

Research Article

## Comparison of Chromatogram Profiles of Red Ginger (*Zingiber officinale* var. *rubrum* Theilade) Rhizome Essential Oil Based on Differences in Growing Places

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### Abstract

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) is a valuable raw material for the pharmaceutical, cosmetic, and food industries, primarily due to its rich essential oil content. This study investigated how cultivation location influences the chromatographic profile of *Z. officinale* var. *rubrum* essential oil. Samples were sourced from two distinct regions in Java, Indonesia: Magelang and Pacitan Regencies. Essential oils were isolated using water distillation, and their chemical compositions were subsequently analyzed via gas chromatography-mass spectrometry (GC-MS). A notable difference in essential oil yield was observed, with Magelang producing 0.1% and Pacitan yielding 0.08%. GC-MS analysis of the Magelang sample identified 16 compounds, prominently featuring E-citral (20.30%), Z-citral (11.31%), geraniol (10.34%), and camphene (8.91%). Conversely, the Pacitan sample contained 15 compounds, with E-citral (24.06%), Z-citral (13.93%), camphene (14.86%), and 1,8-cineole (7.86%) as its major constituents. A Mann-Whitney U test confirmed a statistically significant difference ( $p\text{-value} = 0.025 < 0.05$ ) in the essential oil profiles between the two locations. These variations are likely attributable to diverse environmental factors, including soil composition, altitude, ambient temperature, and rhizome maturity. This research underscores the critical role of cultivation location in shaping the chemical characteristics of *Z. officinale* var. *rubrum* essential oil, providing valuable insights for developing tailored essential oil-based products to meet specific industrial requirements.

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## INTRODUCTION

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) is a highly valued variety of ginger distinguished by its superior concentration of essential oils compared to other cultivars. *Zingiber officinale* var. *rubrum* rhizomes, broadly, are indispensable in the food industry, fragrances, cosmetics, and traditional medicine, providing a distinctive aroma and flavor in culinary applications<sup>1,2</sup>. Scientifically, *Z. officinale* var. *rubrum* diverse phytochemical profile, including monoterpenes and sesquiterpenes, is responsible for its extensive biological properties, such as hepatoprotective, antibacterial, antiviral, antioxidant, and anti-inflammatory effects<sup>3,4</sup>. Consequently, *Z. officinale* var. *rubrum* is recognized as a significant source for natural medicinal ingredients and nutraceutical products<sup>5</sup>.

Optimal *Z. officinale* var. *rubrum* growth requires specific agronomic conditions, including loose, well-aerated, fertile soil with high organic matter, ideally sourced from areas like red-brown latosols or andosols<sup>6,7</sup>. Proper water flow is crucial to

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prevent root rot<sup>8</sup>. *Zingiber officinale* var. *rubrum* is typically harvested when the leaves begin to fall, signifying optimal maturity, which usually occurs between 8 and 10 months of age, ideally before the rainy season to minimize damage<sup>9</sup>. Furthermore, geographic factors significantly influence the composition of plant's bioactive compounds, with *Z. officinale* var. *rubrum* from the highlands of Wonosobo and Semarang, for instance, exhibiting superior antioxidant activity. Principal Component Analysis (PCA) applied to Fourier Transform Infrared (FTIR) spectra has also proven effective in distinguishing Indonesian ginger origins based on unique chemical characteristics<sup>10</sup>.

The volatile essential oil of *Z. officinale* var. *rubrum* contributes significantly to its potent aroma, with gingerol being a primary component known for its ability to inhibit the formation of inflammatory mediators like prostaglandins<sup>11,12</sup>. The essential oil is composed of volatile components, including monoterpenes ( $C_{10}H_{16}$ ), sesquiterpenes ( $C_{15}H_{24}$ ), and non-volatile components like triterpenes ( $C_{30}H_{48}$ ), which are frequently studied for pharmaceutical potential<sup>13,14</sup>. Gas Chromatography-Mass Spectrometry (GC-MS) is the standard technique used to separate and identify these volatile components in complex essential oil mixtures<sup>15</sup>.

