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Variation in the volatile oil composition and antioxidant activity of Zingiberaceae: A comparative investigation

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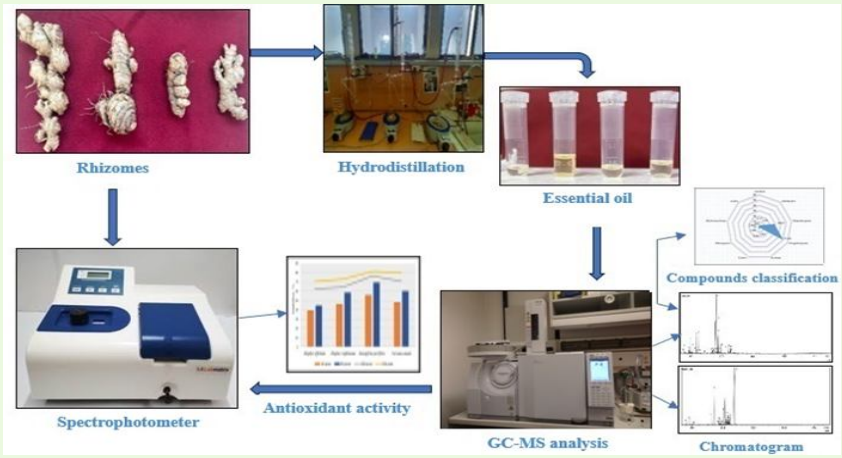
Abstract

An experiment was undertaken to analyze the chemical profile of essential oil and the antioxidant potential of four spices belonging to the Zingiberaceae family such as *Zingiber officinale* Roscoe, *Zingiber wightianum* Thwaites, *Kaempferia parviflora* Wall. ex Baker and *Curcuma amada* Roxb. The essential oil was collected from the rhizomes through hydrodistillation using a Clevenger-type apparatus. The essential oil underwent analysis using gas chromatography-mass spectrometry (GC-MS). The findings unveiled 77 compounds across all species, with terpenoids being the predominant class (51.40%), particularly sesquiterpenes, followed by monoterpenes (28.03%). Each species exhibited a unique chemical profile, with *Z. officinale* containing 28 compounds, *Z. wightianum* with 21 compounds, *K. parviflora* with 24 compounds, and *C. amada* with 35 compounds. Linalool and α-terpineol were identified as common compounds among all four species. However, 11 metabolites in *Z. officinale*, 17 compounds in *C. amada*, 14 in *Z. wightianum*, and 15 in *K. parviflora* were specific to the respective species. Furthermore, the DPPH assay demonstrated significant antioxidant activity, with *K. parviflora* exhibiting the highest activity at 81.15%, followed closely by *C. amada* (80.03%), *Z. wightianum* (73.11%), and *Z. officinale* (70.28%). This species-specific exploration offers scope to the breeders to uncover unique chemical profiles, enabling industries to craft novel products with enriched flavors, therapeutic advantages, and market allure, fueling innovation and competitiveness in the food, pharmaceuticals, and perfumery sectors.



1. Introduction

Medicinal and aromatic plants are integral to the socio-cultural, healthcare, and spiritual practices of rural India, where approximately 80% of the global population relies on traditional medicine systems for their healthcare needs. This preference is driven by safety, accessibility, low cost, and concerns over the adverse effects of conventional medications (Sharma, 2021; Manjider *et al.*, 2022;

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Sundarrajan, 2023). In recent years, there has been a significant surge in the use of essential oils derived from the plants, both in scientific research and industrial applications, with approximately 3000 essential oils identified to date, of which around 300 hold economic importance, particularly in pharmaceuticals and perfumery (Mastan *et al*., 2022).

The Zingiberaceae family, widely distributed in tropical regions worldwide, represents the largest family within the Zingiberales order, comprising numerous genera and over 1200 species. This botanical family is predominantly found in tropical and subtropical regions, including India, Myanmar, Thailand, China, and Malaysia (Kress *et al*., 2002). Plants of this family are renowned for their therapeutic properties, including anti-inflammatory, antiviral, antimicrobial, antioxidant, and anticancer effects, making them valuable targets for drug development (Sofia and Aman, 2021). Rhizomes of various Zingiberaceae species are rich sources of essential oils, known for their pleasant fragrance and therapeutic benefits, commonly used in traditional medicine for a variety of health issues. Some common Zingiberaceae spices in the Indian market include ginger, cardamom, turmeric, and mango ginger, with a few rare and endangered species such as black ginger and mountain ginger (Wen *et al*., 2023). *Z. officinale* is the most widely utilized and studied species under the genus *Zingiber,* extensively employed as a spice to add flavor to food and has a long history of medicinal application in traditional medicine systems to alleviate nausea, common colds, coughs, diarrhea, malaria, fever, arthritis, and inflammation-related disorders (Verma and Bisen, 2022; Jabborova *et al*., 2020). The medicinal benefits of ginger are attributed to its aroma and pungency. Essential oils, which enhance their aroma, carry various advantageous properties such as antibacterial, antifungal, pain relief, antiinflammatory, ulcer prevention, immune system regulation, and relaxation effects (Nishidono, 2020). Another notable species is *C. amada*, commonly known as mango ginger due to the resemblance of its aromatic rhizome to ginger (*Z. officinale*) and its distinctive aroma reminiscent of raw, unripe mango. This plant holds a significant history of usage in folk medicine and as a culinary ingredient to cure diverse digestive maladies and dermatological ailments (Raval *et al*., 2021), whereas its essential oils exhibit antifungal, anti-inflammatory, anticancer, and antihyperglycemic characteristics (Tamta *et al*., 2017). *K. parviflora* stands out as an underutilized medicinal species in this family, renowned for its vibrant, purple-hued rhizome, which has been utilized in traditional medicine to treat erectile dysfunction, gastrointestinal problems, and high blood sugar levels (Prameela and Venkaiah, 2018; Ngoc *et al*., 2021; May San *et al*., 2023). Apart from this, its essential oil have shown anti-inflammatory, antioxidant, anticancer, antiobesity, antimicrobial, and neuroprotective effects (Mekjaruskul *et al*., 2012). Mountain ginger, scientifically known as *Z. wightianum*, is native to the dense evergreen forests of Peninsular Sri Lanka and India, particularly the Western Ghats regions where it is endangered (Meera, 2020). Traditionally used to aid digestion, alleviate fevers, rheumatism, and reduce giddiness, it also exhibits hepatoprotective effects (Nguyen *et al*., 2023). Despite its significance, *Z. wightianum* remains one of the least studied plants in terms of both its chemical composition and biological properties (Sini *et al*., 2021). Essential oils derived from Zingiberaceae herbs contain a variety of compounds such as terpenoids, aldehydes, hydrocarbons, alcohols, ethers, and ketones, contributing to their medicinal properties (Sharifi-Rad, 2017; Soni *et al*., 2022; Rai *et al*., 2023). The crude extract of these plants are also rich in polyphenols and flavonoids known for their strong antioxidant properties, suggesting their potential to prevent various diseases (Stoilova *et al*., 2007).

