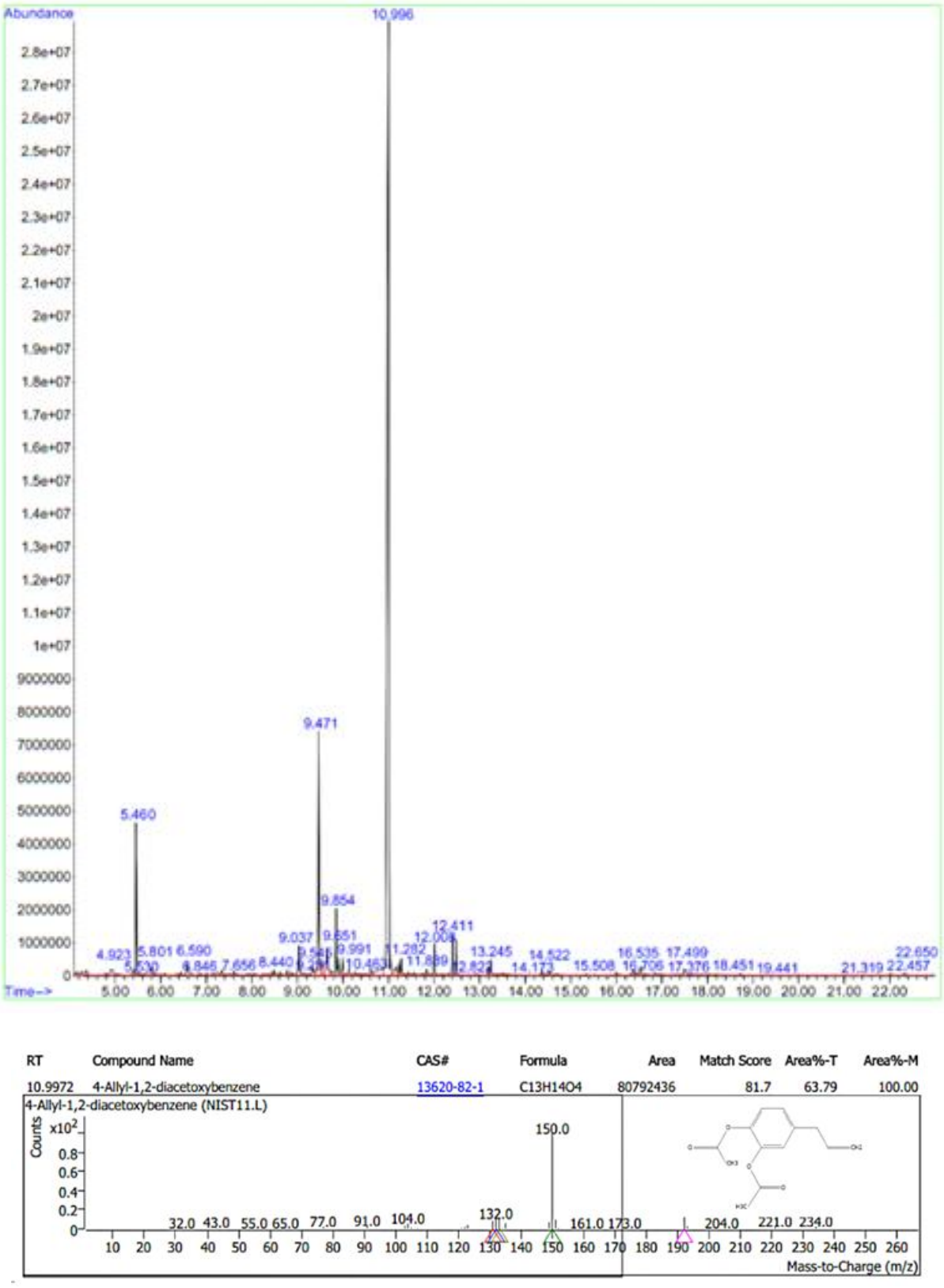

Figure 3: Chromatogram of rhizomic extract of *Z. zerumbet*.

