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Physicochemical Characterization and Chemical Profiling of Essential Oils from Mountainous *Zingiber* Species in Vietnam

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ABSTRACT: This study aims to characterize and compare the physicochemical properties and chemical composition of essential oils derived from seven indigenous *Zingiber* species collected from mountainous regions of Vietnam, with the goal of supporting species differentiation and quality assessment. Essential oils were evaluated through integrated physicochemical characterization and chemical profiling. Key quality indicators, including optical rotation, refractive index, density, acid value, and ethanol solubility, were determined according to national standards. Chemical composition was analyzed using gas chromatography–mass spectrometry (GC–MS). Gas chromatography-mass spectrometry (GC-MS) analysis identified a total of 80 volatile compounds across all samples, with monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons as the predominant classes. Major constituents varied markedly among species, with citronellal (up to ~30%), β-pinene (>20%), γ-terpinene and 1,8-cineole (collectively up to ~30%), and α-pinene (>50% in some species) representing the dominant compounds. Significant variation in both physical and chemical parameters among species enabled effective discrimination and revealed distinct chemical markers such as β-pinene, γ-terpinene, zingiberene, citronellal, and nootkatone. The results provide species-specific chemical fingerprints useful for differentiation and quality assessment of *Zingiber* essential oils. Although pathway-based clustering analysis was beyond the scope of this study, the findings contribute to the development of traceability frameworks and support the valorization of Vietnamese mountainous ginger resources in pharmaceutical, food, and cosmetic applications.

Keywords: Essential oil, ginger, quality index

INTRODUCTION

As demand for natural health-related products continues to increase, scientific interest has intensified in the chemical characterization of bioactive compounds derived from medicinal plants. Within this context, the Zingiberaceae family, particularly species of the genus *Zingiber* has attracted considerable attention due to its long history of use in traditional medicine, food, and aromatic applications. Ginger (*Zingiber officinale*) has been cultivated in Vietnam since at least the second century BC and remains widely used to alleviate digestive disorders, respiratory ailments, joint pain, and nausea. Beyond traditional uses, ginger-derived products are increasingly studied for their pharmacological potential, largely attributed to phenolic compounds such as gingerols and related constituents, which have demonstrated anti-inflammatory, antiemetic, and anticancer activities (Koch et al., 2023; Sharma & Kaur, 2022; Semwal et al., 2015).

In addition to non-volatile constituents, ginger essential oils (GEOs) represent an important fraction of bioactive and aromatic compounds, dominated by monoterpenes and sesquiterpenes. The qualitative and quantitative composition of these volatile compounds is known to vary markedly among *Zingiber* species, reflecting genetic differences as well as environmental influences. Such variability makes essential oil profiling a valuable tool for chemotaxonomic studies, allowing species differentiation based on characteristic chemical fingerprints rather than solely on morphological traits.

Previous studies have reported the chemical composition of GEOs from individual *Zingiber* species or limited geographical locations; however, comparative chemotaxonomic analyses involving multiple indigenous *Zingiber* species from mountainous regions of Vietnam remain scarce (Thao et al., 2023; Wu et al., 2023).

Vietnam's mountainous areas harbor diverse indigenous *Zingiber* species that systematic physicochemical characterization combined with detailed chemical profiling therefore provides an effective approach to elucidate interspecies differences. The present study focuses on the physicochemical properties and volatile chemical composition of essential oils obtained from seven indigenous *Zingiber* species collected from mountainous regions of Central Vietnam. By integrating standard physicochemical parameters with GC-based chemical profiling, this work aims to compare essential oil characteristics across mountaineous *Zingiber* species in Vietnam and identify key compounds contributing to species-level differentiation.