However, due to the similarities in morphology and processing practices like drying and grinding, classification and quality control of these spices pose challenges (Ivanovic *et al*., 2021). Therefore, the comparative analysis of essential oils composition ensures accurate classification, quality control, and authentication, detecting unique aroma markers and optimizing processing for flavor. Additionally, investigating the antioxidant activity provides valuable insights into the potential health benefits. Techniques such as gas chromatography-mass spectrometry (GC-MS) can help unravel their chemical profiles, while antioxidant assays like the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay can assess their ability to combat oxidative stress. The species-specific exploration offers scope to the breeders to uncover unique chemical profiles, enabling industries to craft novel products with enriched flavors, therapeutic advantages, and market allure, fueling innovation and competitiveness in the food, pharmaceuticals, and perfumery sectors. Therefore, the present investigation was undertaken to explore the variations in volatile oil composition and the antioxidant potential among the four Zingiberaceae herbs, including *Z. officinale, C. amada, K. parviflora,* and *Z. wightianum*.

2. Material and Methods

2.1 Plant materials

Fresh rhizomes of *Z. officinale* (Plant authentication number: BSI/SRC/5/23/2024-25/Tech-333) and *C. amada* (BSI/SRC/5/23/2024-25/Tech-335) were procured from the college orchard, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. In contrast, *K. parviflora* (BSI/SRC/5/23/2024-25/Tech-334) and *Z. wightianum* (BSI/SRC/5/23/2024-25/Tech-336) rhizomes were collected from Nagaland and Nilgiris, Tamil Nadu, respectively (Figure 1).

2.2 Chemical and reagents

DPPH was procured from Sigma Aldrich, while all other chemicals used, including solvents, were of analytical grade.

2.3 Extraction of essential oil

The collected rhizomes underwent thorough washing under running tap water before being cut into small pieces. Subsequently, they were dried and pulverized into a powder. 50 grams of powdered samples were weighed and transferred to a round bottom flask containing 500 milliliters of water. Subsequently, the mixture was subjected to the hydrodistillation method using the Clevenger apparatus for about 4 h. At the end of each distillation, the essential oil was collected and treated with anhydrous sodium sulfate to remove any trace amount of water from the essential oil. The appropriate light yellowish essential oil was collected and the percentage (W/W) was calculated (ASTA, 1978). Finally, the essential oil was kept in a sealed vial at 4^0C until GC-MS analysis was performed.

$$\text{Essential oil (\%)} = \frac{\text{Amount of oil collected (ml)}}{\text{Weight of sample (g)}} \times 100$$

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oils of four species underwent analysis using gas chromatography (GC) coupled with a Shimadzu mass spectrometer (MS) (GC-MS-TQ8040 NX SHIMADZU, Shimadzu Corp., Tokyo, Japan) at the Centre of Excellence, Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore. The GC was equipped with a fused silica capillary column, Rix-5 Sil MS, measuring 30 m in length with a diameter of 0.25 mm and a film thickness of 0.25 ml. Injection of the diluted essential oils was performed in split mode (10:1) while maintaining a constant flow rate of 1ml/min of helium gas. The temperature of the oven was set to 70°C for one minute, then was raised to 225°C, followed to 300°C gradually at a rate of 5°C per minute with a total run time of 55 min. The injector port temperature was adjusted to 280°C, while the ion sources were set to 230°C.

2.5 Identification of compounds

The components of the essential oils were identified by comparing their retention indices relative to a series of n-alkanes (C8-C24) under consistent chromatographic conditions. Moreover, retention indices were cross-referenced with those reported in the literature and cataloged in the MS library [NIST 10 (National Institute of Standards and Technology, Gaithersburg, MD, USA)].

2.6 Assessment of antioxidant potential

The DPPH free radical scavenging assay was performed using the spectrophotometric method outlined by Miliauskas *et al*. (2004) and Nguyen *et al*. (2015). In this assay, the DPPH radical undergoes reduction upon interaction with antioxidants capable of hydrogen donation. The alteration in color, transitioning from deep violet to light yellow, was quantified spectrophotometrically at a wavelength of 517 nm. As a benchmark antioxidant, ascorbic acid served as the reference compound. To initiate the assay, one gram of powdered sample from each species was dissolved in 80% methanol and incubated at 60°C for 15 min. Subsequently, centrifugation at 4500 rpm for approximately 20 min was performed, followed by collection of the supernatant. The supernatant was subsequently adjusted to an ultimate volume of 20 ml with 80% methanol. For the assay, 0.1 mL of various sample concentrations was combined with 1 mL of 0.2 mM DPPH solution dissolved in 0.004% methanol. Following an incubation period of 15 min at 25 ± 1°C in darkness, the absorbance at 517 nm was recorded. A blank mixture, containing all reagents except the sample, was also prepared. The scavenging ability against DPPH radicals was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where,

$\text{Abs}_{\text{control}}$ = The absorbance of the DPPH radical in methanol.

$\text{Abs}_{\text{sample}}$ = The absorbance of the DPPH radical solution mixed with samples.

