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Chemical Profiling of Locally Grown *Zingiber officinale* Roscoe (Ginger) Rhizomes using GC-MS and Biological Activities

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Abstract

Ginger (*Zingiber officinale* Roscoe), a widely used rhizomatous species of the family Zingiberaceae, possesses extensive medicinal value owing to its diverse phytochemical composition. This study aimed to characterize the bioactive constituents of locally grown ginger rhizomes using Gas Chromatography–Mass Spectrometry (GC–MS) to elucidate their potential pharmacological activities. GC–MS analysis enabled the detection of volatile and semi-volatile compounds based on retention time and molecular ion fragmentation patterns, providing a comprehensive chemical profile of the extract. A total of 39 compounds were identified, of which 14 exhibited relative abundances exceeding 3%. Major constituents included sesquiterpenes and phenolic derivatives known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. The presence of these bioactive metabolites confirms the therapeutic potential of *Z. officinale* as a natural source of bioactive agents and supports its continued exploration for pharmaceutical and nutraceutical applications.

Keywords: bioactive compounds, GC-MS, natural medicine, rhizome, *Zingiber officinale*

Introduction

Ginger (*Zingiber officinale* Roscoe) is a medicinally and economically important species belonging to the family Zingiberaceae, which comprises approximately 53 genera and more than 1,300 species distributed across tropical and subtropical regions worldwide (Ramdhini et al., 2022). Members of this family are extensively cultivated in Africa, the Americas, South Asia, and particularly in Southeast Asia, where they play an essential role in traditional medicine, culinary practices, and local economies (Windarsih et al., 2021). Indonesia represents one of the major centers of Zingiberaceae diversity, and ginger has been cultivated there for centuries both as a spice and as a therapeutic plant valued for its distinctive flavor, aroma, and curative properties.

In Indonesian ethnomedicine, ginger is an indispensable component of herbal formulations, commonly used in beverages such as wedang jahe and beras kencur, which are believed to warm the body, relieve fatigue, and enhance immune resistance. The rhizome, a modified underground stem, is the principal part utilized for its pharmacological properties and is closely intertwined with Indonesia's ethnobotanical heritage. Its continuous use reflects intergenerational knowledge transmission and cultural adaptation of local biodiversity to health care practices (Sharifi-Rad et al., 2017).

Despite its long-standing traditional application, public and scientific understanding of the specific bioactive constituents responsible for ginger's therapeutic efficacy remains incomplete (Silalahi et al., 2021). Phytochemical analyses have shown that the rhizome is rich in diverse secondary metabolites, including phenolic compounds, terpenoids, and essential oils. Among these, gingerols, shogaols, zingerone, borneol, geraniol, and curcumene are the principal constituents, which are known to exhibit antioxidant, antimicrobial, anti-inflammatory, antiemetic, and anticancer activities (Umeoka, 2024; Moorkoth et al., 2021). The pharmacological relevance of these compounds highlights ginger's potential as a source of bioactive molecules for pharmaceutical and nutraceutical development.

To comprehensively identify and quantify these metabolites, modern analytical platforms are required. Gas chromatography–mass spectrometry (GC–MS) has emerged as one of the most powerful techniques for phytochemical characterization,

enabling the separation and identification of volatile and semi-volatile compounds with high sensitivity and reproducibility (Yu et al., 2022; Spyrou et al., 2024). The GC–MS system operates by separating compounds according to retention time in the chromatographic column and identifying them through mass fragmentation patterns, allowing precise determination of chemical composition. Compared with conventional methods such as thin-layer chromatography or UV–Vis spectrophotometry, GC–MS provides superior structural information and facilitates the detection of minor constituents within complex plant matrices (Zammel et al., 2021).

Therefore, this study aims to profile the chemical composition of locally grown *Z. officinale* rhizomes using GC–MS and to evaluate their potential biological activities. The findings are expected to bridge traditional ethnobotanical knowledge with modern analytical chemistry, providing a scientific foundation for the valorization of ginger as a natural source of therapeutic agents and for the development of standardized herbal products supporting sustainable healthcare systems.

Materials and Methods

Sample Collection

This study was conducted between September and November 2024 in the Pasang Mountain area (445 m a.s.l.), Sumbersari Village, Jember, East Java, Indonesia. Fresh rhizomes of *Z. officinale* Roscoe were harvested from mature plants aged 8–10 months, ensuring uniform physiological conditions. The collected samples were transported in sterile polyethylene bags to the Botany Subdivision, Biology Laboratory, Department of Biology, University of Jember, for further preparation. Voucher specimens were deposited in the departmental herbarium for reference. The GC–MS analysis was performed at the Bioscience Laboratory, Jember State Polytechnic.