Table 1:Phytochemical profile of rhizomic extract of *Z. zerumbet*

S. No.	Compounds	Retention time (min)	Peak area (%)
1	4-Allyl-1,2-diacetoxybenzene	10.9972	63.79
2	Farnesene (E, β)	9.464	7.28
3	Eucalyptol	5.4534	4.64
4	Humulene	9.6418	2.38
5	1-Chlorohexadecane	9.8529	2.15
6	Chavibetol (m-Eugenol)	9.0307	1.24
7	Farnesol, acetate	12.4081	1.15
8	(4-Methoxy-2,6-dimethylphenyl)boronic acid	12.0081	1.09
9	Phenol, 4-(3-hydroxy-1-propenyl)-	9.5418	0.96
10	1,5-Hexadien-3-ol	4.909	0.81
11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.5866	0.78
12	α-Farnesene	9.8973	0.55
13	Acetyleugenol	9.9862	0.55
14	n-Hexadecanoic acid	13.2413	0.55
15	Thymine	5.7978	0.53
16	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	17.4963	0.49
17	Lactonitrile, 3-chloro-, acetate	4.2647	0.40
18	β-Monopalmitin	16.5298	0.39
19	α-Pinene	4.3647	0.32
20	7-Hydroxy-1-indanone	11.1638	0.30
21	5-Hydroxymethylfurfural	7.3421	0.29
22	E-14-hexadecenal	11.2749	0.28
23	D-Limonene	5.3979	0.27
24	3-Deoxy-d-mannoic lactone	10.8638	0.26
25	2-Pentanone, 5-(1,2-propadienyloxy)-	7.2310	0.26
26	Diphenylsulfone	13.1858	0.25
27	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	10.1306	0.24
28	cis-Vaccenic acid	14.3856	0.24
29	Tetradecanoic acid	11.8304	0.23
30	Ethanamine, N-ethyl-N-nitroso-	6.4311	0.22
31	1,2-Heptanediol	9.1307	0.20
32	Stigmasterol	22.3291	0.16

3.2.2 Inflorescence gel

The principal bioactive compounds identified through GC-MS analysis of the gel extract of inflorescence of *Z. zerumbet* include 4-chromanol (10.27%), β-sitosterol (9.07%), d-glycero-d-ido-heptose (7.78%), 2,4,6-decatrienoic acid, ester(7.43%), n-hexadecanoic acid (5.57%), caryophyllene (4.41%), cis-vaccenic acid (4.01%) and eucalyptol (3.58%). A list of all the phytochemical compounds that was screened in the gel extract is presented in Table 2. Chromatogram of gel extract and the peak areas recorded for each phytochemical was displayed in Figure 4 along with the spectral absorbance and chemical structure of 4-chromanol.

