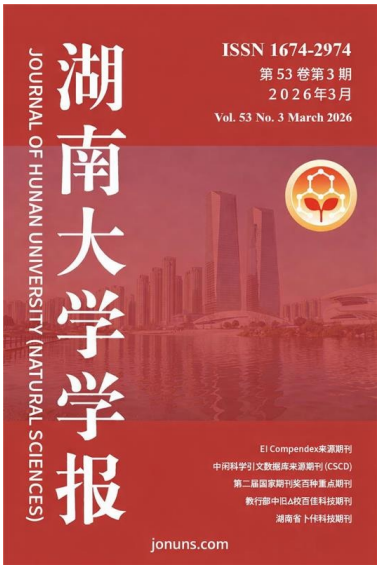

Journal of Hunan University

(Natural Sciences)

Vol. 53 No. 3

March 2026

Available online at

https://jonuns.com

Open Access Article

https://doi.org/10.55463/issn.1674-2974.53.3.11

Volatile Components and Antioxidant Properties of the Essential Oils and Hydrosols from Three Ginger Variants

Ika Oktavianawati [1]*, Ersya Yanu Ramadhani [1], Dina Trianggaluh Fauziah [2], Atok Ainur Ridho [3], Shahana Afrose Chowdhury [4], Wuriyanti Handayani [1]

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember. Jl. Kalimantan III/25 Jember 68121, East Java, Indonesia,
[2] Department of Pharmacy, Faculty of Health Sciences, Universitas dr, Soebandi. Jl. Dr. Soebandi No. 99 Jember 68111, East Java, Indonesia,
[3] Department of Agribusiness, Faculty of Agriculture, Universitas Muhammadiyah Jember. Jl. Karimata No. 49 Jember 68121, East Java, Indonesia,
[4] Department of Environmental Studies and Sustainability, University of Liberal Arts Bangladesh 688, Beribad Road, Mohammadpur, Dhaka-1207, Bangladesh,

* Corresponding author: ika.fmipa@unej.ac.id

Article History:
Received: February 9, 2026
Revised: March 17, 2026
Accepted: March 25, 2026
Published: March 31, 2026

Abstract: This study aims to investigate the antioxidant potential of essential oils and hydrosols derived from three ginger (Zingiber officinale) varieties based on their chemical profiles. Hydrodistillation was conducted for 5 hours to obtain both essential oils and hydrosols. The highest yields were observed in red ginger, followed by emprit ginger and elephant ginger.

Gas chromatography–mass spectrometry (GC–MS) analysis revealed that the major phytochemical constituents of both essential oils and hydrosols include camphene, cis-citral, trans-citral, α-curcumene, zingiberene, α-farnesene, β-bisabolene, and β-sesquiphellandrene. Red ginger oil was characterized by the presence of geranyl acetate and citronellyl acetate as marker compounds, while elephant ginger oil contained β-phellandrene and citronellal as distinguishing components. In contrast, emprit ginger did not exhibit unique marker compounds significantly different from the other varieties.

Antioxidant activity, assessed using IC_{50} values, indicated that red ginger essential oil exhibited the strongest activity (IC_{50} = 49.9 ppm) compared to other samples. However, Pearson correlation analysis suggested that functional groups such as conjugated double bonds, carbonyl groups, and hydroxyl groups were not significantly associated with antioxidant activity.

Furthermore, molecular docking analysis of zingiberene—a major constituent of ginger essential oils and hydrosols—demonstrated favorable binding affinities toward NADPH oxidase and inducible nitric oxide synthase (iNOS), with binding energies of −7.30 and −8.87, respectively. Key amino acid interactions were identified at Tyr88, Val124, Met120, Arg381, Trp463, Phe476, and Glu479, indicating potential mechanisms underlying its bioactivity.

Overall, the findings highlight the potential of ginger-derived essential oils, particularly red ginger, as natural antioxidant sources with possible pharmacological relevance.

Keywords: Ginger; Essential oils; Hydrosols; Antioxidant activity; Molecular docking.