Given the established link between geographical and cultivation factors and the phytochemical profile of *Z. officinale* var. *rubrum*, this study aims to conduct a comprehensive comparative analysis of the chromatogram profile of *Z. officinale* var. *rubrum* essential oil sourced from Magelang and Pacitan. We will investigate how variations in environmental factors, including altitude, air temperature, and harvest age, influence the chemical composition of the essential oils. By employing GC-MS, this research seeks to identify and compare the distinct chemical signatures of *Z. officinale* var. *rubrum* essential oil from these two regions, thereby providing valuable insights for optimizing cultivation practices and enhancing the quality of this important natural product<sup>1</sup>.

## MATERIALS AND METHODS

### Materials

The study utilized a range of equipment and materials for distillation, analysis, and formulation. Essential oil distillation was conducted using standard water distillation apparatus, including an electromantel heater, a round-bottom flask (IWAKI), a condenser, an adapter, and a 1 mL burette. Necessary glassware comprised 500 mL beaker glass (Iwaki), 100 mL measuring cups (Pyrex), 250 mL Erlenmeyer flasks (Iwaki), two vial tubes, a 250 mL separatory funnel (Iwaki), a thermometer, and an analytical balance. For the GC-MS analysis, a dedicated GC-MS instrument was employed.

The plant material used was 1800 g of *Z. officinale* var. *rubrum* rhizomes sourced from the Pacitan and Magelang Regencies in Central Java, Indonesia. The identity of the rhizomes was confirmed by the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. Additional reagents included 8 L of aquadestilata (PT Brataco Chemical), 100 mL of 96% ethanol (PT Smart Lab Indonesia), and 100 g of vaseline album (PT Kimia Farma). All reagents were of analytical grade and stored under appropriate laboratory conditions. Quantitative essential oil distillation was performed at the Pharmaceutical Analysis Laboratory of Universitas Muhammadiyah Klaten, Central Java, Indonesia, while the GC-MS analysis was conducted at the Integrated Faculty of Pharmacy Research and Testing Laboratory of Universitas Ahmad Dahlan, Yogyakarta.

### Methods

#### Study design

This observational study employed quantitative analysis to examine and compare the chromatogram profiles of *Z. officinale* var. *rubrum* essential oil sourced from diverse geographic locations. This methodological approach was designed to precisely characterize the differences in the composition of the essential oil's chemical compounds, which are fundamentally influenced by environmental factors such as soil quality, climate, and altitude<sup>16</sup>. By systematically assessing these variations, this research aims to provide a comprehensive and detailed understanding of how a *Z. officinale* var. *rubrum* growing location impacts the final essential oil composition, thereby enhancing quality control and standardization efforts.

#### Distillation of essential oils

The essential oil from *Z. officinale* var. *rubrum* rhizomes was extracted using the steam distillation method (**Figure 1**). Initially, fresh *Z. officinale* var. *rubrum* rhizomes were carefully sliced crosswise to a uniform thickness of approximately  $\pm 2$  mm using

a precision cutting tool. These slices were then weighed to ensure a consistent starting mass of 900 g per batch of raw simplicia material. For the distillation process, three 1000 mL round-bottom flasks were each charged with 900 g of the prepared *Z. officinale* var. *rubrum* slices, followed by the addition of 500 mL of distilled water per flask.



Figure 1. Distillation process.

Steam distillation was conducted for approximately four hours. The extraction was deemed complete when the volume of essential oil collected in the burette ceased to increase, indicating maximum oil recovery. Following distillation, the system was allowed a 15-minute incubation period to ensure the complete settling and collection of residual essential oil within the apparatus. The final volume of the collected essential oil was then precisely measured and recorded. The oil was subsequently transferred to a clean, airtight storage bottle to protect it from contamination and preserve its quality. Finally, the percent yield of *Z. officinale* var. *rubrum* essential oil was calculated based on the initial weight of the raw simplicia material.

