

Chemical Composition and Biological Activities of Vietnamese *Zingiber montanum* (Koenig) Link ex A. Dietr Essential Oil in Vitro

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Abstract: The chemical composition and biological activities of the essential oil isolated from leaves of *Zingiber montanum* (Koenig) Link ex A. Dietr collected in Thanh Hoa Province, Vietnam, were investigated using integrated *in vitro* approaches. Gas chromatography-mass spectrometry (GC-MS) analysis revealed 23 were tentatively identified, representing 99.59% of the total oil composition. The essential oil was characterized mainly by terpenoid constituents, particularly isopropyl myristate (13.85%), (E)-Cinnamaldehyde (23.37%), Methyl salicylate (16.84%), (-)-Menthyl (35.95%), α -Campholenal (1.1%), and trans-sabinene hydrate (3.87%). The essential oil exhibited notable antioxidant activity in the DPPH radical scavenging assay with an IC₅₀ value of 0.100 $\pm$ 0.007 mg/mL, which was slightly strong than that of Trolox (IC₅₀ = 0.342 $\pm$ 0.003 mg/mL). Selective antimicrobial activity was observed, particularly against *Escherichia coli*, with an inhibition zone diameter of 96.667 $\pm$ 5.774 mm at 0.020 mg/mL. However, these computational results should be considered preliminary and supportive rather than direct evidence of target-specific inhibition. Overall, the obtained findings suggest that *Zingiber montanum* essential oil represents a promising natural source of multifunctional bioactive constituents with potential pharmaceutical and functional applications.

Keywords: Thanh Hoa, *Zingiber montanum*, essential oil, antioxidant, antimicrobial.

INTRODUCTION

In traditional Vietnamese medicine, *Zingiber montanum* (Koenig) Link ex A. Dietr is characterized by its bitter taste, neutral properties, and spicy flavor. It is valued for its ability to expel wind and cold, alleviate pain, and improve blood circulation, making it a common choice for remedies aimed at stimulating, toning, and detoxifying the body. *Zingiber montanum* is also used to treat ailments such as dizziness, nausea, fainting, and windstroke. It is particularly recognized for its detoxifying effects, postnatal recovery benefits, and its role in enhancing digestion [Loi, D. T. 2004; Bhuiyan, M. N. I. *et al.*, 2009].

From the pharmacological point of view, *Zingiber montanum* has been reported to inhibit colon and lung carcinogenesis in mice [Zakaria, Z. A. *et al.*, 2010; Chien, T. Y. *et al.*, 200; Jantan, I. *et al.*, 2008], CXCL12-induced invasion of breast and pancreatic tumor cells [Harborne, A. J. 1998; Jantan, I., *et al.*, 2008; Abdelwahab, S. I., *et al.*, 2012], apoptosis in liver cancer [Bhuiyan, M. N. I. *et al.*, 2009], suppress phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells and Epstein-Barr Virus activation [Zakaria, Z. A. *et al.*, 2010; Zakaria Z. A. *et al.*, 2011; Sulaiman, M. R. *et al.*, 2010]. Previous studies on the chemical composition of *Zingiber montanum* have identified various bioactive compounds such as: zerumbone, borneol, α -pinene, β -pinene, camphor, linalool,

limonene, α -curcumene, α -humulene, humulene monoxide, humulene dioxide, β -caryophyllene, camphene, cineole [Kim, M. *et al.*, 2009] and new sesquiterpenoids such as humulene epoxide I, humulene epoxide II, humulene epoxide III, humulenol I, humulenol II, and caryophyllene oxide found in the essential oil of Indian *Zingiber montanum* [Sung, B. *et al.*, 2008]. Additionally, some new compounds have been identified in the essential oil of *Z. zerumbet* including *cis*-nerolidol, α -phellandrene, terpinolene, *p*-cymene, caryophyllene oxide, δ -cardinene, fenchone, and bornyl acetate [Sharifah Sakinah, S. A. *et al.*, 2007]. Phenolic compounds and glycosides have also been found, including zerumbone, 3-*O*-methyl kaempferol, kaempferol-3-*O*-(2,4-di-*O*-acetyl- α -L-rhamnopyranoside), and kaempferol-3-*O*-(3,4-di-*O*-acetyl- α -L-rhamnopyranoside) from ginger rhizomes [Vimala, S. *et al.*, 1999; Nigam, I. C., & Levi, L. 1963]. Among the bioactive compound isolated from *Z. montanum* *Z. zerumbet*, zerumbone is the only compound that has been studied extensively. Zerumbone revealed *in vivo* antinociceptive and anti-inflammatory and antiplatelet aggregation activities.

