

Chemical Profiling of Essential Oils of *Zingiber ottensii* Valetton Collected from Yala Province, Southern Thailand

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Abstract

Zingiber ottensii Valetton is a medicinal plant that belongs to the Zingiberaceae family. It has been used as traditional medicine to relieve pain and inflammation in many local communities, especially in the lower southern region of Thailand. Three accessions of *Z. ottensii*, including Yala-1, Yala-2, and Yala-3, were collected from three different sub-districts of Than To District, Yala Province. The rhizome oils of three accessions were extracted using the hydrodistillation method before being analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Twenty-four chemical compounds representing 88.77–94.89% of essential oils were identified, which consisted of zerumbone, terpinene-4-ol, α -humulene, and sabinene as major constituents. Zerumbone is a monocyclic sesquiterpene that was found in the highest percentages of rhizome oils of Yala-1 (51.26%), Yala-2 (46.32%), and Yala-3 (36.71%). In addition, the soil of three cultivation areas was analyzed for plant nutrients, which included organic carbon (OC), organic matter (OM), nitrogen (N), phosphorus (P), and potassium (K). Soil samples from the Yala-1 cultivation area exhibited the highest percentages of OC, OM, N, and K. These results may indicate that soil fertility has a positive effect on the rate of zerumbone in *Z. ottensii*'s rhizome oil, which would provide the benefits of soil-improving management for maximizing the yields of *Z. ottensii* cultivation and zerumbone contents.

Keywords: *Zingiber ottensii*, lower southern region, Gas Chromatography-Mass Spectrometry, soil fertility

Introduction

Zingiber ottensii Valetton (also known as Plai-Dum in Thai) is a perennial, aromatic plant that belongs to the Zingiberaceae family. This species is native to Southeast Asia, which was first recorded in Java, Indonesia, by Valetton (Valetton, 1918). It was observed in its distribution in Malaysia (Ridley, 1924), Singapore (Holtum, 1950), Thailand (Theilade, 1999), Vietnam (Ngoc-Sam *et al.*, 2016), and Myanmar (Aung and Tanaka, 2019). In Thailand, *Z. ottensii* has been primarily distributed through cultivation; however, it can also be found in its natural habitat, especially in the southern region. Kittipanangkul and Ngamriabsakul (2008) reported the distribution of *Z. ottensii* at altitudes of 90–300 meters in Khao Nan and Khao Luang National Parks, Nakhon Si Thammarat.

The rhizome of *Z. ottensii* has a pungent smell, and its color occurs in various purple shades, such as dark, greyish, light, and pinkish purple. It has been used as an ingredient in Thai folk medicine for relieving pain, sprains, and inflammation. In addition to human treatment, it has been used to cure the broken bones of the cockfighting roosters in the local southern communities of Thailand. Many previous studies demonstrated the potential bioactivities of *Z. ottensii*'s rhizome, such as antimicrobial, antioxidant, α -glucosidase inhibition, nitric oxide production inhibition, and anti-inflammatory effects (Habsah et al., 2000; Tiengburanatam et al., 2010; Chantaranonthai et al., 2013; Thitinarongwate et al., 2022). Those pharmacological activities were related to the phytochemical compounds in *Z. ottensii*'s rhizome.

Essential oil is one of the sources of bioactive compounds in Zingiber plants. The rhizome of *Z. ottensii* contained the essential oil that is mainly represented by the monoterpenes, sesquiterpenes, and their oxygenated derivatives. Studies by other researchers demonstrated that *Z. ottensii*'s rhizome oil from Malaysia consisted of zerumbone, terpinen-4-ol, α -humulene, and sabinene as the major components (Sirat and Nordin, 1994). The five highest constituents of the volatile oils of *Z. ottensii*'s rhizome from Indonesia were found to be 1-4-terpineol, zerumbone, sabinene, 1,8-cineole, and γ -terpinene, respectively (Marliani et al., 2018). Moreover, the rhizome oil of *Z. ottensii* collected from Thailand showed high amounts of zerumbone, terpinene-4-ol, *p*-cymene, sabinene, and humulene (Thubthimthed et al., 2005).