#### GC-MS analysis

The chemical composition of *Z. officinale* var. *rubrum* essential oil was determined using GC-MS. A 500  $\mu$ L sample of the essential oil was injected into the GC-MS system. To ensure consistent sample handling, the injector and detector temperatures were maintained at a constant 175°C. Compound separation began with the GC oven thermostat initially set at 75°C. Helium was used as the carrier gas at a constant flow rate of 3.0 mL/min, achieving a total flow rate of 83.8 mL/min and an operational pressure of 45.9 kPa. For enhanced separation efficiency, the system incorporated a purge flow of 0.80 mL/min and a split ratio of 100. The linear velocity of the carrier gas was measured at 32.9 cm/sec.

The mass spectrometer was operated in absolute gain mode, with the detector voltage set at 0.80 kV to optimize ion signal amplification. The ion source and interface temperatures were maintained at 200°C and 225°C, respectively, ensuring efficient ionization and ion transfer from the GC column. Compound identification was achieved by analyzing the obtained mass spectra and comparing them against the internal MS index library, allowing for the reliable identification of peaks detected in the total ion chromatogram.

#### Data analysis

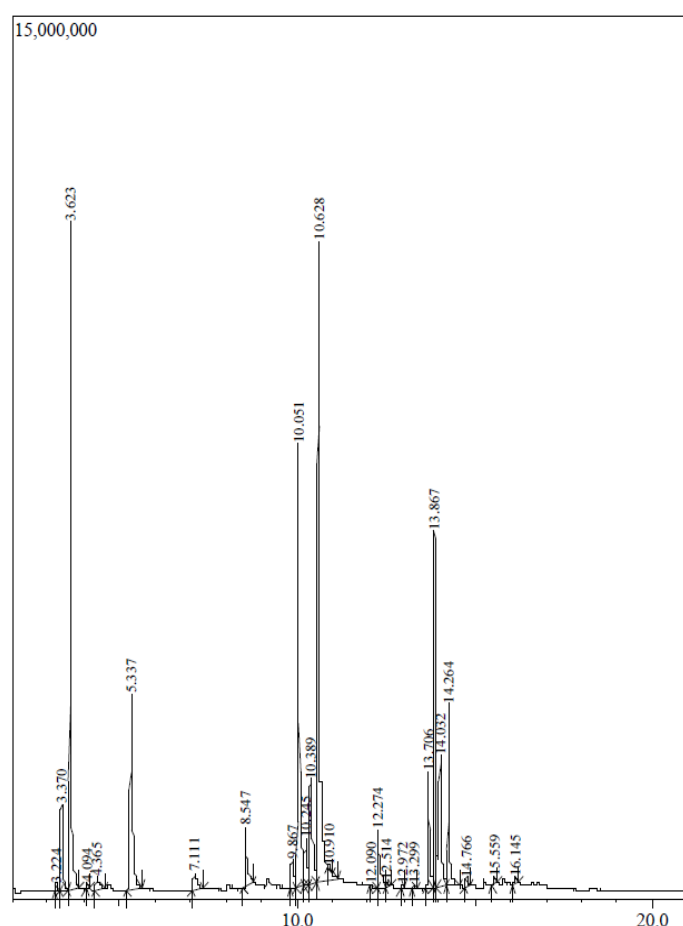
The data analysis for this study was quantitative, focusing on the calculated percentage of essential oil production. Initial assessment of the data for normality was performed using the Shapiro-Wilk test to determine the appropriate subsequent statistical procedure. As the data did not meet the assumptions of parametric testing, the Mann-Whitney U test was then employed for comparative analysis between the experimental groups.

## RESULTS AND DISCUSSION

The essential oil characteristics demonstrated clear regional variations, even within the same species, underscoring the significant influence of environmental factors on essential oil quality and yield. Essential oil distilled from *Z. officinale* var. *rubrum* rhizomes sourced from Pacitan Regency (lithosol soil, ambient temperature 27–32°C) yielded 1.6 mL of oil from 1,800 g of rhizome, resulting in a 0.08% yield. This oil was characterized by a clear, bright yellow color and a soft, refreshing aroma, attributes associated with optimal maturity and suitability for high-end applications in the pharmaceutical, cosmetic, and aromatherapy industries<sup>17</sup>.