EXPERIMENTAL SECTION

Materials

Seven samples tubers of *Zingiber cochinchinensis*, *Zingiber gramineum*, *Zingiber rufopilosum*, *Zingiber zerumbet*, *Zingiber rubens*, *Zingiber collinsii*, *Zingiber officinale* were collected from mountains in Nghe An to Quang Binh provinces, where indigenous mountainous *Zingiber* species are predominantly distributed in. Specimens of all these species have been identified and compared with standard specimens kept at the Plant Specimen Museum, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. After collection, fresh rhizomes were cleaned, air-dried at environmental temperature. The specimens are kept at the Laboratory of the Department of Botany - Biology - Institute of Natural Education - Vinh University.

Instrumental Analysis

Gas Chromatography: Performed on an Agilent Technologies HP 6890N Plus attached to an FID detector from Agilent Technologies, USA. HP-5MS chromatography column with a length of 30 m, inner diameter of 0.25 mm, a thin film layer of 0.25 um was used. Carrying gas was He. Injector and detector temperatures were 260°C. Temperature program of oven was as following 60°C (2 minutes), increased by 4°C/minute until 220°C, stop at this temperature for 10 minutes (Do et al., 2013).

Essential oils were extracted by hydrodistillation using a Clevenger-type apparatus designed for light essential oils (density < 1). Briefly, 500 g of finely ground fresh ginger rhizome was mixed with 2500 mL distilled water for distilation in 5 hrs. After distillation, the essential oil layer was collected, dried over anhydrous sodium sulfate, and stored at 4°C in sealed amber vials until analysis.

Gas chromatography-mass spectrometry (GC/MS): Qualitative analysis is performed on the Agilent Technologies HP 6890N GC/MS gas chromatography and spectrometry system. The Agilent Technologies HP 6890N pairs with the Agilent HP 5973 MSD Mass Selective Detector. The HP-5MS column measures 0.25 um x 30 m x 0.25 mm. Temperature program at 60°C/2 minutes; increase the temperature by 4°C/1 minute until 220°C, then increase the temperature again by 20°/min until 260°C; with He as the carrier gas. Validation of the compound identifications is performed by comparing their MS spectral data with the published standard spectra available in the HP Willey/Chemstation library (Do et al., 2013).

The essential oil was characterized following Vietnamese national standards (TCVN). Sensory evaluation was conducted according to TCVN 8460:2010. Physicochemical properties were determined as follows: density (TCVN 8444:2010), refractive index (TCVN 8445:2010), and optical rotation (TCVN 8446:2010). The acid value and ester content were measured based on TCVN 8450:2010 and TCVN 8451:2010, respectively. The aldehyde content, with cinnamaldehyde as a reference compound, was analyzed by the sodium sulfate method following TCVN 189:1993. Ethanol solubility was assessed according to TCVN 8449:2010.

RESULTS AND DISSCUSSION

Physicochemical Properties of Ginger Essential Oils

The comparison of physicochemical and sensory attributes among essential oils extracted from different *Zingiber* species (**Table 1**) reveals significant variation that can serve as a basis for authenticity determination. All samples exhibited clarity appearance, but noticeable differences were observed in color, odor, and taste. For instance, *Z. officinale* showed a deep lemon-yellow color and a strong spicy aroma, typical of commercial ginger essential oil, whereas *Z. rubens* displayed a pale-yellow tone and a more pungent scent. Similarly, taste intensity varied, with *Z. gramineum*, *Z. zerumbet*, *Z. rubens*, and *Z. officinale* expressing stronger pungency than others like *Z. cochinchinensis* or *Z. collinsii*, which were milder.

The specific gravity (d at 20°C) ranged from 0.867 in *Z. cochinchinensis* to 0.985 in *Z. rufopilosum*, suggesting variation in the concentration of volatile constituents. Optical rotation (α^{t}_{D}) also differed among species, with *Z. officinale* showing the highest value (12.5°), which can potentially serve as a fingerprint parameter for authenticating commercial ginger oil. The refractive index varied notably across samples, with *Z.*

gramineum exhibiting a significantly lower value (1.3359), possibly indicating compositional differences or dilution. Acid values ranged from 16.83 mg KOH/g (*Z. rufopilosum*) to 33.67 mg KOH/g (*Z. rubens*), reflecting variability in free fatty acid content, which may relate to oxidative stability and freshness of the oil (Dang et al., 2022).