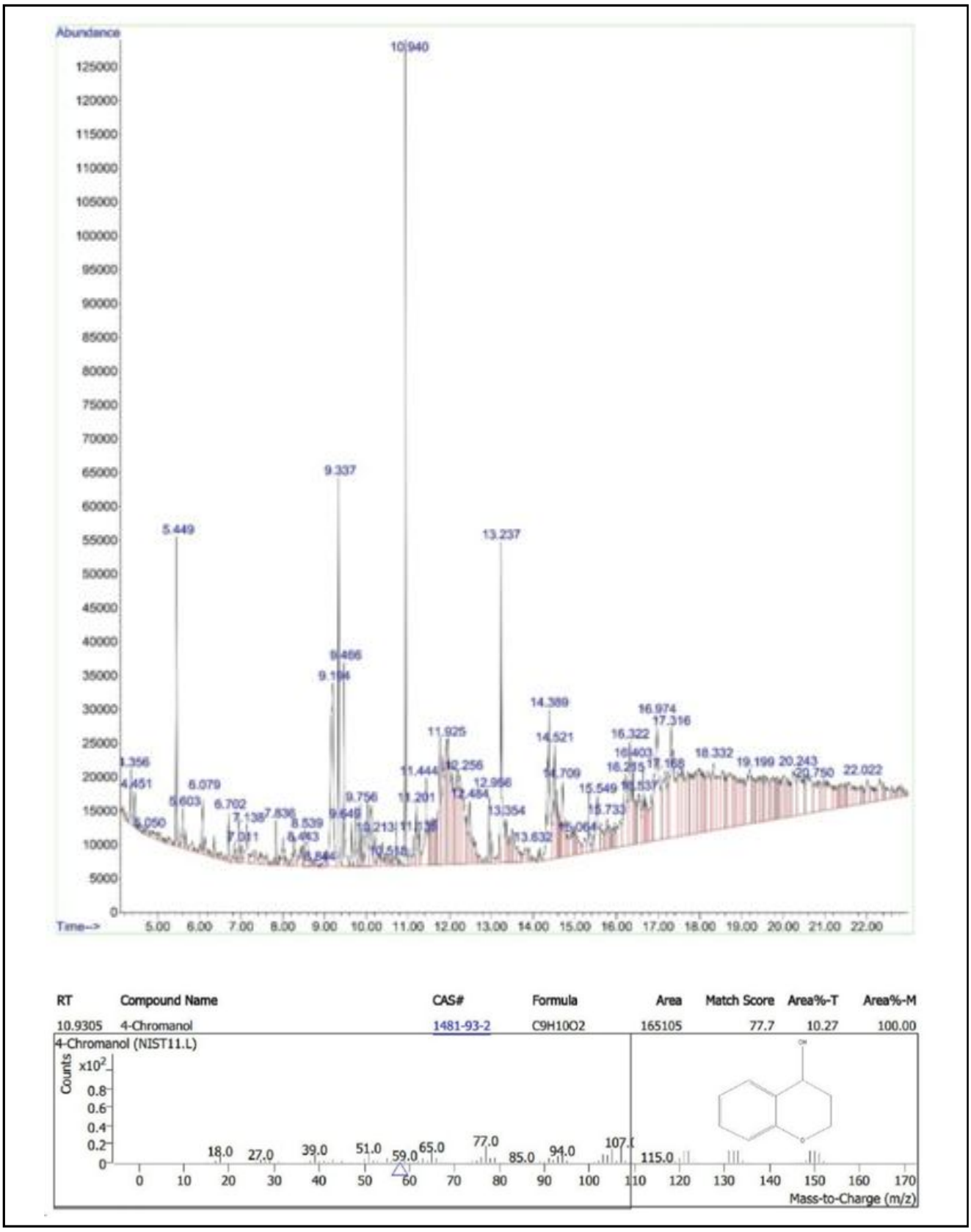

Figure 4: Chromatogram of inflorescence gel extract of *Z. zerumbet*.

Table 2: Phytochemical profile of inflorescence gel extract of *Z. zerumbet*

S. No.	Compounds	Retention time (min)	Peak area (%)
1	4-Chromanol	10.9305	10.27
2	β-Sitosterol	11.9526	9.07
3	d-Glycero-d-ido-heptose	9.1863	7.78
4	2,4,6-Decatrienoic acid, ester	12.1859	7.43
5	n-Hexadecanoic acid	13.2302	5.57
6	Caryophyllene	9.3307	4.41
7	cis-Vaccenic acid	14.3856	4.01
8	Eucalyptol	5.4423	3.58
9	2,4,6,8,10-Tetradecapentaenoic acid	12.0637	3.31
10	Stigmastan-3,5-diene	16.9742	3.22
11	(E)-β-famesene	9.464	2.62
12	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	16.3187	2.37
13	cis-5,8,11-Eicosatrienoic acid, trimethylsilyl ester	17.3075	2.16
14	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	11.7637	1.78
15	9,19-Cyclolanost-24-en-3-ol,acetate, (3)-	16.2076	1.75
16	Stearic acid, 2-hydroxy-1-methylpropyl ester	8.5308	1.73
17	Heptacosane, 1-chloro-	10.1306	1.73
18	Benzene, 1-ethenyl-4-ethyl-	10.0306	1.68
19	Octadecanal, 2-bromo-	15.541	1.45
20	Octadecanoic acid	14.5189	1.38
21	1H-Indene, 1-hexadecyl-2,3-dihydro-	16.3965	1.00
22	1,6-Octadien-3-ol, 3,7-dimethyl-	6.0756	0.94
23	Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl-α-D-allopyranosyl)oxy] -1,14-dihydroxy-,(1α,3β)-	12.4748	0.88
24	Ethyl iso-allocholate	13.4969	0.84
25	α-Terpineol	7.131	0.82
26	Phenol, 4,4'-(1-methylethylidene)bis-	14.7078	0.82
27	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	11.1971	0.78
28	17-Pentatriacontene	16.6409	0.72
29	Cyclohexasiloxane, dodecamethyl-	7.9975	0.7
30	Heptadecane, 2,6,10,14-tetramethyl-	8.2642	0.68
31	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	15.3411	0.66
32	2-Butene, 1,4-diethoxy-	4.3536	0.64
33	endo-Borneol	6.9421	0.48
34	Humulene	9.6418	0.43
35	1b,4a-Epoxy-2Hcyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5 (1aH)-one, 2,7,9,10-tetrakis(acetyloxy)decahydro-3,6,8,8,10apentamethyl-	16.5298	0.43
36	(+)-2-Bornanone	6.6977	0.42
37	α-Farnesene	9.8973	0.36
38	Caryophyllene oxide	10.7194	0.35
39	Tetrapentacontane, 1,54-dibromo-	15.7743	0.32
40	Cyclopentasiloxane, decamethyl-	6.3422	0.31
41	2-Nonadecanone 2,4-dinitrophenylhydrazine	11.2749	0.24