Nowadays, the studies of phytochemicals and bioactivities of *Z. ottensii* have been gradually gaining interest because of its potential pharmacological applications. The plant sources were collected from different locations in Thailand, such as Phetchaburi (Thubthimthed et al., 2005), Bangkok (Chantaranonthai et al., 2013), and Chiang Mai (Thitinarongwate et al., 2022). However, there has been no previous research relating essential oil concentration to soil nutrients in *Z. ottensii* from Yala province. In this study, the essential oils of *Z. ottensii*'s rhizomes collected from three sub-districts in Yala province were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) technique. Plant nutrients in the soil samples of their cultivation areas were also determined to look for the relationship between soil nutrients and the percentage of zerumbone in *Z. ottensii*'s rhizome oil.

Materials and Methods

1. Plant materials and isolation of essential oils

The samples of 2-year-old fresh rhizomes of *Z. ottensii* at the mature stage were collected from three different locations in the Than To district, Yala province, Thailand, in August 2022. The rhizomes of each sample (300 g) were chopped and blended into small pieces before being extracted by the hydrodistillation method using a Clevenger-type apparatus. The isolated essential oils were dried using anhydrous sodium sulphate before being subjected to GC-MS analysis.

2. GC-MS analysis

The obtained essential oils were filtrated through a 0.2 μ m membrane and then subjected to GC-MS analysis using an Agilent Technologies Model 6890 N gas chromatograph (Palo Alto, CA, USA) equipped with an HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μ m), quadrupole analyzer, and 5973 inert mass selective detector (MSD). The column temperature was initially held at 50°C and then increased by 4°C/min to 230°C. The injector was set at 230°C and performed in pulse split mode with a split ratio of 20 volumes per 1 μ L. Helium was used as

a carrier gas at a pressure of 11.01 psi and a flow rate of 1.2 mL/min. Mass spectra were obtained in the electron impact mode at 70 eV with masses ranging from 40 to 400 amu. GC-MS analysis was done in duplicate. The volatile constituents were identified by comparison of their retention indices (RI) and mass spectra fragmentation with those stored in the Wiley7n mass spectral library, with data published in the literature (Adams, 2001). The percentages of identified components were calculated as peak areas to the total peak area.

3. Soil nutrient analysis

Soil samples from three *Z. ottensii* cultivation areas were collected according to the procedure of the Office of Agricultural Research and Development Region 8, Department of Agriculture (2025). Soil nutrients, which consisted of the percentages of organic carbon (%OC), organic matter (%OM), nitrogen (%N), available phosphorus (%P), and available potassium (%K), were measured in triplicate using in-house methods based on Methods of Soil Analysis (Sparks et al., 1996) and Official Methods of Analysis (AOAC, 2016).

Results and Discussion

This was the first study on volatile oil profiling of *Z. ottensii* collected from Yala province, one of three southern border provinces of Thailand that has beautiful nature and high values of biodiversity. Three accessions of *Z. ottensii* were collected from three sub-districts of Than To district, Yala province, which included Yala-1 (Than To sub-district), Yala-2 (Mae Wat sub-district), and Yala-3 (Khiri Khet sub-district). They showed no difference in growth and morphology among the three samples. Also, rhizomes of three samples were of similar shapes with the light shade of pinkish purple color (**Figure 1**), while their essential oils were clear and pale yellow. The yields of volatile oils from Yala-1, Yala-2, and Yala-3 were 0.25%, 0.36%, and 0.59%, respectively. Similarly, previous reports have shown essential oil contents ranging from 0.38% to 0.86%, extracted from the rhizomes of *Z. ottensii* from different countries (Sirat and Nordin, 1994; Malek *et al.*, 2005; Thubthimthed *et al.*, 2005).