The observation that ester content remained relatively consistent across *Zingiber* species suggests limited variation in esterified compounds. In contrast, the aldehyde content exhibited notable differences, ranging from 7.43% in *Z. zerumbet* to 15.71% in *Z. gramineum* and *Z. rubens*, indicating its potential as a chemical marker for species differenciation as also reported in previous chemotaxonomic studies of *Zingiber* essential oils (Do et al., 2013; Theanphong et al., 2016). Previous studies have demonstrated that aldehyde-rich essential oils, particularly those high in citral or related compounds, often possess strong antimicrobial and antioxidant activities (Do et al., 2013; Theanphong et al., 2016; Nguyen et al., 2018). For instance, essential oils from *Z. officinale* with high aldehyde content showed significant free radical scavenging capacity and antibacterial properties (Dang et al., 2022; Nguyen et al., 2018). Similarly, essential oils of *Z. striolatum* exhibited MIC values as low as 0.78 mg/mL against Gram-positive bacteria, supporting the functional relevance of aldehyde compounds (Ma et al., 2019). These findings suggest that aldehyde concentration not only supports chemical authentication but also provides an early indicator of the biological potential of GEOs. Ethanol solubility is another differentiating factor; while most oils showed solubility at a ratio of 1:1.8, oils from *Z. zerumbet*, *Z. rubens*, and *Z. collinsii* required higher ethanol volumes for complete dissolution, indicating differences in polarity or molecular composition.

The results indicate that several physicochemical parameters, particularly optical rotation, aldehyde content, color, and ethanol solubility, can be utilized as distinguishing markers for the authenticity of GEOs. These indicators provide foundational data for the development of a standardized database for traceability and quality control of Zingiberaceae essential oils in Vietnam.

Chemical Composition of Ginger Essential Oils

The essential oil compositions among the seven ginger species (**Table 2**) exhibit notable differences, especially in key biomarker compounds that help distinguish each species. *Z. cochinchinensis* is characterized by its high β-pinene content, accounting for 27.61%, along with moderate levels of caryophyllene at 6.3%, camphene at 3.99%, and bicyclogermacrene at 2.93%.

The notably elevated β-pinene serves as a distinctive biomarker, making it a key feature for the identification of this species. *Z. gramineum* is notable for its high levels of γ-terpinene (17.9%) and 1,8-cineole (12.8%), which serve as key biomarkers for this species. Additionally, it has a significant citronellal content of 26.1%, contributing to a lemony aroma profile, while α-pinene (4.1%) and geraniol (0.8%) are present in moderate amounts.

Z. rufopilosum is characterized by elevated levels of camphene (17.0%) and citronellal (26.1%), which are its major biomarkers. It also contains a low amount of α-phellandrene (0.4%) and β-bisabolene (1.9%), indicating a chemical profile that predominantly favors camphene and citronellal. *Z. zerumbet* is distinguished by high levels of camphene (16.3%), zingiberene (7.2%), bornyl acetate (6.6%), and citronellal (26.1%). The elevated amounts of zingiberene and bornyl acetate make this species unique, with citronellal adding to its distinctive scent profile. Zingiber rubens features prominent citronellal (30.1%) and significant geraniol (6.6%), along with zingiberene (5.3%) and α-cadinol (1.2%). The combination of high citronellal and notable geraniol levels gives this species its distinctive aromatic signature. *Z. collinsii* contains zingiberene at negligible levels, with some samples showing citronellal around 1.9%, nootkatone at 1.4%, and benzyl benzoate at 0.9%. The presence of nootkatone as a biomarker highlights its uniqueness among ginger species, along with a variety of minor compounds. *Z. officinale* (common ginger) is characterized by high citronellal content (30.6%), along with moderate amounts of geraniol (4.6%), zingiberene (4.0%), and benzyl benzoate (0.9%), which collectively serve as typical markers for this widely used specie. From the composition of seven *Zingiber* species presented in **Table 2**, specific volatile compounds were identified as potential chemical markers (Table 3) for distinguishing among the species.