In contrast, *Z. officinale* var. *rubrum* rhizomes obtained from the Magelang Regency (841 m above sea level, regosol soil, temperature 14.3–26.5°C) produced 1.8 mL of essential oil from 1,800 g of rhizome, yielding 0.1%. This oil presented with a slightly different profile: a concentrated yellow color, suggesting a higher concentration of bioactive components, and a sharper, stronger aroma. These more dominant aromatic characteristics make the Magelang oil suitable for products requiring a more robust scent, such as massage oils or balms<sup>18</sup>. The observed differences in essential oil yield (0.1% vs. 0.08%) and aromatic profiles are most likely a direct consequence of the contrasting geographical location, soil type (regosol vs. lithosol), and cultivation temperature between the two regions. This comparative analysis strongly suggests that the growing location of *Z. officinale* var. *rubrum* plays a critical role in determining both the quantity and desirable characteristics of the resulting essential oil<sup>19</sup>.

The chromatographic analysis of *Z. officinale* var. *rubrum* essential oil, obtained via water distillation from the Magelang Regency, successfully identified sixteen distinct components, as visually represented in **Figure 2**. To meticulously characterize the chemical composition of this essential oil, GC-MS was employed. As detailed in **Table I**, every peak observed in the chromatogram corresponds to an identified compound within *Z. officinale* var. *rubrum* essential oil, confirming the rich and diverse nature of its chemical profile.



**Figure 2.** Chromatogram of *Z. officinale* var. *rubrum* essential oil components from Magelang Regency.

**Table I.** The main content of *Z. officinale* var. *rubrum* essential oil from GC-MS found in Magelang Regency.

Number	Peak index	Area (%)	Molecular formula	Identified compound
1	2	1.44	C <sub>10</sub> H <sub>16</sub>	α-Pinene ((-)-isomer)
2	3	8.91	C <sub>10</sub> H <sub>16</sub>	Camphene
3	5	0.66	C <sub>10</sub> H <sub>16</sub>	β-Myrcene
4	6	6.22	C <sub>10</sub> H <sub>18</sub> O	1,8-Cineole
5	7	1.55	C <sub>10</sub> H <sub>18</sub> O	Linalool
6	8	2.12	C <sub>10</sub> H <sub>18</sub> O	Endo-borneol
7	11	1.14	C <sub>10</sub> H <sub>20</sub> O	β-Citronellol
8	12	11.31	C <sub>10</sub> H <sub>16</sub> O	Z-Citral (Neral)
9	14	10.14	C <sub>10</sub> H <sub>18</sub> O	Geraniol
10	15	20.30	C <sub>10</sub> H <sub>16</sub> O	E-Citral (Geranial)
11	19	9.34	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	Geranyl acetate
12	22	2.95	C <sub>15</sub> H <sub>22</sub>	ar-Curcumene
13	23	7.86	C <sub>15</sub> H <sub>24</sub>	Zingiberene
14	24	2.55	C <sub>15</sub> H <sub>24</sub>	Farnesene
15	25	3.45	C <sub>15</sub> H <sub>24</sub>	β-Bisabolene
16	26	4.48	C <sub>15</sub> H <sub>24</sub>	β-Sesquiphellandrene
<b>Total</b>		<b>100</b>		

Gas chromatography-mass spectrometry analysis of *Z. officinale* var. *rubrum* essential oil from the Magelang Regency revealed that components with a peak area greater than 0.5% were analytically significant, as minor compounds below this threshold generally do not contribute meaningfully to the overall chemical profile<sup>20</sup>. The dominant compound identified was E-citral at 20.30%, followed by Z-citral (11.31%), geraniol (10.34%), and camphene (8.91%). This composition establishes E-citral as the primary component, which strongly influences the essential oil's characteristics and functional activity.