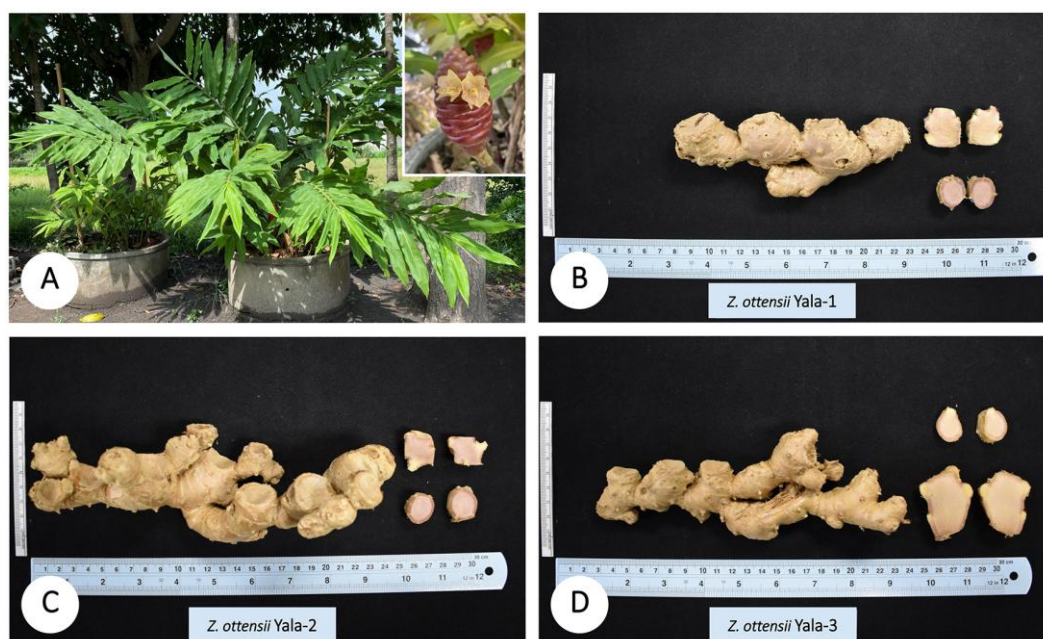


Figure 1 *Zingiber ottensii* with its flowers (A) and the rhizomes of Yala-1 (B), Yala-2 (C), and Yala-3 (D) collected from three different sub-districts of Than To district, Yala province

The chemical profiling of *Z. ottensii*'s rhizome oils was identified by the GC-MS technique, and the result showed that total ion chromatograms (TIC) of the three samples had a similar pattern but differences in peak heights and peak areas (Figure 2). A total of 24 constituents could be identified in the rhizome oils, representing 88.77-94.89% of the total rhizome oils (Table 1). Total detected components were classified as monoterpenes, sesquiterpenes, and their derivatives. Seven dominant constituents of *Z. ottensii*'s essential oils consisted of zerumbone, terpinene-4-ol, α -humulene, sabinene, zingiberene, β -pinene, and *p*-cymene. Zerumbone, the oxygenated sesquiterpene, was observed in the highest percentage in the essential oils of Yala-1 (51.26%), Yala-2 (46.32%), and Yala-3 (36.71%). Many previous works reported that the most abundant compound of *Z. ottensii*'s rhizome oil was zerumbone, representing 25.6-40.1% of the total oil, while other major compounds were sabinene, terpinen-4-ol, α -humulene, *p*-cymene, γ -terpinene, and 1,8-cineole (Sirat and Nordin, 1994; Malek et al., 2005; Thubthimthed et al., 2005; Marliani et al., 2018). Interestingly, Yala-1 and Yala-2 cultivars showed the percentages of zerumbone in rhizome oils superior to those in previous research.

Table 1 Essential oil compositions (% of the total) of *Zingiber ottensii*'s rhizomes collected from three locations in the Than To district, Yala province

No.	RT	Compounds	% Composition in each accession		
			Yala-1	Yala-2	Yala-3
1	6.15	α -thujene	-	0.31	0.46
2	6.33	α -pinene	-	1.56	1.69
3	7.47	sabinene	2.28	9.06	12.33
4	7.56	β -pinene	0.90	4.63	5.34
5	7.93	β -myrcene	-	0.47	0.68
6	8.72	α -terpinene	-	1.57	2.10
7	8.98	<i>p</i> -cymene	4.20	0.83	0.69
8	9.11	limonene	-	0.51	0.68
9	9.25	1,8-cineole	1.34	1.53	1.63
10	10.07	γ -terpinene	-	3.10	3.68
11	11.04	α -terpinolene	-	0.57	0.82
12	11.52	linalool	-	0.15	0.51
13	12.88	1-terpineol	-	-	0.35
14	13.78	borneol	-	-	0.25
15	14.23	terpinene-4-ol	12.30	10.89	11.94
16	14.62	α -terpineol	0.92	0.64	0.92
17	21.99	<i>trans</i> -caryophyllene	-	0.53	0.55
18	23.11	α -humulene	3.27	10.18	7.82
19	27.00	caryophyllene oxide	1.77	0.39	0.61
20	27.82	zingiberene	8.47	-	-
21	28.39	γ -eudesmol	0.74	0.51	0.86
22	28.92	β -eudesmol	0.82	0.56	0.85
23	29.00	α -eudesmol	0.50	0.58	0.76
24	31.46	Zerumbone	51.26	46.32	36.71
		Others	11.23	5.11	7.77