Thanh Hoa Province, located in the Central region of Vietnam, possesses unique ecological conditions characterized by basaltic soils, prolonged dry seasons, and substantial day-night temperature fluctuations. These environmental factors may affect the biosynthesis and

accumulation of volatile metabolites in aromatic plants, potentially resulting in distinctive chemotypic characteristics. However, the chemical composition and biological potential of *Zingiber montanum* essential oil from this region have not yet been comprehensively investigated.

Therefore, the present study aimed to characterize the chemical composition of the essential oil extracted from *Zingiber montanum* leaves collected in Thanh Hoa Province, Vietnam, using gas chromatography-mass spectrometry (GC-MS) [Sahingil, D. 2019]. The antioxidant, antibacterial, inhibitory activities of the essential oil were also evaluated using *in vitro* assays [Nguyen, Q. V., & Eun, J. B. 2014; Wayne, P. A. 2008]. Collectively, this study contributes to the phytochemical characterization and biological evaluation of Vietnamese *Zingiber montanum* essential oil and highlights its potential as a natural source of bioactive compounds for pharmaceutical and functional applications.

MATERIALS AND METHODS

Chemicals

A homologous series of *n*-alkanes (C7–C30) employed for retention index determination, together with Tween 80, Trolox, and Gentamicin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All solvents and reagents used in the experiments were of analytical grade, including methanol, diethyl ether, and anhydrous sodium sulfate (Na_2SO_4). Microbiological culture media and ciprofloxacin standard discs utilized for antimicrobial assays were purchased from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

Plant Material

The whole plant (leaves and stems) of *Zingiber montanum* were collected in January 2026 from Ben En National Park, Thanh Hoa Province, Vietnam. Botanical identification of the plant material was performed, and a voucher specimen (No. GN-TH-01) was deposited at the Faculty of Natural Science and Technology, Hong Duc University, Thanh Hoa Province, Vietnam.

Essential Oil Isolation

Leaves and stems of *Zingiber montanum* were cleaned to eliminate adhering impurities and gently rinsed with distilled water. The samples were kept at room temperature to remove excess surface moisture, followed by brief air-drying prior to extraction. Subsequently, 100 g of finely cut leaves were introduced into a Clevenger-type

distillation system containing 1000 mL of distilled water. Hydrodistillation was carried out for 3 h under continuous heating to obtain the volatile oil fraction. The condensed distillate separated into aqueous and organic phases, and the essential oil portion was recovered using diethyl ether. Residual moisture was eliminated with anhydrous sodium sulfate (Na_2SO_4), after which the solvent was evaporated under reduced pressure to yield the purified essential oil.

Chemical Profiling of the Essential Oil by GC-MS

The chemical composition of the essential oil from *Zingiber montanum* was analyzed using a Trace GC Ultra gas chromatograph coupled to an ITQ900 mass spectrometer (Thermo Fisher Scientific, MA, USA). Chromatographic data acquisition and spectral interpretation were performed with MassFinder software version 4.0. Separation of volatile constituents was achieved on a TG-SQC fused-silica capillary column (30 m $\times$ 0.25 mm i.d.; film thickness 0.25 μm). The injector temperature was maintained at 250 $^{\circ}\text{C}$, while the oven temperature was programmed from 60 to 260 $^{\circ}\text{C}$ at a heating rate of 4 $^{\circ}\text{C}/\text{min}$. Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. A 1 μL aliquot of the sample was injected under split conditions with a split ratio of 1:10.

Mass spectrometric analysis was conducted in electron ionization (EI) mode at 70 eV. The interface, ion source, and quadrupole temperatures were set at 280, 230, and 200 $^{\circ}\text{C}$, respectively, and mass spectra were recorded within an *m/z* range of 35–650 [Sahingil, D. 2019]. Identification of individual constituents was accomplished by comparison of their mass fragmentation patterns with those available in the NIST 20 spectral database, together with comparison of reported retention index values from the literature obtained under comparable chromatographic conditions. Since a homologous series of *n*-alkanes was not analyzed under identical experimental conditions in the present study, experimental retention indices could not be calculated. Therefore, only literature retention indices were considered to support compound assignment. Relative percentages of the identified compounds were estimated from normalized peak areas in the total ion chromatogram without applying response correction factors.