Sample Extraction

The rhizomes were washed under running tap water, air-dried, and cut into small slices. A total of 30 g of fresh rhizome was macerated with 300 mL of analytical-grade methanol (96%) in a sealed Erlenmeyer flask at room temperature (26 ± 1 °C) for 72 h, with occasional shaking to enhance solvent penetration. Maceration was selected for its efficiency in preserving thermolabile compounds. The extract was filtered through Whatman No. 1 filter paper, and the residue was re-macerated once to maximize compound recovery. The combined filtrate was concentrated using a rotary evaporator (Heidolph Laborota 4000) at 30 °C for 60 min under reduced pressure to yield a crude methanolic extract, which was stored at 4 °C until analysis (modified from Ulum et al., 2023).

GC–MS Analysis

The chemical composition of the methanolic extract was analyzed using a Shimadzu GC–MS QP2010 Plus system equipped with a Restek Rtx-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm; Crossbond® 5% phenyl – 50% methyl polysiloxane). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1} and inlet pressure of 64.1 kPa. The injection volume was 1 µL (split ratio 10:1), and the injector temperature was 280 °C. The oven temperature was programmed from 80 °C (2 min hold) to 280 °C at 10 °C min^{-1}, held for 8 min at the final temperature. Electron impact ionization (EI) was performed at 70 eV, with the MS source temperature at 200 °C and the interface at 250 °C. The mass scan range (m/z 40–650) was used to detect volatile constituents. Compound identification was performed by comparing mass spectra with the NIST14.LIB and Wiley9.LIB databases.

Data Processing and Compound Identification

Chromatographic data were presented with retention time, peak area, and mass spectral pattern were recorded for each compound. Tentatively identified compounds were classified based on functional groups (terpenoids, phenolics, aldehydes, ketones, etc.), and their biological relevance was verified through literature searches in Scopus, PubChem, and Google Scholar databases.

Results and Discussion

Gas chromatography–mass spectrometry (GC–MS) analysis of the methanolic extract of *Z. officinale* rhizome revealed 39 distinct bioactive compounds, eluting within a total retention time of 45 minutes. The identified metabolites comprised mainly phenolic derivatives, terpenoids, esters, fatty acids, and hydrocarbons, consistent with previous reports on ginger phytochemistry (Premram et al., 2018; Bawadood et al., 2020; Ghavam et al., 2021). Among the total compounds, 14 constituents exhibited relative area percentages exceeding 3%, indicating their predominance in the extract. Major constituents included hexadecanoic acid, methyl ester (CAS), 9-octadecenoic acid (Z)-, methyl ester (CAS), octacosane (CAS), and cis-6-shogaol, which collectively accounted for approximately 72% of the total chromatographic area.

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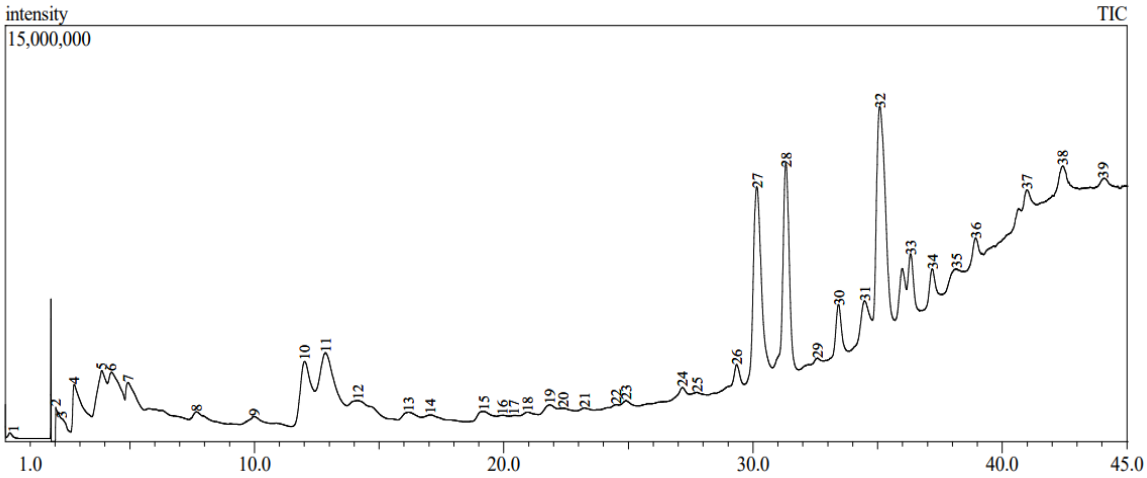

Figure 1. GC–MS total ion chromatogram (TIC) of the methanolic extract of *Zingiber officinale* rhizome

The detection of these abundant components highlights the chemical richness and pharmacological potential of the locally grown ginger rhizome. Each compound's retention time, molecular ion peak, and fragmentation pattern were cross-referenced with the NIST14.LIB and Wiley9.LIB databases, ensuring accurate compound identification. The predominance of fatty acid methyl esters and shogaol-type phenolics aligns with the biochemical roles of these metabolites in plant defense and their established antioxidant, antimicrobial, and anti-inflammatory properties.