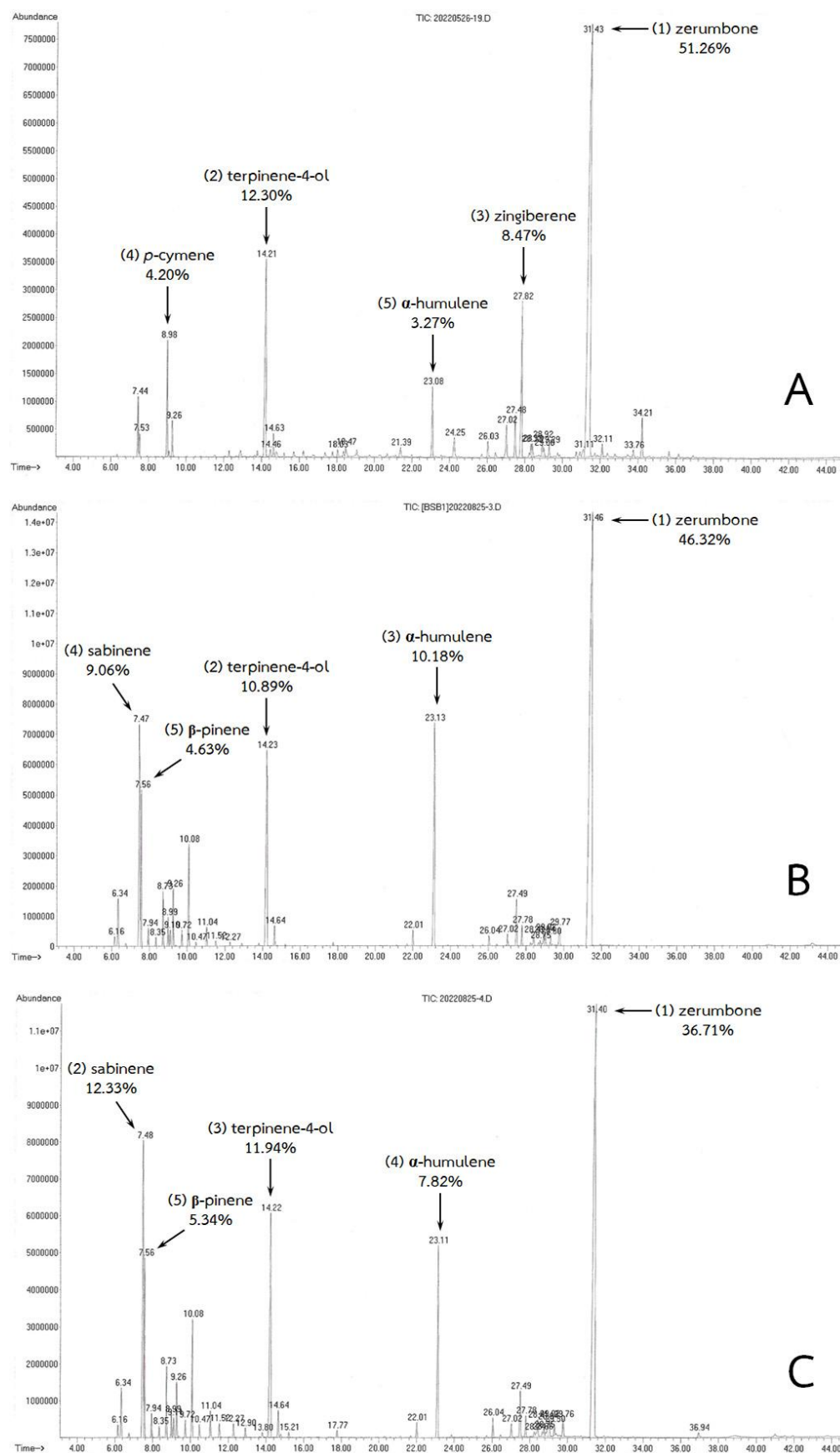


Figure 2 TIC chromatograms of the essential oils extracted from *Zingiber ottensii*'s rhizomes from three locations in the Than To district, Yala province, A: Yala-1; B: Yala-2, and C: Yala-3

Zerumbone is the potential bioactive compound that possesses various biological activities such as anti-inflammatory, antioxidant, antimicrobial, antitumor, anti-hyperalgesia, antibiofilm, anticancer, and herbicidal activity (Ibáñez *et al.*, 2023). The previous study demonstrated that zerumbone showed the inhibitory effect against colon carcinoma SW480 cells (Sadhu *et al.*, 2007). Moreover, this compound could reduce osteoclastogenesis and suppress human breast cancer-induced bone loss in mice, which might be further applied to the therapeutic agent for osteoporosis and cancer-associated bone loss (Sung *et al.*, 2009).

In addition to zerumbone, other major constituents identified in *Z. ottensii*'s rhizome oils have been reported for their biological activities. Sabinene, a bicyclic monoterpene, showed antibacterial, antifungal, antioxidant, anti-inflammation capacities and prevented skeletal muscle atrophy by inhibiting the MAPK-MuRF-1 pathway in rats (Ryu *et al.*, 2019; Sharma *et al.*, 2019). β -pinene could serve as a miracle gift of nature because of its diverse therapeutic properties, such as antimicrobial, antitumor, anticonvulsant, and antidepressant (da Silva *et al.*, 2012; Guzmán-Gutiérrez *et al.*, 2015; Salehi *et al.*, 2019). *p*-cymene has shown remarkable analgesic, antioxidant, anti-inflammatory, antidiabetic, antinociceptive, and neuroprotective agents, which could be a potential source of drug discovery for human healthcare and industrial applications (Balahbib *et al.