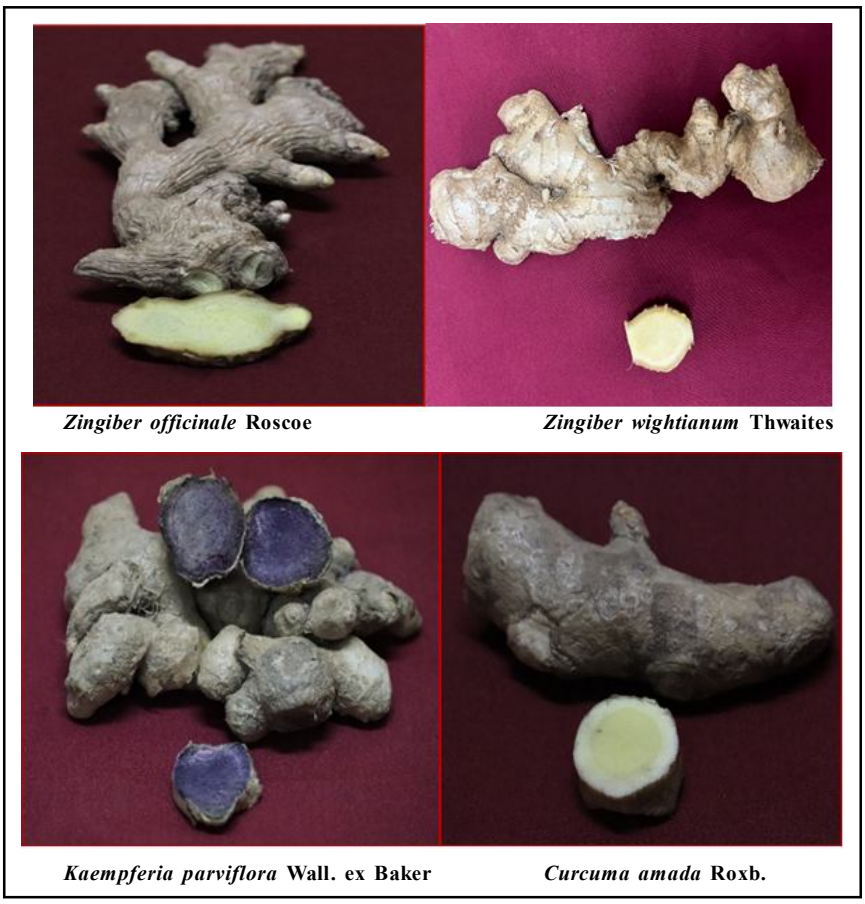


Figure 1: Rhizome of different Zingiberaceae spices.

3. Results

3.1 Identification of compounds

GC-MS analysis of essential oils extracted from four Zingiberaceae species such as *Z. officinale*, *Z. wightianum*, *K. parviflora*, and *C. amada* revealed the identification of 77 metabolites, with 28, 21, 24, and 35 volatile compounds detected in each species, respectively (Table 1). These compounds encompassed a diverse array of terpenoids, aldehydes, esters, alcohols, ketones, hydrocarbons, and other metabolites. Notably, terpenoids emerged as the predominant fraction across all samples, with sesquiterpenes comprising the most significant proportion at 51.40%, followed by monoterpenes at 28.03%, 6.54% of esters, aldehydes (3.38%), 2.80% of ketones and hydrocarbons, alcohol (2.77%) and 0.93% of acids were observed (Figure 2). This indicates the crucial role of terpenoids within Zingiberaceae species.

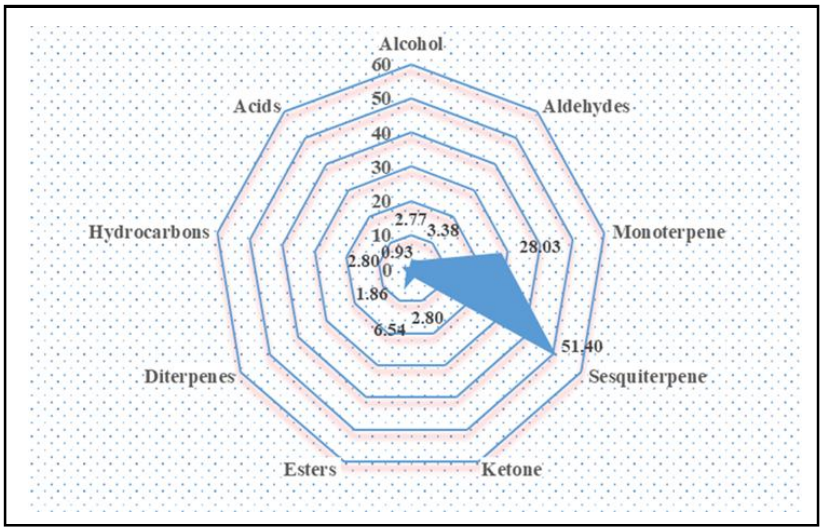


Figure 2: The compound classification diagram of four Zingiberaceae spices.