Table 3: Pharmacological importance of predominant phytochemicals in *Z. zerumbet*

S. No.	Compounds	Molecular weight (g/mol)	Pharmacological properties	References
1	4-allyl-1,2-diacetoxybenzene.	234.25	Antibacterial, bactericidal effects, bacteriostatic effects and antibiofilm activity	Saeloh and Visutthi, 2021
2	4-chromanol	150.17	Antimicrobial, antibacterial, antiseptic and anti-candidal effects	Poolkerd *et al.*, 2023
3	β-sitosterol	14.71	Anti-inflammatory, angiogenic, antibacterial, antinociceptive, antifertility, immunomodulatory, anticancer, diabetes-fighting, antioxidant	Dwivedi *et al.*, 2024
4	d-glycero-d-ido-heptose	210.18	Antimicrobial	Gharari *et al.*, 2022
5	Farnesene (E, β)	204.35	Modulator of human neutrophils, anti-inflammatory and antinecrotic	Schepetkin *et al.*, 2022; Arslan *et al.*, 2021
6	Eucalyptol	154.25	Anti-inflammatory, antimicrobial,vasodilating effect, respiratory disorders	Campos and Berteina-Raboin, 2022
7	Caryophyllene	204.35	Insecticidal agent and antibacterial	Tsigoriyna *et al.*, 2024
8	cis-vaccenic acid	282.46	Anti-inflammatory properties	Ogar *et al.*, 2025
9	Stigmastan-3,5-diene	396.69	Anticancer, antimicrobial and antinociceptive	Narayanan *et al.*, 2025
10	Humulene	204.35	Antigastritis, antiallergic, anti-inflammatory, anticancer	Yeo *et al.*, 2021
11	Palmityl chloride	260.89	Antibacterial and antifungal	Bulbul *et al.*, 2021
12	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	310.51	Antibacterial, antioxidant, and antimicrobial properties	Uka *et al.*, 2022
13	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	366.71	Antimicrobial and antifungal	Patiland Singh, 2022
14	Heptacosane, 1-chloro-	415.18	Antifungal and antibacterial	Uddin *et al.*, 2024
15	Benzene, 1-ethenyl-4-ethyl-	132.20	Antibacterial and acaricidal activities	He *et al.*, 2022

Several bioactive compounds were found to occupy significant proportions in both extracts of *Z. zerumbet*. These compounds were observed to possess useful pharmacological applications. These phytochemicals, along with their pharmacological properties, are listed in Table 3.

4. Discussion

The comparative phytochemical profiling of *Z. zerumbet* (shampoo ginger) reveals a distinct compartmentalization of major compounds between its reproductive (rhizome) and vegetative (inflorescence) structures. The inflorescence is dominated by 4-chromanol, followed by β-sitosterol and d-glycero-d-ido-heptose, whereas the rhizome is characterized by high concentrations of 4-allyl-1,2-diacetoxybenzene, farnesene (E, β) and eucalyptol. This differential accumulation is not random but is governed by a combination of ecological, physiological and biosynthetic factors that align with the specific functions of each plant part. This specialization can be traced to distinct biosynthetic origins, with terpenoids like farnesene and eucalyptol synthesized *via* the cytosolic mevalonic acid (MVA) and plastidial methylerythritol phosphate (MEP) pathways, respectively (Lange and Ghassemian, 2003). In contrast, phenylpropanoids like 4-allyl-1,2-diacetoxybenzene originate from the shikimate pathway *via* phenylalanine, demonstrating how different plant organs regulate these complex metabolic networks to fulfill specific ecological roles (Dixon and Paiva, 1995).