三种生姜品种精油及其水提液的挥发性成分与抗氧化特性

摘要：本研究旨在基于化学成分特征，探讨三种生姜（Zingiber officinale）品种精油及其水提液的抗氧化潜力。采用水蒸气蒸馏法提取精油和水提液，蒸馏时间为5小时。结果表明，红姜的产率最高，其次为小姜（emprit ginger）和大姜（elephant ginger）。

气相色谱-质谱联用（GC-MS）分析显示，精油和水提液的主要植物化学成分包括樟脑烯（camphene）、顺式柠檬醛（cis-citral）、反式柠檬醛（trans-citral）、α-姜黄烯（α-curcumene）、姜烯（zingiberene）、α-法呢烯（α-farnesene）、β-倍半檀烯（β-bisabolene）以及β-倍半萜烯（β-sesquiphellandrene）。其中，红姜精油以乙酸香叶酯（geranyl acetate）和乙酸香茅酯（citronellyl acetate）为特征标志物；大姜精油则含有β-菲兰烯（β-phellandrene）和香茅醛（citronellal）等特征成分。而小姜未表现出明显区别于其他品种的特征性标志物。

抗氧化活性通过IC_{50}值进行评估，结果表明红姜精油具有最强的抗氧化能力（IC_{50} = 49.9 ppm）。然而，Pearson相关分析结果显示，共轭双键、羰基和羟基等官能团与抗氧化活性之间不存在显著相关性。

此外，对姜烯（zingiberene）进行分子对接分析结果表明，其与NADPH氧化酶和诱导型一氧化氮合酶（iNOS）具有良好的结合能力，结合能分别为-7.30和-8.87。关键氨基酸作用位点包括Tyr88、Val124、Met120、Arg381、Trp463、Phe476和Glu479，表明其可能的生物活性机制。

总体而言，本研究结果表明生姜精油，尤其是红姜精油，具有作为天然抗氧化剂的潜力，并具有潜在的药用价值。

关键词：生姜；精油；水提液；抗氧化活性；分子对接

1. Introduction

Ginger (*Zingiber officinale*) is one of Indonesia's primary commodities due to its high economic value [1–4]. Three varieties of ginger cultivated in Indonesia are emprit ginger, gajah (elephant) ginger, and red ginger [5,6]. Ginger rhizomes (roots) are widely used in many products, including essential oil, which is generally present in dry ginger at 1 to 15% [7–9]. Hydrodistillation is one method for extracting ginger essential oil and hydrosol. Hydrosol is a low-value byproduct. In fact, this aqueous phase from distillates

commonly contains a small amount of essential oil components based on its partition ability in water. Therefore, hydrosol may exhibit biological activity, though it is less active than essential oil. Research investigating the hydrosol part would be required to increase its potency [10].

The yield and chemical components of the essential oil can also be affected by storage conditions, post-harvest handling, planting location, ripeness level, harvest age, plant part, harvest season, and other environmental factors [11]. Moreover, ginger varieties could affect the profile of ginger essential oil. The average yields of emprit ginger, elephant ginger, and red ginger essential oils from that research, in consecutive order, are 0.675%, 0.454%, and 0.887%, with zingiberene contents of 32.7197%, 31.8688%, and 41.4799%, respectively [12]. Ginger oil has various medical benefits, including treating malaria, cough, rheumatism, toothache, and constipation. This oil can also act as an antioxidant, helping prevent and neutralize the chain oxidation process initiated by free radicals, which can lead to various diseases, such as coronary heart disease and cancer [13,14].

Previous research mainly focuses on ginger extracts without considering ginger variants [14,15]. In fact, species diversity may influence the chemical content of plants [16,17]. In addition, the extraction method is a crucial factor in obtaining the targeted phytochemicals associated with bioactivities. Distillation of essential oil is a popular method for producing a safe, functional ginger extract. Commonly, most papers only investigate the essential oil product, but not the hydrosol.

Accordingly, this research investigates the profiles of both essential oils and hydrosol extracts from three ginger varieties, including their yields and chemical compositions. The ginger essential oils were collected by hydrodistillation, while the hydrosol extracts were obtained by solvent extraction. The antioxidant activities of the essential oils and hydrosol extracts from three varieties of ginger were examined using the DPPH method. In silico molecular docking is also performed to analyze amino acid residues in the active sites of protein targets, which correlate with the antioxidant properties of major phytochemicals in ginger.

2. Materials and Methods

2.1 Materials

The emprit ginger, elephant ginger, and red ginger (approximately one year old) were obtained from Baban Timur, Mulyorejo Village (600 mdpl), Silo District, Jember Regency, East Java, and were identified by LIPI Kebun Raya Purwodadi as *Zingiber officinale* var. amarum, *Zingiber officinale* var. officinale, and *Zingiber officinale* var. rubrum, respectively (Figure 1). Diethyl ether p.a, $MgSO_4$ anhydrous, ascorbic acid, ethanol p.a, and DPPH were obtained from Sigma Aldrich.