While *Z. officinale* var. *rubrum* characteristic odor is attributed to zingiberene<sup>21</sup>, its spicy, pungent, and fiery flavors are typically derived from gingerol, shogaol, and zingerone<sup>22</sup>. Interestingly, chromatographic analysis detected zingiberene at a concentration of 7.86%, confirming the expected warm aroma. However, the key pungent compounds—gingerol, shogaol, and zingerone—were undetected. This suggests that the essential oil possesses the distinctive *Z. officinale* var. *rubrum* aroma but lacks the characteristic hot flavor. This compositional profile provides vital information for its potential application, indicating that this essential oil may be more suitable for applications relying on its fragrance, such as aromatherapy or perfumery, rather than as a food additive requiring the typical spicy notes of fresh *Z. officinale* var. *rubrum*<sup>23</sup>.

The absence of gingerol, shogaol, and zingerone in the chromatogram is likely due to the degradation of these compounds during processing and analysis. One primary factor is the high temperature inherent in the GC-MS method, which can cause direct thermal destruction and degradation of gingerol before detection. This degradation was likely initiated or exacerbated by exposure to excessive heat during the steam distillation process<sup>24</sup>. Furthermore, storing the essential oil at ambient room temperature for four days may have accelerated the chemical alteration and decomposition of these key *Z. officinale* var. *rubrum* components. These observations underscore the high thermolability of *Z. officinale* var. *rubrum* active compounds and necessitate further research. Optimal analytical conditions, including more precise temperature control and minimal pre-treatment of the essential oil, are required to preserve the integrity of these characteristic compounds and ensure more accurate data on the overall chemical composition. Understanding the stability of these active compounds is crucial for determining the full therapeutic and commercial potential of *Z. officinale* var. *rubrum* essential oil, which includes sesquiterpene compounds like geranyl acetate (anti-inflammatory)<sup>25</sup>, benzene compounds (antioxidants)<sup>26</sup>, farnesene (aromatic)<sup>17</sup>, β-bisabolene (aromatherapy)<sup>27</sup>, and β-sesquiphellandrene (antimicrobial)<sup>28</sup>.

The chromatographic analysis of *Z. officinale* var. *rubrum* essential oil, obtained via water distillation from samples sourced in the Pacitan Regency, revealed the presence of 15 distinct chemical components (Figure 3). The unique soil characteristics of this district are known to support the cultivation of high-quality *Z. officinale* var. *rubrum*, likely influencing this rich phytochemical profile<sup>29</sup>. As detailed in Table II, each discernible peak in the chromatogram corresponds to a specific volatile compound within the essential oil. Further structural identification was precisely carried out using GC-MS, which leveraged both mass fragmentation patterns and retention times for accurate component determination. The comprehensive results from this test confirm the existence of a diverse range of volatile compounds. The results, detailing the percentage composition, reveal that E-citral is the dominant component, accounting for 24.06% of the total oil. This is followed by camphene at 14.86% and Z-citral at 13.93%. Other notable components include zingiberene at 7.22%, while β-myrcene constitutes a much smaller fraction at 0.73%.