The compartmentalized phytochemistry in *Z. zerumbet* is a direct manifestation of spatially and temporally regulated biosynthetic pathways. The terpenoid constituents, which form a significant portion of the identified volatiles, are primarily synthesized through two independent yet sometimes interconnected pathways: the mevalonic acid (MVA) pathway in the cytosol and the methylery thritol phosphate (MEP) pathway in plastids (Vranová *et al.*, 2013). Sesquiterpenes like farnesene (E, β), dominant in the rhizome, are quintessential products of the cytosolic MVA pathway. This pathway begins with the condensation of acetyl-CoA molecules to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is then reduced to mevalonic acid. Subsequent phosphorylation and decarboxylation steps yield isopentenyl diphosphate (IPP). The sesquiterpene synthase enzyme farnesene synthase catalyzes the cyclization of farnesyl diphosphate (FPP), derived from the head-to-tail condensation of three IPP units, to produce farnesene (Degenhardt *et al.*, 2009). The rhizome's investment in this pathway aligns with its role as a perennial storage and defensive organ, as sesquiterpenes are often involved in direct antimicrobial defense and below-ground communication (Bennett and Wallsgrove, 1994).

In contrast, monoterpenes like eucalyptol (1,8-cineole), found in both rhizome and inflorescence, are synthesized *via* the plastidial MEP pathway. This pathway starts with the condensation of pyruvate and glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose-5-phosphate (DXP), which is then converted to MEP and

subsequently to IPP (Rodríguez-Concepción and Boronat, 2002). The enzyme cineole synthase then catalyzes the cyclization of the monoterpene precursor geranyl diphosphate (GPP) to form eucalyptol. The presence of eucalyptol across tissues suggests a broad defensive role, possibly induced or amplified by environmental cues, as its biosynthesis can be upregulated in response to biotic stress or interspecific interactions (Chen *et al.*, 2011). The co-occurrence of compounds from both pathways highlights the metabolic versatility of *Z. zerumbet* and the organ-specific regulation of these biosynthetic fluxes.

The biosynthesis of 4-allyl-1,2-diacetoxybenzene, a key phenylpropanoid in the rhizome, follows the general phenylpropanoid pathway originating from the shikimate pathway. This compound's specific accumulation in the rhizome suggests a targeted defense strategy, as phenylpropanoids are often synthesized in response to pathogen challenge and soil-borne stress (Dixon, 2001).

The major inflorescence compound, 4-chromanol (a tocopherol-like antioxidant), is biosynthesized *via* the plastidial pathway that combines precursors from the shikimate and MEP pathways. Homogentisic acid, derived from tyrosine (shikimate pathway), is condensed with phytyl diphosphate (derived from the MEP pathway) to form 2-methyl-6-phytyl-1,4-benzoquinol, which is then cyclized to form the chromanol ring (Mène-Saffrané and DellaPenna, 2010). Its high concentration in the inflorescence underscores the organ's high demand for lipophilic antioxidants to protect reproductive tissues and the mucilaginous gel from photo-oxidative damage, a critical requirement for reproductive success.

The phytochemical profile of a plant is not a static trait but a dynamic interface between its genotype and the environment. The significant presence of eucalyptol in both examined tissues of *Z. zerumbet* in this study strongly suggests an environmental induction, likely linked to cultivation practices. As noted, agroforestry systems foster complex allelopathic interactions. Eucalyptus species are renowned for their prolific release of volatile terpenes, including eucalyptol, into the soil and atmosphere through leaf litter decomposition, root exudation, and volatilization (Xu *et al*., 2023). These released compounds can act as environmental signals, potentially inducing the expression of terpene synthase genes in neighboring plants like *Z. zerumbet* through a phenomenon known as "eavesdropping" or heterologous induction (Kegge and Pierik, 2010). This cross-kingdom signaling can prime the plant's defense responses, leading to the constitutive or induced accumulation of similar compounds as a pre-emptive or responsive defense strategy (Baldwin, 2010). Therefore, the phytochemistry reported here may represent a "phenotype" specific to a particular agro-ecological context, highlighting the importance of documenting cultivation conditions in phytochemical studies.

Beyond allelopathy, other ecological factors undoubtedly shape the metabolite profile. Light intensity and spectral quality can dramatically influence the flux through the MEP and phenylpropanoid pathways. Higher light levels typically increase the production of photoprotective compounds like flavonoids and antioxidants (*e.g.,* 4-chromanol) in aerial parts (Sabarivasan *et al.*, 2024). Soil composition, moisture and microbial community structure in the rhizosphere can selectively pressure the rhizome to produce specific antimicrobial agents like 4-allyl-1,2-diacetoxybenzene and farnesene.