*, 2021). Terpinen-4-ol was found in high abundance in three samples (10.89-12.30%). It exhibited many biological activities that could be a novel and promising therapeutic agent for human gastrointestinal cancers (Shapira *et al.*, 2016). Interestingly, zingiberene could only be detected in Yala-1's essential oil. A previous study demonstrated that this monocyclic sesquiterpene could elevate the antioxidant level and suppress the inflammatory markers. Also, it showed anticancer potential against tumorigenesis *in vivo*, which could be a promising chemotherapeutic agent (Seshadri *et al.*, 2022). Another major compound is α -humulene, which displayed the inhibitory effects in the inflammatory experimental models in mice and rats, which might be an alternative compound for the treatment of inflammatory diseases (Fernandes *et al.*, 2007).

This might suggest that the multifunctional bioactivities of major constituents detected from the rhizome oils of *Z. ottensii* are implicated in the therapeutic properties of this medicinal plant. However, pharmacological studies should be performed to prove their bioactivities. Although the rhizome oils of three cultivars showed similar chemical profiles, their component percentages were different, and some components were unique to each cultivar (Figure 2; Table 1). Yala-1 had the highest abundance of zerumbone content, whereas Yala-3 showed numerous chemical compounds. The differences in essential oil compositions among the three samples probably resulted in the varied therapeutic potential in each *Z. ottensii* cultivar.