Antioxidant Activity Assay

The antioxidant potential of *Zingiber montanum* essential oil was evaluated using the 2,2-diphenyl-

1-picrylhydrazyl (DPPH) radical scavenging assay according to a modified method described by Nguyen and Eun (2013) [Nguyen, Q. V., & Eun, J. B. 20141]. Essential oil samples were dissolved in methanol to obtain final concentrations of 30, 15, 7.5, 3.75, and 1.875 mg/mL. For each assay, 1 mL of the prepared sample solution was mixed with 5 mL of DPPH solution and thoroughly vortexed to ensure complete mixing. The reaction mixtures were subsequently incubated in the dark at room temperature for 30 min prior to absorbance measurement at 517 nm using a UV-Vis spectrophotometer (Jenway 6305, UK). A control sample containing methanol in place of the essential oil was prepared under identical conditions.

The percentage of DPPH radical scavenging activity was calculated according to Equation (1):

$$\text{Antioxidant (\%)} = \frac{AB-AA}{AB} \times 100\% \quad (1)$$

where AB represents the absorbance of the control solution and AA corresponds to the absorbance of the reaction mixture containing the essential oil sample. Antioxidant capacity was expressed as the half maximal inhibitory concentration (IC_{50}), determined from the corresponding concentration-response curve.

Antibacterial Activity Assay

The antibacterial activity of *Zingiber montanum* essential oil was evaluated using the agar disc diffusion method with minor modifications based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020) [Wayne, P. A. 2008]. To improve the dispersion of the hydrophobic essential oil, sample solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 2.00, 1.75, 1.50, 1.25, 1.00, 0.75, and 0.50 mg/mL. The final DMSO concentration in all assays was maintained below 1% (v/v), and a solvent control containing DMSO alone was included to verify the absence of inhibitory effects on bacterial growth. Sterile paper discs (6 mm in diameter) were

impregnated with 50 μ L of each sample solution and allowed to dry under aseptic conditions prior to use.

A bacterial suspension of *E. coli* ATCC 25922 (approximately 10^6 CFU/mL) was evenly spread onto tryptic soy agar (TSA) plates supplemented with 2% NaCl using a sterile spreader. After allowing the inoculated plates to dry for 3–5 min, the prepared discs were carefully placed on the agar surface. The plates were then incubated at 28 °C for 24 h, after which inhibition zone diameters were measured to assess antibacterial activity. The incubation temperature was selected to maintain uniform experimental conditions for all tested microorganisms. It should be noted that this temperature is lower than the optimal growth temperature for *E. coli* (37 °C) and may therefore affect bacterial growth kinetics. Standard antibiotic discs containing ciprofloxacin (0.05 mg/mL) were used as positive controls for antibacterial evaluation against *E. coli*.

All experiments were conducted in triplicate to ensure reproducibility. Antibacterial activity was evaluated by measuring the diameters of the inhibition zones (IZDs) formed around the discs after incubation at 28 °C for 24 h.

Statistical Analysis

All measurements represent technical triplicates ($n = 3$), and results are expressed as mean $\pm$ standard deviation (SD) based on three independent measurements.

RESULTS

Chemical Profile of *Zingiber Montanum* Essential Oil

Hydrodistillation of fresh leaves from *Zingiber montanum* afforded an essential oil yield of 0.38 % (w/w) based on fresh plant material. The GC-MS total ion chromatogram of the obtained essential oil is presented in Fig. 1, while the identified volatile constituents and their relative abundances are summarized in Table 1.