Table 1. Metabolites identified in *Zingiber officinale* rhizome extract by GC–MS and their potential biological activities

Peak	***R.Time***	**Area%**	**Name**	**Potential**	**References**
5	3.86	3.34	Acetic acid, anhydride with formic acid	6	Myllys et al., 2024
6	4.25	3.75	2,4-Pentanediamine, 2-methyl- (CAS)	4	Bošković et al., 2020
10	12.02	4.70	2-Propanol, 1,1'-oxybis- (CAS)	2,3	Setyati et al., 2024
11	12.85	8.13	1-Propanol, 2-(2-hydroxypropoxy)- (CAS)	2,3	Setyati et al., 2024
12	14.16	4.21	1,1-Dodecanediol, diacetate (CAS)	2	Okukawa et al., 2021
27	30.17	10.55	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	4	Kure et al., 2021
28	31.34	9.12	Hexadecanoic acid, methyl ester (CAS)	1,2	Purushothaman et al., 2024
31	34.50	2.58	Pentadecanoic acid (CAS)	4	To et al, 2020
32	35.11	13.91	9-Octadecenoic acid (Z)-, methyl ester (CAS)	2,5	Ghavam et al., 2021; Xie et al., 2022
33	36.34	5.64	Octadecanoic acid, ethyl ester (CAS)	2,5	Ghavam et al., 2021; Xie et al., 2022
34	37.21	3.09	Hexacosane (CAS)	7	-
35	38.17	3.56	9-Octadecenoic acid (Z)- (CAS)	1,2,3	Ghavam et al., 2021; Premram et al., 2018
36	38.95	5.29	Octacosane (CAS)	1	Balachandran et al., 2023
38	42.42	3.13	cis-6-shogaol	1,2,4	Bawadood et al., 2020

Dominant bioactive compounds with Area (%) ≥ 3%

The antimicrobial potential (antibacterial and antifungal) exhibited by ginger rhizome extract is attributed to the presence of several bioactive compounds, including 2-Propanol, 1,1'-oxybis- (CAS), 1-Propanol, 2-(2-hydroxypropoxy)- (CAS), 1,1-Dodecanediol, diacetate (CAS), Hexadecanoic acid, methyl ester (CAS), 9-Octadecenoic acid (Z)-, methyl ester (CAS), Octadecanoic acid, ethyl ester

(CAS), 9-Octadecenoic acid (Z)- (CAS), and cis-6-shogaol (Premram et al., 2018; Bawadood et al., 2020; Okukawa et al., 2021; Ghavam et al., 2021; Xie et al., 2022; Setyati et al., 2024; Purushothaman et al., 2024). Previous studies indicate that ginger rhizome extract possesses antibacterial activity against isolates such as *S. aureus, B. cereus, M. luteus, L. monocytogenes, P. aeruginosa, En. cloacae, S. typhimurium,* and *E. coli*. The inhibitory effect on bacterial growth is influenced by the choice of solvent used, specifically ethyl acetate and ethanol. The inhibition of bacterial growth is evidenced by the presence of clear zones on growth media (Yousfi et a*l.,* 2021). Ginger rhizome extract has potential as an antifungal, as shown by previous studies indicating its ability to inhibit the growth of pathogenic fungi, including *Alternaria alternata* (Rizwana, 2015). *Alternaria alternata* is a pathogenic fungus associated with respiratory diseases and allergies. Research by Kipkogei et al. (2019) revealed that methanolic ginger rhizome extract at a concentration of 50 mg/mL effectively inhibited the growth of this fungus. Significant inhibition was observed between days 8 and 16 of treatment. Therefore, ginger rhizome extract holds promise as a potential treatment for diseases caused by *Alternaria alternata*.

The rhizome extract of *Zingiber officinale* (ginger) exhibits potential anticancer properties, supported by the presence of bioactive compounds such as 2,4-Pentanediamine, 2-methyl- (CAS), 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-, Pentadecanoic acid (CAS), and cis-6-shogaol, senyawa bioaktif tersebut sangat potensial sebagai antikanker (Bošković et al., 2020; Bawadood et al., 2020; Kure et al., 2021). A study conducted by Ansari et al. (2016) investigated the effects of methanolic ginger rhizome extract on HeLa cells and observed a reduction in both the number and size of colonies. The methanolic extract exhibited optimal efficacy in reducing HeLa cell counts at concentrations of 46.5 µg/mL after 24 hours and 37.5 µg/mL after 48 hours. These findings suggest that ginger rhizome extract holds promise as a treatment for cervical and breast cancer, although further clinical studies are essential for validation.

The study highlights that ginger rhizome extract is a rich source of bioactive compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 39 total compounds, with 14 predominant compounds contributing more than 3% to the total area. These dominant compounds exhibit potential pharmacological applications, including antioxidant, antibacterial, antifungal, anti-inflammatory, and anticancer activities. Further clinical research is imperative to substantiate these findings and ensure their practical applicability in medicinal development.

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