Across all investigated *Zingiber* species, essential oils were dominated by terpenoid compounds, with sesquiterpene hydrocarbons and monoterpene hydrocarbons as the principal chemical classes. However, the relative contribution of each class varied markedly among species, forming distinct chemotaxonomic patterns. Although a total of numerous volatile compounds were identified across the seven *Zingiber* species, chemotaxonomic interpretation was primarily based on dominant constituents and their distribution patterns. The essential oils could be grouped into distinct chemotypes. *Z. collinsii* and *Z. cochinchinensis* exhibited a pinene-rich chemotype characterized by high levels of α-pinene and β-pinene, distinguishing them from the other species.

Table 1. Physicochemical properties of ginger essential oils

	Physico chemical properties	Essential oils						
		Z. cochinchinensis	*Z. gramineum*	*Z. rufopilosum*	*Z. zerumbet*	*Z. rubens*	*Z. collinsii*	*Z. officinale*
1	Oil clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear
2	Color of essential oil	Bright yellow	Light bright yellow	Deep bright yellow	Deep bright yellow	Light yellow	Dark yellow	Dark lemon yellow
3	Aroma of essential oil	Pleasant aroma	Mild fragrant	Mild, slightly pungent	Fragrant, slightly sharp	Strong, pungent	Mild aroma	Strong, pungent
4	Taste of essential oil	Slightly spicy	Spicy tendency	Slightly spicy	Spicy tendency	Spicy tendency	Slightly spicy	Spicy tendency
5	Density d at 20°C	0.867	0.975	0.985	0.943	0.939	0.920	0.974
6	Optical rotation α^{t}_{D}	7°	11°	9°	9°	2.5°	10°	12.5°
7	Refractive index n^{20}_{D}	1.4619	1.3359	1.4851	1.4811	1.4631	1.4818	1.4810
8	Acid value (mg KOH/g)	28.05	22.44	16.83	28.05	33.67	22.44	28.05
9	Ester value (mg KOH/g)	1.6	1.4	1.5	1.7	1.5	1.5	1.7
10	Aldehyde content (%)	14.4	15.71	8.56	7.43	15.71	12.9	14.31
11	Solubility in ethanol (μl)	1: 1.8	1: 1.8	1: 1.8	1: 2.2	1: 3.6	1: 3.6	1: 1.8

Table 2. Chemical composition of *Zingiber* species' essential oils by GC MS

Composition	*Z. cochinchinnensis*	*Z. gramineum*	*Z. rufopilosum*	*Z. zerumbet*	*Z. rubens*	*Z. collinsii*	*Z. officinale*
Tricyclene	0.51	-	0.1	0.2	-	-	0.2
α-Pinene	-	4.1	10.0	3.3	2.6	50.2	3.1
Camphene	3.99	1.7	17.0	16.3	9.7	2.3	12.8
Sabinene	-	-	-	14.6	-	-	0.1
β-Pinene	27.61	7.2	6.2	0.5	0.4	23.6	0.4
β-Myrcene	2.28	3.0	0.6	-	-	2.9	-
α-Phellandrene	-	0.8	0.4	0.7	0.4	1.3	0.6
α-Terpinene	1.29	17.1	0.2	-	-	0.3	-
o-Cymene	-	0.1	-	-	-	-	-
Limonene	5.00	-	4.0	-	-	5.3	-