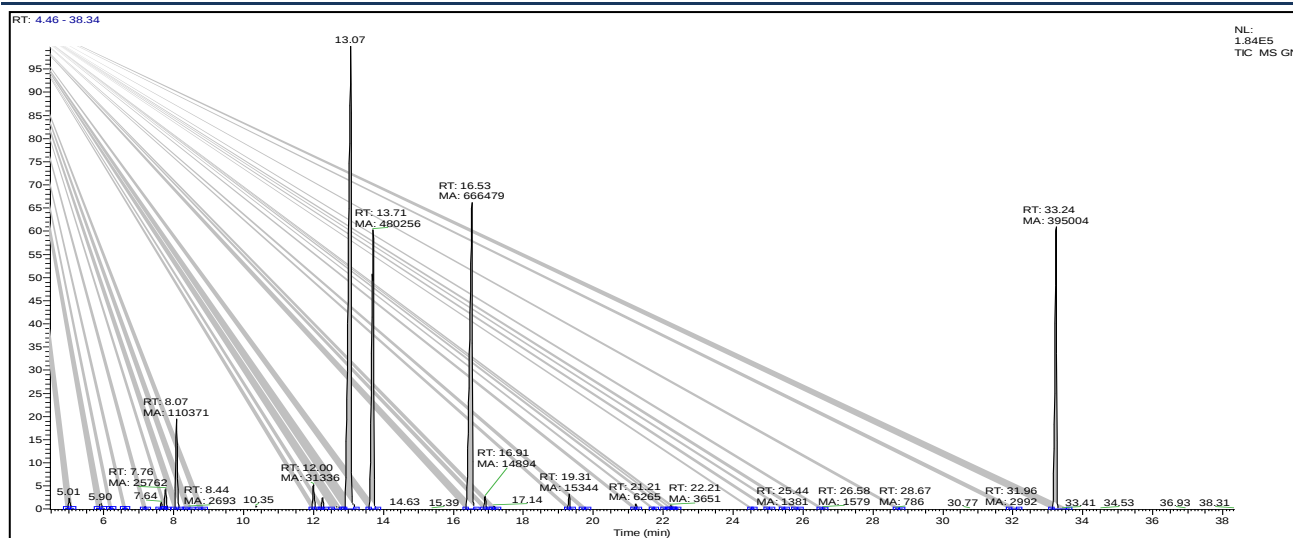


Fig. 1: GC-MS total ion chromatogram of *Zingiber montanum* essential oil.

Hydrodistillation of fresh leaves from *Zingiber montanum* yielded an essential oil with a characteristic aromatic odor. GC-MS analysis revealed 23 constituents, of which it constituents were tentatively identified, collectively accounting for 99.59% of the total essential oil composition. Compound identification was performed by

comparison of mass spectral fragmentation patterns with the NIST 20 library, together with literature retention indices reported under comparable chromatographic conditions. Detailed compositional data and the corresponding peak assignments are summarized in Table 1 and Supplementary Material.

Table 1: Chemical profile of *Zingiber montanum* essential oil.

Peak	Retention time (min)	Compound	Relative amount (%)
1	5.01	α -Thujene	0.47
2	5.9	Benzoyl chloride	0.29
3	6.18	Sabinene	0.13
4	7.18	β -Phellandrene	0.06
5	7.64	<i>p</i> -cymene	0.22
6	7.76	Delta-3-Carene	0.9
7	8.07	Trans-Sabinene hydrate	3.87
8	8.44	l-Phellandrene	0.09
9	12	α -Campholenal	1.1
10	12.26	Cis-Cyclohexanone-5-methyl-2-(1-methylethyl)	0.5
11	12.69	l-Menthone	0.28
12	13.07	(-)-Methol	35.95
13	13.71	Methyl salicylate	16.84
14	16.53	(E)-Cinnamaldehyde-	23.37
15	16.91	2-Propenoic acid, 3-phenyl methyl ester	0.52
16	17.14	Terpineol	0.11
17	19.31	Cis-isoeugenol	0.54
18	19.75	6-Methylchromone	0.04
19	21.21	Trans-Caryophyllene	0.22
20	22.07	2H-1-Benzopyran-2-one	0.05
21	22.21	2-Propen-1-ol-3-phenylacetate	0.13
22	26.58	Trans-Vaccenic acid	0.06
23	33.24	Isopropyl myristate	13.85

Compounds were tentatively identified based on mass spectral data and chromatographic characteristics. Relative percentages were

calculated from GC peak areas without applying correction factors.

The remaining fraction consisted primarily of unidentified minor components. Among the identified compounds, Isopropyl myristate (13.85%), (E)-Cinnamaldehyde (23.37%), Methyl salicylate (16.84%), (-)-Menthol (35.95%), α -Campholenal (1.1%), and trans-sabinene hydrate (3.87%) were detected as the predominant volatile constituents. The predominance of monoterpene hydrocarbons and oxygenated sesquiterpenes may contribute significantly to the characteristic chemical profile and biological properties of the essential oil.

Antioxidant Capacity of *Zingiber Montanum* Essential Oil

The antioxidant activity of *Zingiber montanum* essential oil was evaluated using the DPPH radical scavenging assay, and the obtained results are summarized in Table 2. The essential oil exhibited concentration-dependent radical scavenging activity, with the percentage inhibition increasing progressively at higher concentrations. At 0.625 mg/mL, the essential oil showed strong antioxidant activity with a scavenging effect of $85.307 \pm 3.355\%$, whereas strong concentrations produced more moderate inhibitory effects.

Table 2: DPPH radical scavenging capacity of *Zingiber montanum* essential oil.