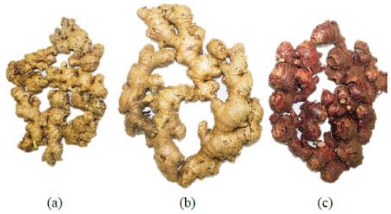

Figure 1. Three varieties of ginger: (a) emprit ginger (*Zingiber officinale* var. amarum); (b) elephant ginger (*Zingiber officinale* var. officinale); and red ginger (*Zingiber officinale* var. rubrum).

2.2 Methods

2.2.1 Hydrodistillation of Ginger Essential Oil and Extraction of Ginger Hydrosol

Ginger roots (rhizomes) were cleaned, dried, and weighed to 500 grams, then hydrodistilled in water for 5 hours using the Clevenger apparatus. The distillate collected in the first hour was further separated to obtain the oil and the aqueous phase containing the hydrosol. The ginger essential oil from a 5-hour distillation was collected and dried over anhydrous $MgSO_4$. At the same time, the hydrosol of the first hour of distillation was collected and extracted with diethyl ether at a 2:1 ratio using a separating funnel. The upper phase containing the diethyl ether extract was collected and vacuum-evaporated to produce the hydrosol extract. The essential oils and hydrosol extracts were stored in the fridge prior to GC-MS analysis.

2.2.2 GC-MS Analysis

GC-MS analysis was carried out using GC-MS-QP2010S Shimadzu equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 mm). The gas carrier was Helium with ionization EI 70 eV. GC-2010 has a column oven and injection temperatures of 70 °C and 300 °C. The pressure was adjusted to 30 kPa, with total and column flow of 35.6 and 0.65 mL/min, respectively. The linear velocity resulted in 29.6 cm/sec with a purge flow and split ratio of 3 mL/min and 49. The initial temperature of 70 °C was held for 5 minutes, followed by a temperature of 300 °C for 19 minutes. The program was set to 250 °C for the ion source temperature and 305 °C for the interface temperature, with a solvent cut time of 3 minutes. The MS started at 3.20 minutes and lasted 70 minutes. The event time was 0.5 seconds with a scan speed of 1250. The scanned molecular weight/charge (m/z) range was 28 to 600. Spectra and their fragmentations obtained from GC-MS analysis were matched to the spectra in the Mass Spectral Libraries of WILEY229 and

NIST62. The instrument is regularly standardized using a reference mass of PEG-PPG-Raffinose.

2.2.3 Antioxidant Activity Test

Antioxidant activity determination of essential oil and hydrosol extract of ginger was based on the DPPH method [18–20]. One mL of testing solution (essential oils or hydrosol extracts) at concentrations of 16, 32, 48, 64, and 80 ppm, along with 2 mL of DPPH solution, was added to the reaction tube. The mixture was homogenised and incubated at room temperature for 30 minutes, and the absorbance was measured at 515 a using a spectrophotometer. Absorption value from each concentration variation was used next to calculate the %inhibition value using this equation:

$$\%\text{Inhibition} = 1 - \frac{\text{Testing solution absorbation}}{\text{DPPH absorbtion}} \times 100\%$$

The %inhibition values for each concentration were then plotted in a linear regression. The IC_{50} value is determined from the x-value in the linear regression after substituting y with a value of 50. The IC_{50} value is compared with that of vitamin C, which is tested with the same process.

2.2.4 Statistical Analysis

Statistical analysis using Pearson's correlation analysis was performed with XLSTAT 2021.2.2.1141 to produce a correlation matrix, p-values, and coefficient of determination.

2.2.5 Molecular Docking

The molecular docking analysis consisted of three steps: ligand preparation, protein preparation, and docking simulation. The ligands, major phytochemicals in ginger essential oil, were prepared by drawing their structures in ChemDraw Ultra 8.0 and further processed in Avogadro 1.2.0 and OpenBabel 3.1.1 to obtain optimised 3D chemical structures in *.pdbqt* format. The protein structures were retrieved from the PDB website, with accession codes 2CDU (https://www.rcsb.org/structure/2CDU) and 4NOS (https://www.rcsb.org/structure/4NOS) corresponding to the X-ray crystal structures of NAD(P)H oxidase and human inducible nitric oxide synthase, respectively. The proteins were prepared directly in AutoDockTools-1.5.7 by removing water, adding the missing hydrogen atoms, and correcting charges and valences.