1.8-Cineole	-	12.8	-	0.1	-	-	7.3
γ-Terpinene	-	17.9	0.4	0.1	-	1.0	-
α-Terpinolene	1.11	6.6	0.3	0.4	-	0.5	0.4
Linalool	0.31	-	0.8	-	1.6	0.2	0.1
Borneol	0.61	0.1	0.2	2.0	1.3	-	2.0
Terpinen-4-ol	-	13.0	0.1	-	-	0.1	-
α-Terpineol	-	1.5	0.2	-	1.1	-	-
Bornyl acetate	1.20	0.5	6.6	-	1.6	0.4	0.3
β-Elemene	0.29	0.6	0.3	0.2	-	0.1	0.2
Zingiberene	-	0.9	-	7.2	5.3	-	4.0
Caryophyllene	6.30	0.3	1.0	-	-	-	0.1
Caryophyllene oxide	1.93	0.1	0.6	-	0.1	-	-
δ-Cadinene	2.80	-	-	-	-	-	0.3
α-Cadinol	0.50	-	4.9	-	1.2	0.1	-
Spathulenol	-	0.1	0.7	-	0.9	0.2	1.0
Geraniol	-	0.8	0.8	0.8	6.6	-	4.6
Geranyl acetate	-	0.7	-	1.4	1.3	-	1.6
Citronellal	-	26.1	-	26.1	30.1	-	30.6
Z-Citral	-	0.1	-	0.1	0.4	-	0.3
Citronellyl acetate	-	-	1.9	-	1.9	0.4	1.7
Bicyclogermacrene	2.93	-	1.3	-	-	-	1.0
β-Bisabolene	-	0.3	-	1.9	1.6	-	1.8
Valencene	-	-	0.2	1.9	-	-	-
Nootkatone	-	-	1.4	-	0.2	-	0.2
Phytol	-	-	0.2	-	-	-	0.1
Benzyl benzoate	-	0.1	0.9	-	0.1	-	0.9
Elemol	-	-	0.4	-	-	0.1	1.0
α-Gurjunene	-	-	-	0.3	-	-	0.3
Germacrene D	-	-	1.2	0.2	-	0.3	-
β-Eudesmol	-	-	0.3	0.7	1.2	-	-

Table 3. Compound-marker of *Zingiber* species' essential oils

Species	Dominant compounds' chemotype	Major marker compounds	Chemotaxonomic significance
Z. cochinchinensis	β-Pinene	β-Pinene (27.6), Camphene (4.0)	Distinct monoterpene-hydrocarbon profile
Z. collinsii	α-/β-Pinene	α-Pinene (50.2), β-Pinene (23.6)	Strongly differentiated from other species
Z. rufopilosum	Camphene-rich	Camphene (17.0), α-Pinene (10.0)	Monoterpene-dominant profile
Z. gramineum	Citronellal	Citronellal (26.1), γ-Terpinene (17.9)	Oxygenated monoterpene
Z. zerumbet	Citronellal + esquiterpene	Citronellal (26.1), Zingiberene (7.2)	
Z. rubens	Citronellal +zingiberene	Citronellal (30.1), Zingiberene (5.3)	Citronellal and Ginger-like sesquiterpene
Z. officinale	Citronellal + camphene	Citronellal (30.6), Camphene (12.8), Zingiberene (4.0)	

A camphene-dominant profile was observed in *Z. rufopilosum*, *Z. zerumbet*, *Z. rubens*, and *Z. officinale*, suggesting close chemotaxonomic affinity. Notably, *Z. gramineum*, *Z. zerumbet*, *Z. rubens*, and *Z. officinale* showed a citronellal-dominant chemotype, with citronellal accounting for more than 25% of the total composition, indicating its potential as a diagnostic marker. In addition, sesquiterpenes such as zingiberene, caryophyllene, and cadinol derivatives further contributed to species differentiation. These compositional patterns highlight clear chemotaxonomic relationships among Vietnamese mountainous *Zingiber* species