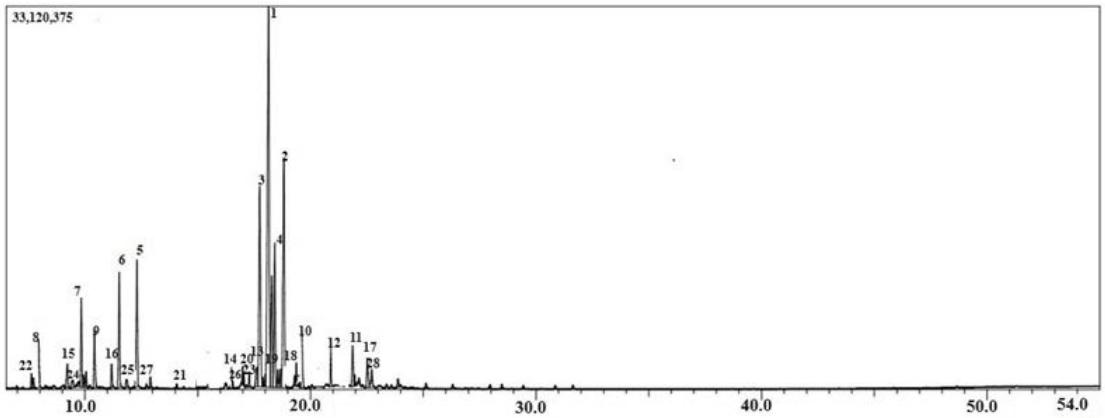
The detailed presence of volatile compounds from all four species is depicted in Table 1 and further illustrated in Figure 3. The volatile components of *Z. officinale* included, terpinolene, linalool, citronellal, isoneral, borneol, α-terpineol, citronellol, nerol, (Z,E)-farnesol, citral, 2-undecanone, α-copaene, γ-elemene, β-farnesene, alloaromadendrene, γ-muurolene, α-curcumene, β-selinene, zingiberene, β-bisabolene, β-myrcene, β-sesquiphellandrene, elemol ,trans nerolidol, zingiberenol, α-springene, β-eudesmol and α-bergamotenol. Out of these compounds, the maximum peak area was obtained by zingiberene (22.81%) followed by β-sesquiphellandrene (10.92%), α-curcumene (9.95%), citral (5.45%), and nerol (4.70%) whereas the minimum peak area was obtained by α-bergamotenol (0.34%). The volatile oil compounds in *Z. wightianum* were linalool, camphor, α-terpineol, β-caryophyllene, humulene, β-elemene, bulnesol, trans nerolidol, caryophyllene oxide, α-bisabolene, alloaromadendrene oxide, β-gurjunen, 1,4,4-trimethyl bicyclononane, eudesma-4, β-eudesmol, aR-turmerone, hydroxy caryophyllene, ylangena, longipinane and zerumbone. The compound with the maximum peak area was identified as zerumbone (59.09%), followed by humulene (10.57%), α-bisabolene (5.43%), alloaromadendrene oxide (4.00%), and longipinane (3.21%), whereas the compound with minimum peak area was α-terpineol (0.22%). In *K. parviflora* essential oil, the detected compounds were 2-carene, linalool, borneol, α-terpineol, bornyl acetate, α-terpinene, α-copaene, β-elemene, β-caryophyllene, γ-elemene, cis-muurola-3-5-diene, D-germacrene, zingiberene, bicyclo germacrene, isoledene, β-sesquiphellandrene, T-muurolol, methyl nervonate, methyl myristoleate, methyl palmitoleate, methyl cis-10-heptadecenoate, methyl oleate, methyl tricosanoate, and lauric acid. In *K. parviflora,* the major compound was methyl nervonate (12.20%), followed by borneol (11.12%), linalool (10.68), methyl oleate (7.59%) and methyl tricosanoate (4.81%), whereas minimum peak area was obtained by α-terpineol (0.32%). The chemical constituents in *C. amada* essential oil were β-ocimene, 3-carene, 2-Methyl-endo norbornanol, 1,5,9-undecatriene, 8-hydroxylinalool, borneol, 4-terpineol, α-terpineol, citronellol, neral, citral, 2-undecanone, β-elemene, β-caryophyllene, humulene, α-curcumene, zingiberene, isolongifolol, α-farnesene, β-bisabolene, β-sesquiphellandrene, trans-nerolidol, humulenol II, zingiberenol, epicurzerenone, culmorin, β-eudesmol, longifolenaldehyde, boronal, ambrial, α-camphorene, (-)-copalol, γ-camphorene, geranylgeraniol, β-farnesene and coronarin E. In *C. amada* essential oil, the major compound was β-ocimene (9.38% peak area) followed by ambrial (7.84%), 1,5,9-undecatriene (7.29%), α-curcumene (6.25%) and β-caryophyllene (4.89%) whereas the minor compounds observed was coronarin E (0.30%) and β-elemene (0.32%).

The existing research study has shown 11 compounds are specific in *Z. officinale,* 17 in *C. amada*, 14 in *Z. wightianum*, and 15 in *K. parviflora*. The common compounds that are identified in *Z. officinale* and *K. parviflora* were α-terpineol, borneol, linalool, α-copaene, γ-elemene, zingiberene, and β-sesquiphellandrene. Whereas, the compounds that are common in *Z. officinale* and *Z. wightianum* were α-terpineol, linalool, trans nerolidol, and β- eudesmol. *Z. officinale* and *C. amada* exhibited considerable similarity in their chemical compositions, with a total of 15 compounds common between them. The compound zingiberene is common in *Z. officinale, K. parviflora,* and *C. amada*. Whereas, β-caryophyllene and β-elemene are common in *Z. wightianum, K. parviflora,* and *C. amada*. Identification of common compounds across different ginger species helps to know the targeted breeding strategies for enhancing desired traits like medicinal properties, flavor profiles, and environmental adaptation.

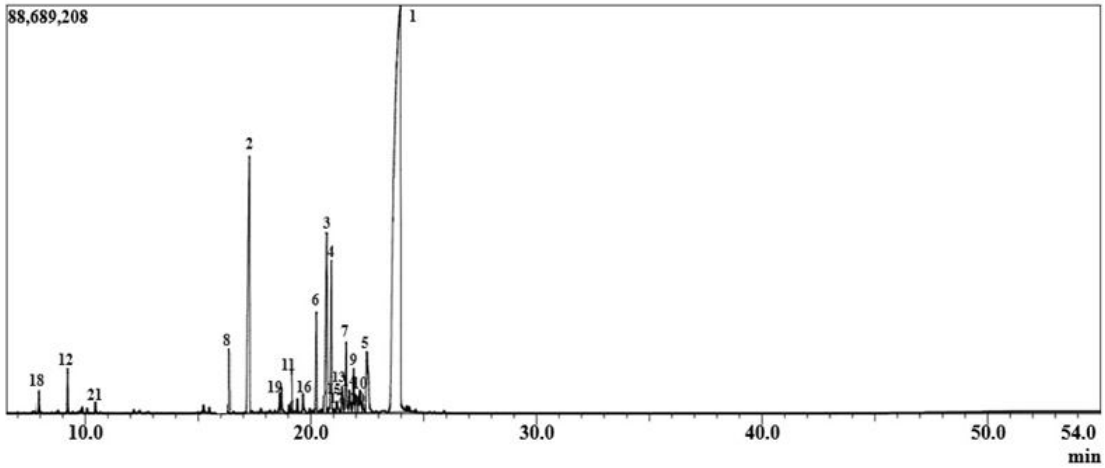
Table 1: Chemical composition of essential oil from four Zingiberaceae spices