The docking simulations of 2CDU and 4NOS were adjusted and run based on the parameters shown in Table 1. This parameter was initially verified during the redocking procedure, which involves docking a native ligand into a targeted protein. The optimised parameter was used as the default for subsequent docking simulations on test compounds whenever the RMSD was less than 2.0. The ligand-protein complex's optimal conformation was achieved by the binding energies with the lowest value in kcal/mol, which represented the highest rank of the listed binding energies. Pymol Molecular Graphics System v2.5.8 and Biovia Discovery Studio v24.1.0.23298 were used to visualise this compound.

Table 1. Parameters for molecular docking.

Parameters	Value	
	2CDU	4NOS
Number of GA runs	50	50
Population size	300	300
Number of evaluations	2500000	2500000
Center grid box:		
x center	18.264	8.762
y center	-6.355	97.427
z center	1.535	11.762
Dimensions *x* x *y* x *z*	40 x 40 x 40	40 x 40 x 40

3. Results and Discussion

3.1. Yield of the Essential Oils and Hydrosol Extracts

The average yield of emprit ginger, elephant ginger, and red ginger essential oil was illustrated in Figure 2. Those yield values show a similar trend to the previous research [12], in which the order of essential oil yield, from highest to lowest, was red ginger, emprit ginger, and elephant ginger, i.e., 2.58-3.90%, 1.50-3.50%, and 0.82-1.66%, respectively. Meanwhile, the yields of hydrosol extracts from three varieties of ginger are lower than those of their essential oils because most hydrosol constituents are water-soluble essential oil compounds.

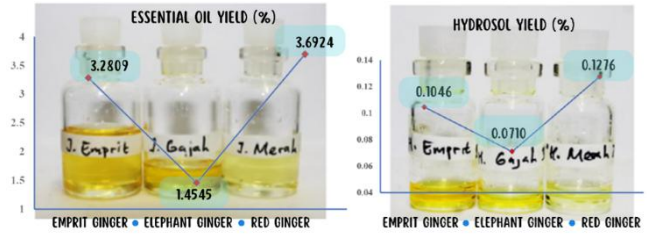

Figure 2. The essential oil yields from emprit, elephant, and red gingers.

3.2 Chemical Components of Essential Oils and Hydrosol Extracts of Gingers

The GC-MS results indicated that the total number of compounds in the essential oils of emprit, elephant, and red gingers is 26, 28, and 31, respectively. In comparison, the hydrosol extracts of emprit, elephant, and red gingers are 29, 28, and 27 compounds, respectively. Figure 3 illustrates the chromatograms of the chemical components of ginger essential oils and hydrosols.

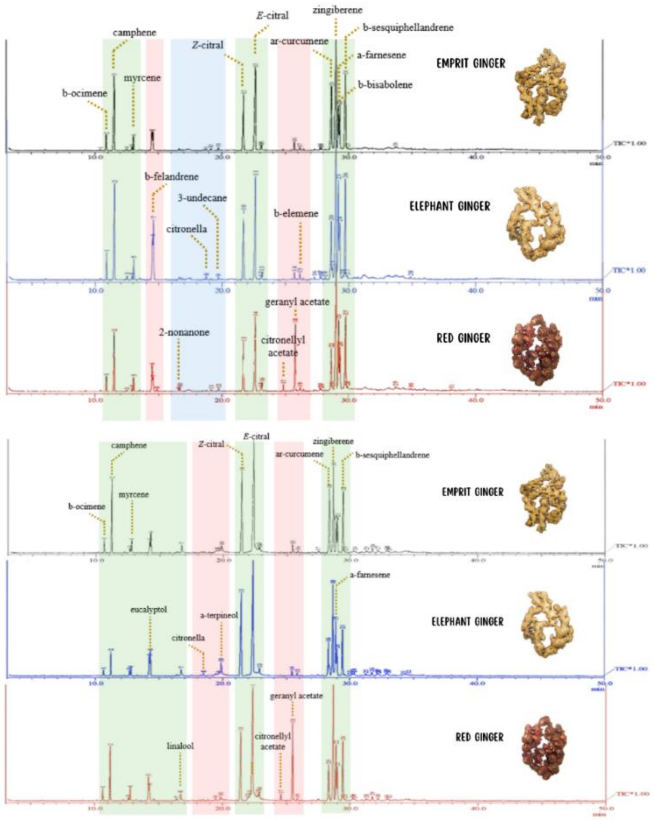

Figure 3. Chromatograms of the essential oils (top image) and hydrosol extracts (bottom image) from three varieties of ginger: emprit, elephant, and red gingers.