Table 3 highlight distinct volatile profiles among *Zingiber* species, with key compounds such as citronellal, β-pinene, α-pinene, γ-terpinene, 1,8 cineole, zingiberene, and nootkatone serving as potential chemotaxonomic markers. These findings are consistent with the chemotaxonomic analysis of volatile oils from rhizomes of nine *Zingiber* species in Thailand, where species differentiation was effectively achieved based on monoterpene and sesquiterpene composition (Theanphong et al., 2016). In particular, β pinene was found at levels above 20% in *Z. cochinchinensis*, confirming its diagnostic value, as similarly reported in Vietnamese *Zingiber* species rich in pinene derivatives with larvicidal and antimicrobial activity (Roach et al., 2020).

Relation Between Geographical Condition and Chemical Composition

Figure 1 presents a finger print of relative peak area percentages of major volatile compounds in essential oils from seven *Zingiber* species collected from mountain in the Central Vietnam. In *Z. gramineum*, the simultaneous presence of γ-terpinene and 1,8-cineole aligns with studies highlighting the anti-inflammatory and antibacterial properties of these oxygenated monoterpenes (Pena-Rivera & Gómez, 2018). Citronellal, which was detected at ≥26% in five species, including *Z. officinale*, has been previously identified as a prominent compound in ginger essential oils from Ecuador and Vietnam, and is often used as a quality marker for origin-based classification (Nguyen et al., 2018; Martínez et al., 2015).

The presence of zingiberene in high amounts in *Z. zerumbet* and *Z. rubens* reflects its well-established role as a major sesquiterpene in ginger, contributing to its spicy aroma and functional properties (Perveen, 2022). Of particular note is the detection of nootkatone in *Z. collinsii*, a compound known for its woody, a citrus aroma and pharmacological relevance, including antimicrobial and insect-repelling activities, although it has rarely been reported in *Zingiber* species to date (Ma et al., 2019). These compositional distinctions not only confirm the taxonomic uniqueness of each species but also underline their potential functional differences. Therefore, the identified marker compounds are valuable for both species authentication and quality control in the development of high-value ginger-based products. These findings demonstrate that the volatile composition of ginger essential oils varies by species, and several compounds, especially citronellal, β-pinene, α-pinene, and zingiberene can serve as reliable chemical markers for species authentication and geographical traceability. In term of geography, **Table 4**, present main information realting to Nghe An and Quang Binh province climate, where the *Zingiber* species were collected.

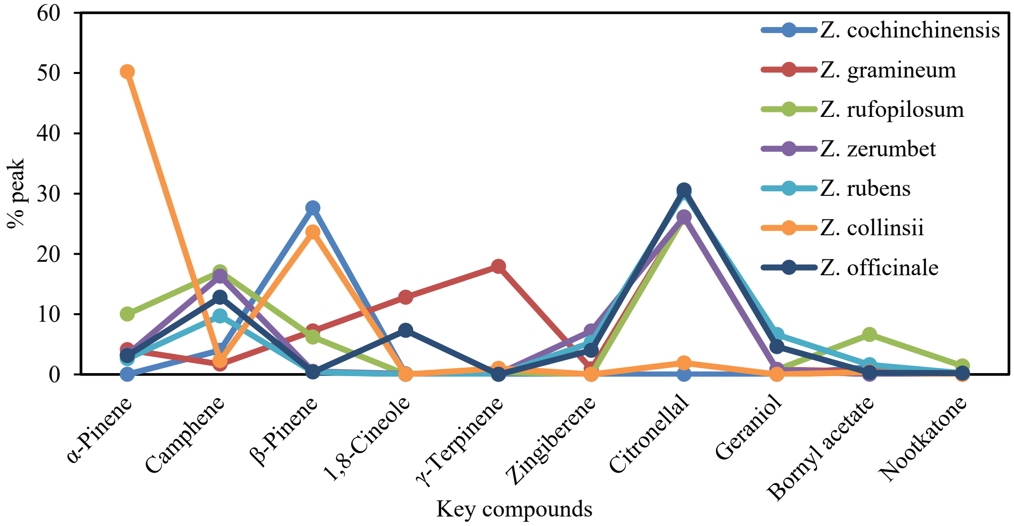

Figure 1. Finger print of relative peak area percentages of major volatile compounds in essential oils from seven *Zingiber* species collected from mountain in the Central Vietnam