Essential oil (mg/mL)	% Inhibition	IC ₅₀ (mg/mL)
0.625	85.307 ± 3.355	0.100 ± 0.007
0.313	60.971 ± 0.637	
0.156	55.955 ± 0.826	
0.078	42.783 ± 0.742	
0.039	31.197 ± 1.237	
0.020	22.816 ± 1.604	
0.010	15.411 ± 2.228	
0.005	15.728 ± 1.78	
0.0025	15.955 ± 1.893	
Trolox ^a		0.342 ± 0.003

^a Positive control for antioxidant activity. Values are expressed as mean $\pm$ SD (n = 3). IC₅₀ values were calculated from dose-response curves.

The IC₅₀ value of the essential oil was determined to be 0.100 ± 0.007 mg/mL, indicating considerable antioxidant potential. This activity was comparable to that of the reference antioxidant compound Trolox, which exhibited an IC₅₀ value of 0.342 ± 0.003 mg/mL. These findings suggest that the essential oil possesses substantial free radical scavenging capacity and may represent a promising natural source of antioxidant constituents.

Antibacterial and Antimicrobial Activities of *Zingiber Montanum* Essential Oil

The antibacterial activity of *C. operculatus* essential oil against *E. coli* was evaluated using the agar disc diffusion method [20], and the obtained results are presented in Table 3 and Fig. 2. The essential oil exhibited concentration-dependent antibacterial activity, as reflected by the gradual increase in IZD with increasing sample concentrations. At the highest tested concentration (0.02 mg/mL), the essential oil produced a clear inhibition zone of 96.667 ± 5.774 mm, the antibacterial activity observed for the reference antibiotic Gentamicin.



Fig. 2: Disc diffusion assay demonstrating the antibacterial activity of *Zingiber montanum* essential oil against *E. coli* in comparison with the reference antibiotic. (1): essential oil at 0.020 mg/mL; (2): essential oil at 0.010 mg/mL; (3): essential oil at 0.005 mg/mL; (4): essential oil at 0.003 mg/mL; (5): essential oil at 0.001 mg/mL and (6): Gentamicin (0.05 mg/mL, positive control).

A progressive reduction in antibacterial effect was observed at lower concentrations, with IZDs decreasing from 60 ± 0 mm at 0.001 mg/mL to complete loss of detectable activity at 0.020

mg/mL. Gentamicin, used as the positive control at 0.05 mg/mL, exhibited a larger inhibition zone diameter of 191.667 ± 2.887 mm under the same experimental conditions.

Table 3 Inhibition zones of *Zingiber montanum* essential oil against *E. coli*.

Bacterial density	Essential oil (mg/mL)	IZD (mm)
10^6 CFU/mL	0.02	96.667 ± 5.774
	0.01	80 ± 0
	0.005	65 ± 0
	0.003	60 ± 0
	0.001	60 ± 0
	Gentamicin ^a	191.667 ± 2.887

Reference antibiotic used as the positive control. IZD: inhibition zone diameter. Values are expressed as mean $\pm$ SD (n = 3). Relative activity (%) was calculated based on inhibition zone diameter relative to ciprofloxacin.

The results indicate that the essential oil possesses appreciable antibacterial activity against *E. coli*, although its efficacy remained lower than that of the reference antibiotic. Since the disc diffusion assay mainly provides a preliminary estimation of antibacterial potential, the antimicrobial activity of the essential oil was further investigated by MIC determination. It should also be noted that inhibition zone diameters obtained from diffusion-based assays may not directly reflect the intrinsic antimicrobial potency of hydrophobic essential oils because their diffusion through agar media is often limited.

Overall, these findings suggest that the essential oil of *Zingiber montanum* exhibits selective antibacterial activity, particularly against *E. coli*, and may represent a potential natural source of bioactive compounds with antimicrobial properties.

CONCLUSION

In conclusion, the essential oil of *Zingiber montanum* collected in Thanh Hoa Province, Vietnam, was characterized by a terpene-rich composition dominated by Isopropyl myristate, (E)-Cinnamaldehyde, Methyl salicylate, (-)-Methol, α -Campholenal, and trans-sabinene hydrate. The obtained chemical profile differed from previously reported *Zingiber montanum* essential oils from other geographical regions, suggesting considerable chemotypic variability influenced by environmental and ecological

factors. The essential oil exhibited notable antioxidant activity, selective antibacterial effects. Overall, the present findings suggest that *Zingiber montanum* essential oil from Thanh Hoa may represent a promising natural source of biologically active terpenoid constituents with potential applications in food, pharmaceutical, and functional product development. However, further studies involving isolated compounds, detailed mechanistic investigations, and *in vivo* evaluations are necessary to validate the biological activities and safety profiles of the essential oil.

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