Most GC-MS studies on *Z. zerumbet* rhizome oil consistently report zerumbone as the major constituent, often comprising 20-60% of the total oil, alongside variable amounts of humulene, caryophyllene and camphene (Chane-Ming *et al.*, 2003; Bhuiyan *et al.*, 2009). In contrast, GC-MS profiles of the closely related and economically dominant *Z. officinale* (common ginger) are markedly different. Ginger rhizome oil is typically dominated by monoterpenes like α-zingiberene, ar-curcumene, and β-sesquiphellandrene, with only trace amounts of zerumbone (Uddin *et al.*, 2020). This highlights a divergent evolutionary trajectory in terpenoid biosynthesis within the genus. Similarly, *Curcuma longa* (turmeric) essential oil is characterized by high proportions of turmerone and curlone, with significant antifungal and anti-inflammatory activities (Duong *et al.*, 2022; Naikodi *et al.*, 2021), analogous to the defensive role proposed for *Z. zerumbet* rhizome compounds.

The inflorescence of *Z. zerumbet* exhibits a specialized phytochemical composition, predominantly featuring 4-chromanol, β-sitosterol and d-glycero-d-ido-heptose, which are integral to its structural integrity and defensive strategies. The high concentration of 4-chromanol, a potent lipophilic antioxidant, provides critical protection for reproductive tissues against photo-oxidative stress and alongside its established antimicrobial properties, preserves the mucilaginous gel from microbial spoilage (Gharari *et al.*, 2022). This compound is similarly prevalent in other Zingiberaceae genera, such as certain *Alpinia* species, which also utilize chromanol-type antioxidants for the protection of floral parts. The abundance of β-sitosterol, a significant phytosterol, facilitates the extensive cellular proliferation and differentiation required for inflorescence development (Dwivedi *et al.*, 2024). This is a ubiquitous constituent across the family, found in significant quantities in the rhizomes of turmeric (*Curcuma longa*) (Akter *et al.*, 2019) and ginger (*Zingiber officinale*) (Singh *et al.*, 2012), underscoring its essential role in plant cell architecture. Complementing these, d-glycero-d-ido-heptose is inferred to act as an osmoprotectant and a structural moiety within the gel, aiding in hydric balance. The synthesis of such specialized sugars is a metabolic feature observed in other plant families for stress adaptation, suggesting a convergent evolutionary strategy. This distinct combination of compounds underscores an evolutionary refinement in *Z. zerumbet*, tailoring its chemical arsenal to ensure the defense and viability of its reproductive organs.

The rhizome of *Z. zerumbet* synthesizes a specialized defense-oriented phytochemical profile, dominated by 4-allyl-1,2-diacetoxybenzene, farnesene (E, β) and eucalyptol, which collectively protect the perennial storage organ from soil-borne threats. The prevalence of 4-allyl-1,2-diacetoxybenzene, a potent phenylpropanoid, provides a primary antimicrobial defense. This mirrors the defense strategy in *Piper betle* (betel leaf), where structurally similar phenylpropanoids like chavibetol and eugenol demonstrate strong antibacterial and antifungal activities (Poolkerd *et al.*, 2023). The sesquiterpene farnesene (E, β) acts as a volatile semiochemical, creating a defensive zone in the rhizosphere. Similar sesquiterpene hydrocarbons are major constituents in the essential oil of *Curcuma aeruginosa* (a wild turmeric species), contributing to its documented antifungal properties (Sari and Supratman, 2022). Complementing these, eucalyptol serves as a broad-spectrum antimicrobial shield, a role it also plays in the essential oil of *Alpinia nigra*, where its presence is correlated with significant antibacterial efficacy (Saikia *et al.*, 2023). This strategic combination of compounds underscores an evolutionary adaptation for subterranean defense, a theme common among storage organs within the Zingiberaceae family.