S. No.	Compound name	Molecular formula	Molecular weight	Retention indices (RI) literature	Retention indices (RI) exper-imental	Peak area (%)			
						Zingiber officinale	*Zingiber wightianum*	*Kamperia parviflora*	*Curcuma amada*
1	β-ocimene	$C_{10}H_{16}$	136	1015	976	-	-	-	9.38
2	3-carene	$C_{10}H_{16}$	136	993	948	-	-	-	1.24
3	Terpinolene	$C_{10}H_{16}$	136	1061	1023	0.50	-	-	-
4	2-carene	$C_{10}H_{16}$	136	1000	948	-	-	0.52	-
5	2-methyl-endo -norbornanol	$C_{18}H_{12}O3$	156	1232	1224	-	-	-	1.72
6	1,5,9-undecatriene	$C_{14}H_{24}$	192	1345	1350	-	-	-	7.92
7	Linalool	$C_{10}H_{18}O$	154	1082	1082	2.99	0.40	10.68	0.43
8	Camphor	$C_{10}H_{16}O$	152	1121	1121	-	0.86	-	-
9	Citronellal	$C_{10}H_{18}O$	154	1128	1125	1.04	-	-	-
10	Isoneral	$C_{10}H_{18}O$	154	1151	1174	0.46	-	-	-
11	Borneol	$C_{10}H_{18}O$	154	1146	1138	3.19	-	11.12	1.34
12	4-terpineol	$C_{10}H_{18}O$	154	1143	1137	-	-	-	0.69
13	α-terpineol	$C_{10}H_{18}O$	154	1144	1143	2.18	0.22	0.32	1.20
14	Citronellol	$C_{10}H_{20}O$	156	1207	1179	0.83	-	-	0.42
15	Nerol	$C_{10}H_{16}O$	152	1207	1174	4.70	-	-	2.46
16	(Z,E)-farnesol	$C_{15}H_{26}O$	222	1705	1710	0.43	-	-	-
17	Citral	$C_{10}H_{16}O$	152	1223	1174	5.45	-	-	3.43
18	Bornyl acetate	$C_{12}H_{20}O_2$	196	1277	1277	-	-	1.35	-
19	2-undecanone	$C_{10}H_{18}O_2$	170	1270	1251	0.37	-	-	0.54
20	α-terpinene	$C_{10}H_{16}$	136	1000	998	-	-	0.82	-
21	α-copaene	$C_{15}H_{24}$	204	1423	1221	0.54	-	1.48	-
22	β-caryophyllene	$C_{15}H_{24}$	204	1468	1494	-	1.51	1.43	4.98
23	γ-elemene	$C_{15}H_{24}$	204	1430	1431	1.11	-	0.45	-
24	β-farnesene	$C_{15}H_{24}$	204	1441	1440	0.62	-	-	2.77
25	Alloaromadendrene	$C_{15}H_{24}$	204	1419	1386	0.50	-	-	-
26	Humulene	$C_{15}H_{24}$	204	1488	1579	-	10.57	-	0.45
27	Cis-muurola-3-5 -diene	$C_{15}H_{24}$	204	1441	1440	-	-	0.69	-
28	γ-muurolene	$C_{15}H_{24}$	204	1468	1435	1.25	-	-	-
29	α- curcumene	$C_{15}H_{22}$	202	1479	1556	9.95	-	-	6.25
30	D-germacrene	$C_{15}H_{24}$	204	1490	1515	-	-	4.67	-
31	β-selinene	$C_{15}H_{24}$	204	1469	1469	0.41	-	-	-
32	Zingiberene	$C_{15}H_{24}$	204	1492	1451	22.81	-	1.28	2.46
33	Bicyclogermacrene	$C_{15}H_{24}$	204	1500	1499	-	-	0.91	-
34	Isolongifolol	$C_{17}H_{28}O_2$	264	1850	1759	-	-	-	1.07
35	α-farnesene	$C_{15}H_{24}$	204	1494	1458	-	-	-	1.66
36	β-bisabolene	$C_{15}H_{24}$	204	1505	1500	5.64	-	-	2.36
37	β-myrcene	$C_{15}H_{24}$	204	991	1008	0.73	-	-	-