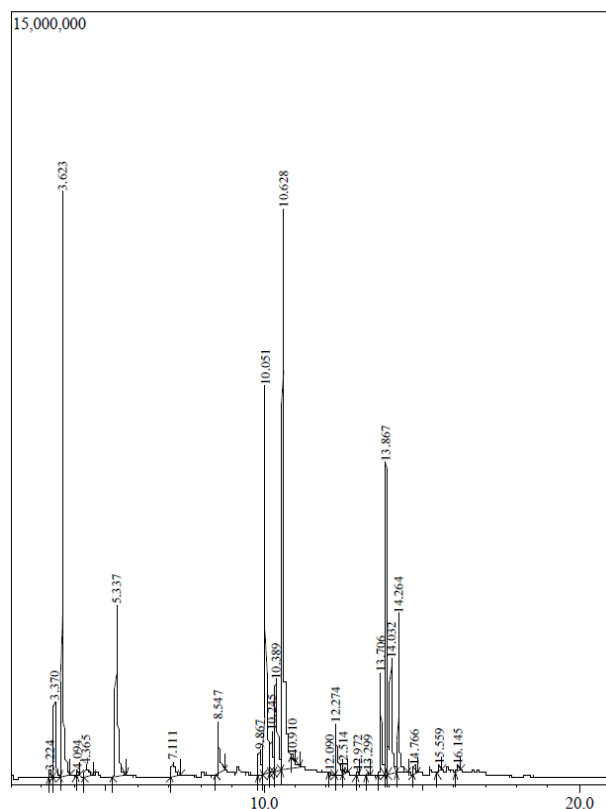


Figure 3. Chromatogram of *Z. officinale* var. *rubrum* essential oil components from Pacitan Regency.

Table II. The main content of *Z. officinale* var. *rubrum* essential oil from GC-MS found in Pacitan Regency.

Number	Peak index	Area (%)	Molecular formula	Identified compound
1	2	2.14	C <sub>10</sub> H <sub>16</sub>	$\alpha$ -Pinene ((-)-isomer)
2	3	14.86	C <sub>10</sub> H <sub>16</sub>	Camphene
3	5	0.73	C <sub>10</sub> H <sub>16</sub>	$\beta$ -Myrcene
4	6	7.86	C <sub>10</sub> H <sub>18</sub>	1,8-Cineole
5	7	1.21	C <sub>10</sub> H <sub>18</sub> O	Linalool
6	8	2.34	C <sub>10</sub> H <sub>18</sub> O	Endo-Borneol
7	9	1.30	C <sub>10</sub> H <sub>20</sub> O	$\beta$ -Citronellol
8	10	13.93	C <sub>10</sub> H <sub>16</sub> O	Z-Citral
9	12	6.05	C <sub>10</sub> H <sub>18</sub> O	Geraniol
10	13	24.06	C <sub>10</sub> H <sub>16</sub> O	E-Citral
11	16	2.34	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	Geranyl acetate
12	20	3.16	C <sub>15</sub> H <sub>22</sub>	ar-Curcumene
13	21	7.22	C <sub>15</sub> H <sub>24</sub>	Zingiberene
14	22	5.11	C <sub>15</sub> H <sub>24</sub>	$\beta$ -Bisabolene
15	23	4.25	C <sub>15</sub> H <sub>24</sub>	$\beta$ -Sesquiphellandrene
Total		100		

A significant observation was the absence of gingerol, shogaol, and zingerone in the chromatogram. The lack of these hallmark *Z. officinale* var. *rubrum* compounds, which typically contribute to the pungent, spicy flavor, suggests that while the essential oil possesses a distinct aroma, it is likely less pungent than other *Z. officinale* var. *rubrum* oils containing these major constituents<sup>30</sup>. The non-detection of these compounds may be attributed to several factors, including the degradation of gingerol into shogaol or zingerone due to excessive heat exposure during the steam distillation process, or further degradation during the four-day storage period at room temperature<sup>24</sup>. Alternatively, the specific assay conditions used in the GC-MS analysis may not have been fully optimized for the detection of these less volatile compounds. Therefore, future research is warranted to refine the GC-MS methodology to conclusively detect and quantify any latent gingerol and its related compounds.

The detected monoterpene and sesquiterpene components endow the Pacitan essential oil with a range of therapeutic and functional properties. For example, the monoterpenes  $\alpha$ -pinene is recognized for its antibiotic effects, camphene is used in flavor and aroma formulations<sup>31</sup>, 1,8-cineole is commonly utilized for treating respiratory and muscular ailments<sup>32</sup>, and both

Z-citral and E-citral demonstrate significant antibacterial activity<sup>33</sup>. Among the sesquiterpenes, zingiberene contributes to the warming sensation and aroma<sup>22</sup>, while  $\beta$ -sesquiphellandrene exhibits antimicrobial properties<sup>28</sup>. These varied chemical properties underscore the potential utility of this *Z. officinale* var. *rubrum* essential oil in pharmaceutical, cosmeceutical, and food industries.