Table 4. *Zingiber* species' cultivation's condition in Nghe An and Quang Binh province (2024) *

Geographical	Provinces with > 80% area with mountain in the Central of Vietnam
Season	West - South dry and hot monsoon (from April to August) and the Northeast monsoon is cold and wet (from November to March year later).
Temperature	Annual high temperature: 25.33°C Annual low temperature 15.78 °C
Air humidity	Average: 83.27%
Rainfall	Number of days with rainfall (≥ 1.0 mm): 191.82 days (52.55%) Days with no rain: 173.18 days (47.45%)

(https://weatherandclimate.com/vietnam)

The cultivation environment, characterized by its mountainous terrain, high annual humidity (83.27%), and significant rainfall distribution (approximately 191.82 rainy days per year), creates favorable ecological niches for the growth of diverse *Zingiber* species (**Table 4**). Seasonal variations, particularly the hot-dry period (April-August) followed by the cold-wet Northeast monsoon (November-March), provide contrasting stress conditions that can influence secondary metabolite biosynthesis. Climatic pressures such as temperature shifts, humidity, and rainfall are known to modulate the biosynthetic pathways of terpenoids and phenolic compounds, enhancing both their diversity and intensity (Pena-Rivera & Gomez, 2018; Perveen, 2022).

The chemical fingerprints of seven *Zingiber* species from these regions (**Figure 1**) reflect clear compositional variations that may be linked to geographical conditions. For instance, high citronellal levels in *Z. zerumbet* and *Z. rubens* could be associated with the cool and humid conditions of the Northeast monsoon, which favor aldehyde biosynthesis. In contrast, the remarkable dominance of α-pinene (>50%) in *Z. collinsii* suggests a possible adaptive response to the drier and hotter season, as monoterpenes such as α-pinene are often linked to plant defense under heat and water stress (Roach et al., 2020). Similarly, β-pinene, abundant in *Z. cochinchinensis*, may represent an adaptation to fluctuating temperature and altitude-related stress, while γ-terpinene and 1,8-cineole in *Z. gramineum* correlate with its strong aromatic and protective cultural perception, consistent with their roles as chemotaxonomic markers (Theanphong et al., 2016).

Together, **Table 4** and **Figure 1** highlight the interplay between geographical conditions and chemical composition, showing that the unique ecological environment of Nghe An and Quang Binh not only supports species diversity but also shapes the essential oil fingerprints that underpin both cultural valuation and scientific authentication. This relationship provides an important scientific basis for linking terroir-like effects to the authenticity and heritage value of indigenous ginger species (Giovannucci et al., 2009).

CONCLUSSIONS

This study highlights the significant diversity and distinctive features of essential oils from various *Zingiber* species based on their chemical and physicochemical profiles. Key compounds such as β-pinene, γ-terpinene, citral, zingiberene, and nootkatone, along with physical parameters including clarity, color, density, refractive index, optical rotation, and acid value, were identified, enabling differentiation and quality assessment of each species. The physicochemical attributes, such as clarity, color, and solubility, show marked differences that reflect the intrinsic properties of each species. Meanwhile, parameters like specific density, refractive index, optical rotation, and acid value provide essential information for standardization, authentication, and optimization of *Zingiber* essential oils. Overall, integrating chemical and physicochemical analyses enhances the ability to trace origin, evaluate quality, and unlock the potential applications of these oils in pharmaceutical, cosmetic, and culinary industries, while also contributing to the conservation of these valuable natural resources.

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