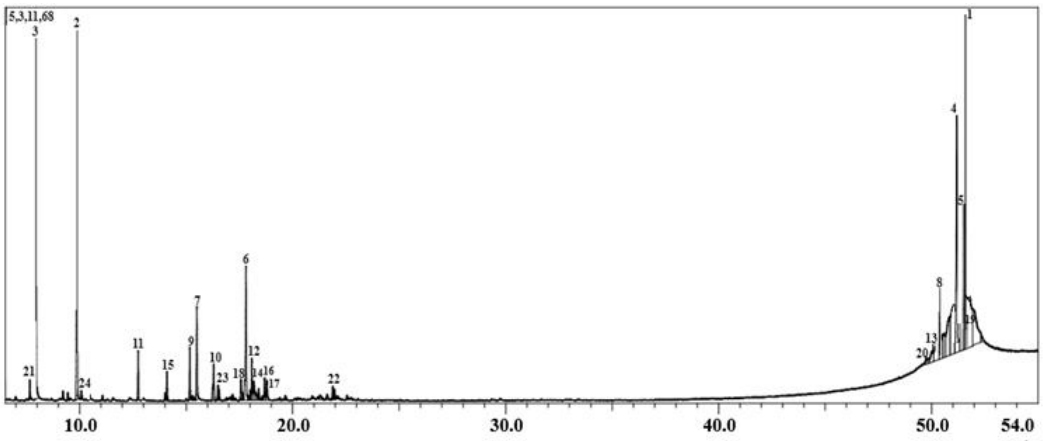
38	Isoledene	$C_{15}H_{24}$	204	1377	1419	-	-	0.70	-
39	β-sesquiphellandrene	$C_{15}H_{24}$	204	1509	1446	10.92	-	0.77	3.94
40	β-elemene	$C_{15}H_{24}$	204	1387	1398	-	0.90	2.88	0.32
41	Elemol	$C_{15}H_{26}O$	222	1529	1522	0.76	-	-	-
42	Bulnesol	$C_{15}H_{26}O$	222	1630	1614	-	0.31	-	-
43	Trans nerolidol	$C_{15}H_{26}O$	222	1564	1564	1.97	0.56	-	1.04
44	Humulenol II	$C_{15}H_{24}O$	220	1632	1762	-	-	-	4.13
45	Caryophyllene oxide	$C_{15}H_{24}O$	220	1581	1507	-	2.34	-	-
46	Epicurzerenone	$C_{15}H_{18}O_2$	230	1601	1704	-	-	-	2.44
47	α-bisabolene	$C_{15}H_{24}$	204	1443	1518	-	5.43	-	-
48	Zingiberenol	$C_{15}H_{26}O$	222	1593	1591	1.32	-	-	0.51
49	Alloaromadendrene oxide	$C_{15}H_{24}O$	220	1419	1462	-	4.00	-	-
50	β-gurjunen	$C_{15}H_{24}$	204	1445	1494	-	0.43	-	-
51	1,4,4-trimethyl bicyclo nonane	$C_{10}H_{18}O_2$	170	1392	1322	-	0.84	-	-
52	Bicyclo[5.2.0] nonane	$C_{15}H_{24}$	204	1401	1407	-	1.94	-	-
53	Eudesma-4	$C_{15}H_{24}O$	220	1670	1636	-	0.76	-	-
54	β -eudesmol	$C_{15}H_{26}O$	222	1611	1598	1.72	1.29	-	0.72
55	T-muuro	lol	$C_{15}H_{26}O$	222	1606	1580	-	-	0.49
56	aR-turmerone	$C_{15}H_{20}O$	216	1664	1660	-	0.73	-	-
57	Hydroxycaryo-phyllene	$C_{15}H_{24}O$	220	1677	1737	-	1.28	-	-
58	Ylangena	$C_{15}H_{22}O$	218	1574	1410	-	0.29	-	-
59	Longifolenaldehyde	$C_{15}H_{24}O$	220	1668	1581	-	-	-	4.09
60	Longipinane	$C_{15}H_{26}$	206	1422	1393	-	3.21	-	-
61	α-springene	$C_{20}H_{32}$	272	1969	1940	0.82	-	-	-
62	Boronal	$C_{14}H_{22}O$	206	1584	1586	-	-	-	1.12
63	α-bergamotenol	$C_{15}H_{24}O$	220	1692	1673	0.34	-	-	-
64	Zerumbone	$C_{15}H_{22}O$	218	1733	1750	-	59.09	-	-
65	Ambrial	$C_{16}H_{26}O$	234	1809	1725	-	-	-	7.84
66	α-camphorene	$C_{20}H_{32}$	272	1986	1982	-	-	-	4.37
67	(-)-copalol	$C_{20}H_{34}O$	290	2154	2162	-	-	-	0.45
68	γ-camphorene	$C_{20}H_{32}$	272	1960	1982	-	-	-	5.93
69	Geranylgeraniol	$C_{20}H_{34}O$	290	2201	2192	-	-	-	1.27
70	Coronarin E	$C_{20}H_{28}O$	284	2135	2066	-	-	-	0.30
71	Methyl myristoleate	$C_{15}H_{28}O_2$	240	2052	2283	-	-	0.66	-
72	Methyl palmitoleate	$C_{17}H_{32}O_2$	268	2245	2478	-	-	1.16	-
73	Methyl oleate	$C_{19}H_{36}O_2$	296	2452	2675	-	-	7.59	-
74	Methyl cis-10-heptadecenoate	$C_{18}H_{34}O_2$	282	2015	2581	-	-	2.62	-
75	Methyl tricosanoate	$C_{24}H_{48}O_2$	368	2632	3102	-	-	4.81	-
76	Methyl nervonate	$C_{25}H_{48}O_2$	380	2709	3263	-	-	12.20	-
77	Lauric acid	$C_{19}H_{38}O_2$	326	1604	1653	-	-	0.67	-



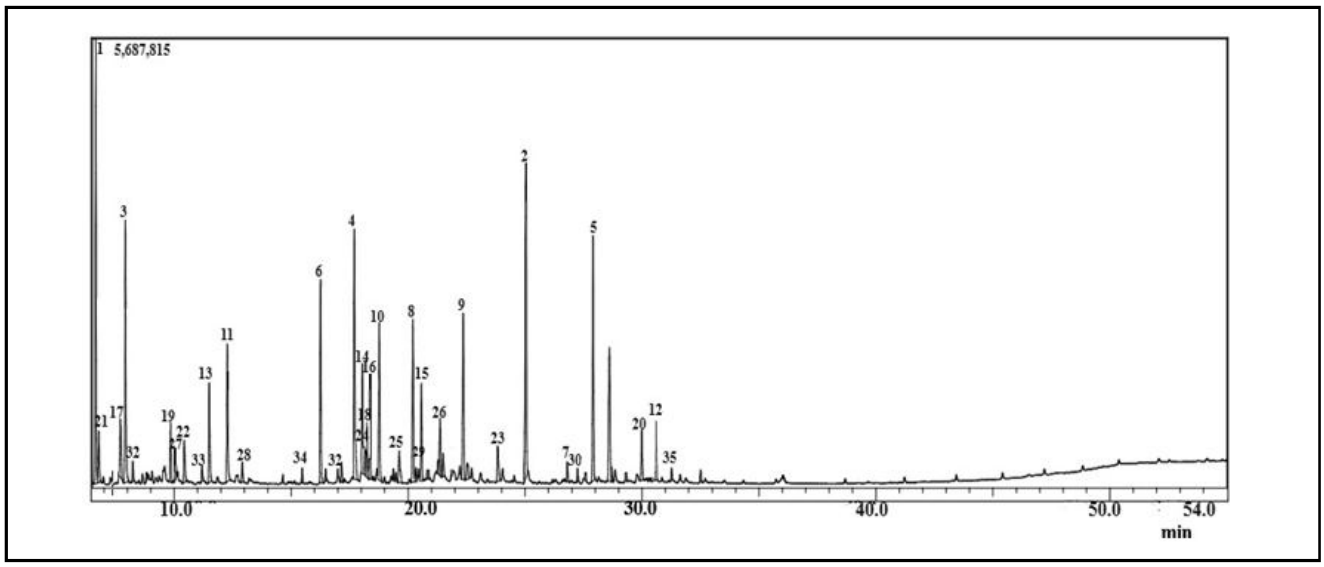
a. Gas chromatogram of essential oil from *Z. officinale*: 1.Zingiberene 2. β-sesquiphellandren 3. α-curcumene 4. β-bisabolene 5. Citral 6. Nerol 7. Borneol 8. Linalool 9. α-terpineol 10. Trans nerolidol 11. β-eudesmol 12. Zingiberenol 13. γ-muurolene 14. γ-elemene 15. Citronellal 16. Citronellol 17. α-springene 18. Elemol 19. β-myrcene 20. β-farnesene 21. α-copaene 22. Terpinolene 23. Alloaromadendrene 24. Isonerol 25. (Z,E)-farnesol 26. β-selinene 27. 2-undecanone 28. α-bergamotenol.