The monoterpene group in both samples included compounds such as  $\alpha$ -pinene, camphene,  $\beta$ -myrcene, 1,8-cineole, linalool, endo-borneol, Z-citral, E-citral, and geraniol. Key sesquiterpene compounds identified included zingiberene, farnesene,  $\beta$ -bisabolene, and  $\beta$ -sesquiphellandrene. Compounds like E-citral, Z-citral, camphene, and 1,8-cineole are of particular importance due to their known potent antibacterial properties, likely functioning by damaging the bacterial cell membrane<sup>31</sup>.

Despite these similarities, distinct differences were observed, primarily influenced by geographical and cultivation factors. The *Z. officinale* var. *rubrum* from Magelang (841 m above sea level, Regosol soil, 10-month harvest age, 14.3–26.5°C) yielded 0.1% oil. The GC-MS analysis showed 35 peaks in the literature standard<sup>34</sup>, with the highest component being E-citral at 20.30%. In contrast, *Z. officinale* var. *rubrum* from Pacitan (964 m above sea level, reddish Lithosol soil, 2-year harvest age, 27–32°C) yielded 0.08% oil. Its GC-MS analysis showed 26 peaks in the literature standard<sup>34</sup>, with E-citral as the highest detected chemical at 24.06%. These variations in chemical profile and yield are attributed to the differences in altitude, soil type, temperature, and rhizome age between the two growth locations.

Statistical analysis of the yield data confirmed a significant difference in essential oil production between the two districts. The Shapiro-Wilk Normality Test showed that the data distribution was irregular ( $p = 0.004 < 0.05$ ), necessitating the use of the non-parametric Mann-Whitney U test. The result of the Mann-Whitney test ( $p = 0.025 < 0.05$ ) indicates a statistically significant difference in essential oil yield between the Magelang and Pacitan samples.

A major limitation of this research lies in the distillation time. The water distillation was conducted for only 3 to 4 hours. This duration is considered suboptimal, as research suggests that a longer distillation period of 4 to 6 hours is typically required to achieve the maximum yield and highest quality of *Z. officinale* var. *rubrum* essential oil<sup>35</sup>. Furthermore, the overall essential oil yield (0.08–0.1%) fell below the general quality standards of the Indonesian Herbal Pharmacopoeia, which typically range from 0.2% to 3%. This suboptimal yield may be due to the short distillation time, coupled with factors like the quality and age of *Z. officinale* var. *rubrum* rhizomes. Future studies should focus on optimizing the distillation duration and implementing precise monitoring of temperature and solvent levels to enhance both the quantity and quality of the extracted essential oil.

## CONCLUSION

The research successfully demonstrated a statistically significant difference in the quantity of *Z. officinale* var. *rubrum* essential oil produced in the Magelang and Pacitan Regencies. Furthermore, GC-MS analysis revealed a variation in the chemical profile, with the Magelang essential oil exhibiting 16 dominant chromatogram peaks compared to 15 in the Pacitan sample, indicating distinct compositional differences. These quantitative and qualitative variations suggest that environmental factors, cultivation practices, or processing methods specific to each region influence the final essential oil yield and chemical makeup. Consequently, further investigation into the specific factors driving these compositional shifts is warranted to optimize the regional cultivation and processing of *Z. officinale* var. *rubrum* for essential oil production.

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## AUTHORS' CONTRIBUTION

**Conceptualization:** Anita Agustina Styawan

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**Formal analysis:** Anita Agustina Styawan

**Funding acquisition:** -

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**Methodology:** Anita Agustina Styawan

**Project administration:** Anita Agustina Styawan

**Resources:** Anita Agustina Styawan, Awal Prichatin Kusumadewi

**Software:** -

**Supervision:** Awal Prichatin Kusumadewi

**Validation:** Anita Agustina Styawan

**Visualization:** -

**Writing - original draft:** Anita Agustina Styawan, Helmi Mustofa, Awal Prichatin Kusumadewi

**Writing - review & editing:** Anita Agustina Styawan

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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