The presence of eucalyptol as a common volatile compound in both the rhizome and inflorescence extracts of *Z. zerumbet* may be attributed to the unique ecological interactions inherent to cultivation under eucalyptus. In agroforestry, plant species are co-cultivated in close proximity, creating a dynamic biochemical environment characterized by the exchange of specialized secondary metabolites known as allelochemicals. This process, known as allelopathy, involves one plant releasing chemicals that influence the growth, survival, or metabolism of neighboring plants (Mushtaq and Fauconnier, 2024). Eucalyptus species are particularly renowned as potent allelopathic agents, with their leaf litter, root exudates and volatilized emissions significantly altering the chemical ecology of their surroundings (Zhang *et al.*, 2010). The primary mechanism involves the release of a complex volatile organic compounds, with monoterpenes like eucalyptol (1,8-cineole) and α-pinene being dominant constituents, which can permeate both the rhizosphere and the phyllosphere (Niu *et al.*, 2024).

It is plausible that the *Z. zerumbet* plants in this study, cultivated alongside or near Eucalyptus species, were influenced by this chemical milieu. The well-documented production and release of eucalyptol into the shared environment could have induced a metabolic response in *Z. zerumbet*. This interspecific chemical interplay represents a form of volatile-mediated plant-plant communication, where VOCs from one species act as environmental cues to trigger defensive or adaptive metabolic changes in a receiver plant (Kegge and Pierik, 2010). This chemical induction is not limited to aerial parts; the rhizosphere serves as a critical hot spot for chemical exchange. Root exudates and decomposing litter from eucalyptus create a soil solution rich in allelochemicals, which can be taken up by neighboring roots or perceived as signals, leading to systemic changes in the secondary metabolism of the receiver plant (Bais *et al.*, 2006). This phenomenon of chemically-induced metabolite profile modulation is supported by analogous studies. For instance, Deepikakrishnaveni *et al.* (2025) reported that the phytochemical profile of *Asparagus sprengeri* was significantly influenced when cultivated near Allspice (*Pimenta dioica*) trees, a change attributed to rhizosphere chemical interactions. Similarly, the essential oil composition of *Mentha piperita* has been shown to alter when grown in association with allelopathic shrubs, demonstrating the broader ecological principle of metabolomic flexibility in plant communities (Li *et al.*, 2022).

The distinct phytochemical profiles of the inflorescence and rhizome of *Z. zerumbet* are a testament to plant organ-specific metabolic specialization, governed by divergent biosynthetic pathway regulation and fine-tuned by ecological pressures. The inflorescence invests in antioxidants (4-chromanol) and structural components (β-sitosterol) to ensure reproductive success, while the rhizome accumulates potent antimicrobial phenylpropanoids and terpenoids (4-allyl-1,2-diacetoxybenzene, farnesene, eucalyptol) for subterranean defense. This functional dichotomy, rooted in the MVA, MEP and shikimate pathways, provides a robust chemical rationale for the traditional uses of different plant parts. Future research integrating transcriptomics and metabolomics will be crucial to fully elucidate the regulatory networks behind this remarkable compartmentalization and its potential manipulation for pharmaceutical and agricultural applications.

5. Conclusion

Z. zerumbet is an underutilized crop. The GC-MS exploration of *Z. zerumbet* revealed numerous bioactive compounds in its rhizome and inflorescence with significant pharmacological properties, including anti-inflammatory, antimicrobial and antioxidant activities. These findings underscore its potential for development in the natural cosmetics and pharmaceutical industries. To harness this potential fully, further biotechnological research focusing on metabolite enhancement is recommended for sustainable commercial utilization.

6. Future research and potential

While this study provides a foundational comparative phytochemical profile of *Z. zerumbet*'s rhizome and inflorescence, further deep insights could be explored through advanced analytical tools and methodologies. An integrated multi-omics approach is essential for pathway elucidation and enhancement. Coupling comprehensive metabolomics (LC-MS, NMR) with transcriptomics (RNA-Seq) and proteomics will map the complete biosynthetic landscape, identifying key rate-limiting enzymes in the MVA, MEP and phenylpropanoid pathways. Using biotic or abiotic elicitors can hyper-induce the production of target compounds like zerumbone and antimicrobial phenolics, potentially boosting yields far beyond field-grown levels.

Furthermore, the distinct bioactive profiles position it as a high-value candidate for multiple sustainable industries. In cosmeceuticals, the inflorescence gel-with its saponins, antioxidants (4-chromanol) and humectants-is ideal for premium "clean beauty" shampoos and scalp treatments, while the antimicrobial rhizome extract serves as a natural preservative and active for acne-prone skincare. Standardized rhizome extracts also hold potential as gastrointestinal health supplements. Realizing this potential necessitates parallel development in bioactive ingredient extraction and agri-technology as well.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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