The distinguished chemical profiling of rhizome oils among three *Z. ottensii* accessions might be caused by the genetic and/or environmental factors. The soil samples, which were collected from Yala-1, Yala-2, and Yala-3 cultivation areas, were subjected to soil nutrient analysis. The result showed that Yala-1's soil sample had the percentages of organic carbon, organic matter, nitrogen, and available potassium superior to the specimens sampled from two other cultivated regions (Figure 3). The rhizome oil of Yala-1 exhibited the highest zerumbone content (51.26%). This might indicate that the soil fertility affected the percentage of zerumbone. In the cultivation of some medicinal plants, soil nutrients could increase oil yield and quality (Jabbari *et al.*, 2011). Another previous study demonstrated that enhanced potassium fertilization could modify the chemical composition of caraway (Ezz El-Din *et al.*, 2010). This research might support the result in our study. However, there were several factors, including

the cultivation method, fertilization, irrigation, and harvesting time of plant materials, that could significantly affect the content and composition of essential oil (Nurzynska-Wierdak, 2013). Identification of volatile constituents of *Z. ottensii*'s rhizomes coupled with soil nutrient analysis was initially carried out in this work, which would need to be further studied to clarify the influence of soil nutrients on the percentage of zerumbone in *Z. ottensii*'s rhizome oil.

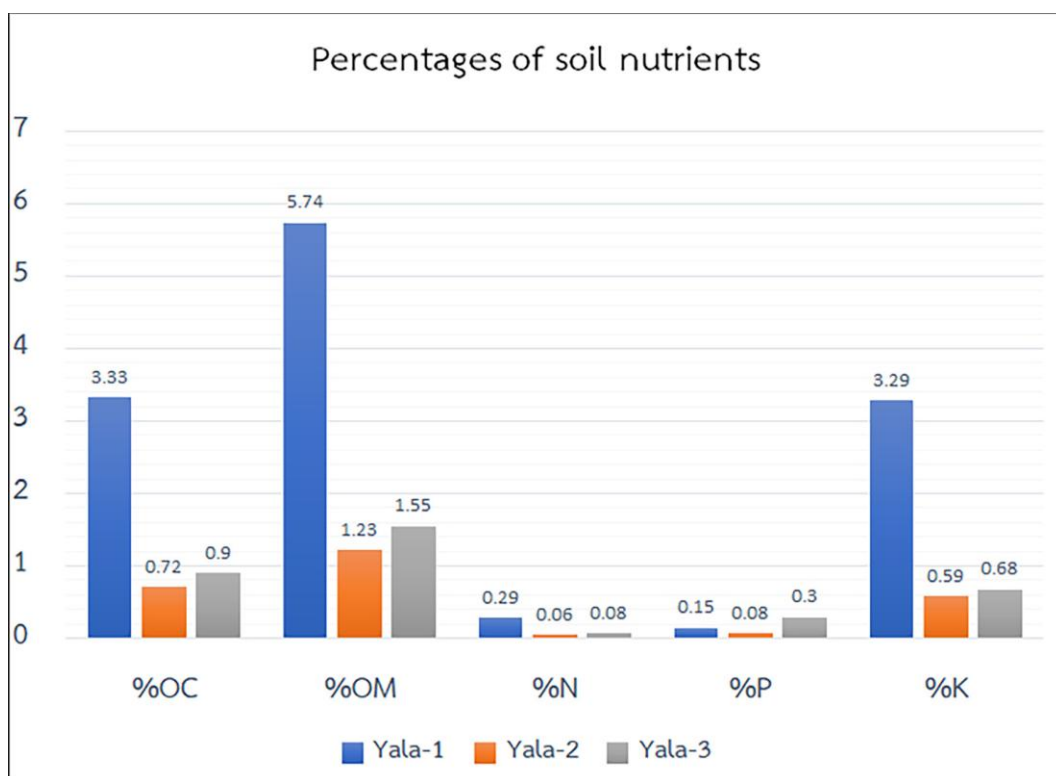


Figure 3 Percentages of organic carbon (OC), organic matter (OM), nitrogen (N), available phosphorus (P), and available potassium (K) in soil samples collected from *Z. ottensii*'s cultivation areas

Conclusions

The study identified key chemical constituents in the rhizome oils of *Zingiber ottensii* using GC-MS analysis, with several compounds potentially linked to the plant's therapeutic properties, including anti-inflammatory, antioxidant, antinociceptive, and antimicrobial effects. Preliminary observations suggest that soil nutrient composition may influence the accumulation of these bioactive compounds, particularly zerumbone. Ongoing research aims to verify this relationship by cultivating different accessions under uniform soil conditions. These findings may contribute to the development of optimized soil management practices to enhance the productivity, quality, and bioactive compound content of *Z. ottensii* for potential pharmaceutical applications.

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