b. Gas Chromatogram of essential oil from Z. *wightianum:* 1. Zerumbone 2. Humulene 3. Bisabolene 4. Alloaromadendrene oxide 5. Longipinane 6. Caryophylleneoxide 7. Bicyclo[5.2.0]nonane 8. β-caryophllene 9. β-eudesmol 10. Hydroxycaryophyllene 11. β-elemene 12. Camphor 13. 1,4,4-trimethyl bicyclo nonane 14. Eudesma-4 15. aR-turmerone 16. Trans nerolidol 17. β-Gurjunen 18. Linalool 19. Bulnesol 20. Ylangena 21. α-terpineol.



c. Gas Chromatogram of essential oil from *K. parviflora*: 1. Methyl nervonate 2. Borneol 3. Linalool 4. Methyl oleate 5. Methyl tricosanoate 6. D-g ermacrene 7. β-elemene 8. Methyl cis-10-heptadecenoate 9. α-Copaene 10. β-caryophyllene 11.Bornyl acetate 12. Zingiberene 13. Methyl palmitoleate 14. Bicyclogermacrene 15. α-terpinene 16. β-sesquiphellandrene 17. Isoledene 18. Cis-muurola-3-5-diene 19. Lauric acid 20. Methyl myristoleate 21. 2-carene 22. T-muurolol 23. γ-elemene 24. α-terpineol.



d. Gas Chromatogram of essential oil from *C. amada* : 1. β-ocimene 2. Ambrial 3.1,5,9-undecatriene 4. α-curcumene 5. γ-camphorene 6. β-caryophllene 7. α-camphorene 8. Humulenol II 9.Longifolenaldehyde 10. β-sesquiphellandrene 11. Citral 12. β-farnesene 13. Nerol 14. Zingiberene 15. Epicurzerenone 16. β-bisabolene 17. 2-methyl-endo-norbornanol 18. α-farnesene 19. Borneol 20. Geranylgeraniol 21. 3-carene 22. α-terpineol 23. Boronal 24. Isolongifolol 25. Trans nerolidol 26. β-eudesmol 27. 4-terpineol 28. 2-undecanone 29. Zingiberenol 30. (-)-copalol 31. Humulene 32. Linalool 33. Citronellol 34. â-elemene 35. Coronarin E.

Figure 3: Gas chromatogram of essential oil of four Zingiberaceae spices.

3.2 Determination of DPPH radical scavenging assay

The current research focused on assessing the antioxidant properties of various ginger extracts through the DPPH assay. DPPH, recognized as a stable free radical, is commonly employed to judge the ability of antioxidants to counteract free radicals. All four species exhibited a significant effect in inhibiting the DPPH radicals (Figure 4 and Table 2). *K. parviflora* exhibited the highest antioxidant activity among the tested species, with percentage inhibition values of 56.02%, 69.25%, 76.41%, and 81.15% at concentrations of 100 µl/ml, 500 µl/ml, 1000 µl/ml, and 1500 µl/ml, respectively. *C. amada* followed, showing inhibition percentages of 48.09, 60.16, 70.60, and 80.03% across the same concentration. Whereas, the scavenging activity of *Z. wightianum* ranged from 46.25% to 73.11% across various concentrations. *Z. officinale* also exhibited notable free radical scavenging activity, with percentages ranging from 39.22% to 70.28% across the tested concentrations.

Table 2: DPPH activity of different Zingiberaceae spices

Concentration	*Zingiber officinale*	*Zingiber whitianum*	*Kamperia parviflora*	*Curcuma amada*
100 µl/ml	39.22	46.25	56.02	48.09
500 µl/ml	44.60	58.57	69.25	60.16
1000 µl/ml	62.16	64.33	76.41	70.60
1500 µl/ml	70.28	73.11	81.15	80.03

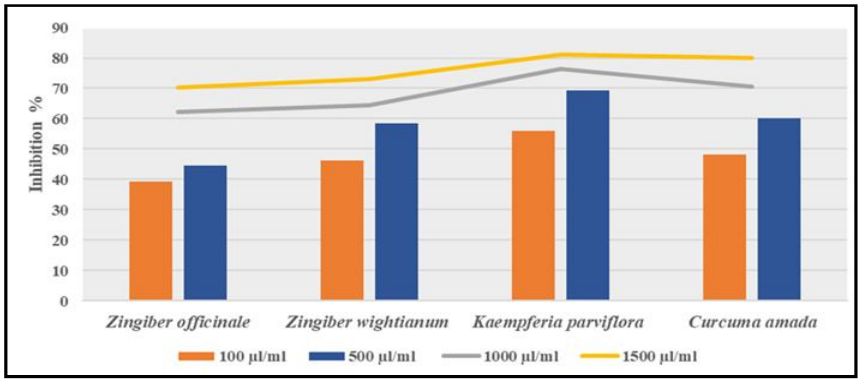


Figure 4: Graphical representation of DPPH assay of four Zingiberaceae spices.

4. Discussion

The findings presented in this study provide valuable insights into the volatile compound profiles of four significant Zingiberaceae species, highlighting the diverse chemical compositions they exhibit. The classification of these compounds emphasizes the prevalence of sesquiterpenoids, followed by monoterpenoids, which are poised to significantly contribute to the aroma, nutritional, and medicinal properties of the four species. This observation is consistent with findings by Wen *et al*. (2023), who similarly underscored the crucial role of terpenoid compounds across a broader spectrum of Zingiberaceae species, affirming their importance. The GC-MS analysis of the essential oil of *Z. officinale* revealed the presence of 28 metabolites, with zingiberene emerging as the predominant compound, constituting 22.81% of the peak area. This finding aligns with the results reported by Akshitha *et al.* (2020), who also identified zingiberene as the principal compound in ginger essential oil, highlighting its significance in the aroma and therapeutic properties. Out of 21 compounds zerumbone (59.09% peak area) was identified as the major metabolite in *Z. wightianum*. Whereas in *K. parviflora* essential oil, out of 24 metabolites methyl nervonate (12.20% peak area) was the predominant compound. Nguyen *et al*. (2023) also found similar constituents from hydrodistillation extract of essential oil in *K. parviflora*. In *C. amada*, β-Ocimene emerged as the primary metabolite, constituting 9.38% of the peak area among the 35 metabolites examined. These findings underscore the key sources of the medicinal properties associated with the species. This discovery resonates with the research conducted by Lenka *et al.* (2023), reinforcing the significance of the metabolites in contributing to the healing properties.

Few compounds were found specific to the respective species, which might be a useful marker for the discrimination of Zingiberaceae species. Mastan *et al*. (2022) also found a similar kind of difference among the three *curcuma* species through GC-MS analysis. Linalool and α-terpineol are prevalent compounds across all four species, but their distribution differs among them. This might be due to the different geographical locations of the species. For instance, in *Z. officinale*, the linalool content is 2.99%, while it spikes to 10.68% in *K. parviflora* and falls to 0.40% in *Z. wightianum* and 0.43% in *C. amada*. This variability in compound percentages suggests that the efficacy of essential oils from each species in treating specific ailments may vary. Wen *et al*. (2023) similarly noted a range of linalool concentrations from 0.61% to 2.46% across different Zingiberaceae species. One notable observation is the presence of common compounds across multiple species, indicating potential phylogenetic relationships or shared biosynthetic pathways. For instance, α-terpineol, borneol, linalool, α-copaene, γ-elemene, and β-sesquiphellandrene are identified as common constituents in both *Z. officinale* and *K. parviflora*. Similarly, α-terpineol, linalool, trans-nerolidol, and β-eudesmol are shared between *Z. officinale* and *Z. wightianum*. Additionally, a significant overlap in chemical composition is observed between *Z. officinale* and *C. amada*, with 15 compounds identified in both species. These shared compounds suggest potential similarities in flavor profiles, medicinal properties, and possibly evolutionary relationships among these species. Understanding these commonalities could inform targeted breeding strategies to enhance desired traits such as medicinal efficacy, flavor characteristics, and environmental adaptability. Furthermore, this comprehensive characterization of volatile compounds lays the foundation for future studies investigating the biological activities and therapeutic potentials of these compounds.

The DPPH assay conducted on crude extracts of Zingiberaceae spices revealed significant radical scavenging activity across all four species. The maximum scavenging activity of 81.15% was exhibited by *K. parviflora*. This finding aligns with a study by Nguyen *et al*. (2023), who reported similar radical scavenging activity ranging from 23.22% to 80.09%. While 80.03 per cent of antioxidant activity was observed in *C. amada*. Annapoorna *et al*. (2021) also found a similar result where *Curcuma amada* exhibited the highest antioxidant activity against other species. *Z. wightianum* showed a scavenging activity of 73.11%, aligning with Meera's (2021) findings of a 70% inhibition rate from a solvent extract. *Z. officinale* exhibited a notable antioxidant potential of 70.28%, this might be due to the presence of phenolic compounds particularly gingerols and shagols, known for their antioxidant and anti-inflammatory properties (Ali *et al.,* 2018; Karpagapandi and Farhat, 2021; Basha *et al*., 2023). Likewise, Silvia *et al.* (2015) highlighted the presence of potent antioxidant phenolic compounds like 6-gingerol, 8-gingerol, and shogaols extracted from ginger. Hence, observed antioxidant activity of these herbal extracts can be attributed to their abundance of phenolic compounds. These compounds are known for their ability to neutralize free radicals and mitigate oxidative stress, thereby conferring protective effects against various diseases and age-related conditions. Further, understanding the structure-activity relationships of these phytochemicals can aid in the development of novel antioxidant agents with enhanced efficacy and safety profiles.

5. Conclusion

GC-MS analysis was employed in the present investigation to examine essential oils from four distinct Zingiberaceae species. The study unveiled a rich diversity of 77 metabolites, primarily terpenoids, with sesquiterpenoids constituting approximately 51.40% of the total. Notably, significant variations in metabolomic compositions were evident among the species. *Z. officinale* predominantly featured zingiberene, while zerumbone was prominent in *Z. wightianum*. *K. parviflora* and *C. amada* exhibited methyl nervonate and β-Ocimene as primary bioactive compounds, respectively. Moreover, all species demonstrated substantial antioxidant activity, ranging from 70.28% to 81.15%, as assessed by the DPPH radical scavenging assay, attributable to their diverse bioactive compounds. This underscores the necessity for species-specific investigations to elucidate the chemical profiles of Zingiberaceae herbs, essential for informed medicinal applications, cultivation strategies, and conservation initiatives. Future studies should explore varied extraction methods to optimize both quality and quantity concurrently. Comparative analysis of essential oil compounds across species is pivotal for industries, offering insights into aroma, flavor, and therapeutic attributes, thus facilitating the selection of suitable species, product enhancement, and market competitiveness in sectors like food, perfumery, and pharmaceuticals.

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Conflict of interest

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

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