The figure shows that elephant ginger oil contains two unique components that are not present in emprit and red gingers, i.e., β-fellandrene, in significant abundance, and citronella, at a low percentage. In comparison, red ginger oil contains three unique components: 2-nonane and citronellyl acetate, in minor amounts, and geranyl acetate in bulky amounts, whereas other ginger oils show it only in minor amounts. However, the chromatogram of emprit ginger oil does not show any significant differences from those of other ginger oils. These patterns are also similar to those present in the ginger's hydrosols. Thus, it can be stated that some chemical components in ginger oils may be defined as chemical markers in each variety of ginger.

Chemical markers in red ginger oil are geranyl acetate and 2-nonane, while in elephant ginger oil are β-fellandrene and citronella. Emprit ginger oil does not show any significant chemical marker when compared to other ginger oils.

Table 1 lists the major components of the essential oils from three varieties of ginger, with mostly similar compounds, except for geranyl acetate, which is abundant in the red ginger essential oil only. In addition, all of the primary compound types in hydrosol extracts are similar to the major compounds of essential oils. The hydrosol extract contained abundant cis-citral and trans-citral, which were higher than zingiberene. It is due to the presence of oxygenated functional groups, which makes them more polar and easier to dissolve in water or hydrosol.

Figure 4 illustrates graph slices in a Venn diagram of volatile components in the essential oils and hydrosol fractions of ginger analyzed by GC-MS. It showed that all compounds listed as major components in ginger oils and hydrosols in Table 2 are included among the 21 compounds identified across all kinds of ginger, as shown in Figure 4.

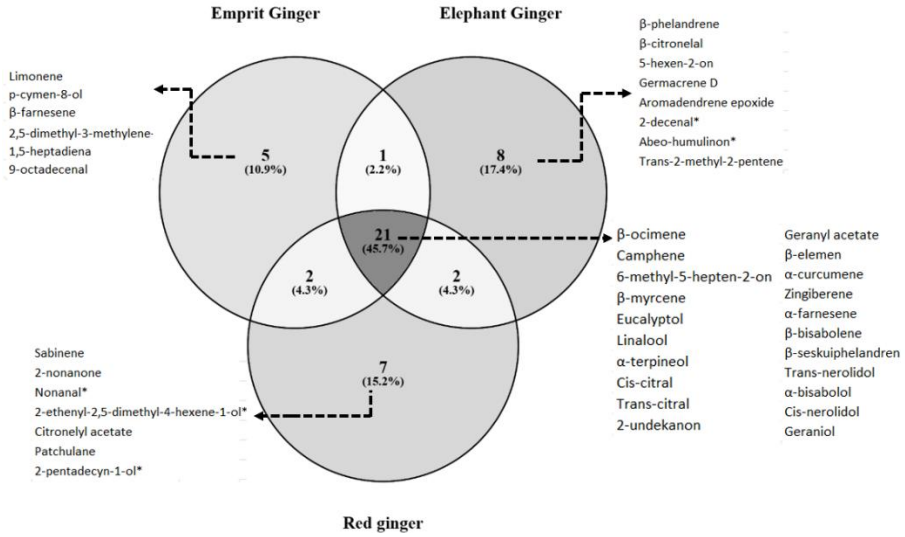

Figure 4. Venn diagram of volatile components inside ginger essential oils from three varieties.

Table 2. Volatile Components in the Essential Oils and Hydrosols from Three Varieties of Ginger

No.	Compound Name	Abundance (%)					
		Emprit Ginger		Elephant Ginger		Red Ginger	
		EO	Hydrosol	EO	Hydrosol	EO	Hydrosol

1.	Camphene	**9.60**	**8.36**	**8.38**	2.97	6.54	5.56
2.	Cis-citral	9.26	**13.42**	7.41	**14.97**	7.04	**9.27**
3.	Trans-citral	**14.87**	**24.92**	**12.46**	**23.03**	**12.18**	**21.74**
4.	Geranyl acetate	1.01	0.92	0.66	0.73	**8.62**	**9.02**
5.	α-curcumene	**10.52**	**8.98**	6.19	5.32	5.55	4.50
6.	Zingiberene	**19.91**	**12.37**	**20.82**	**14.08**	**22.90**	**16.55**
7.	α-farnesene	6.03	4.11	**10.49**	**8.52**	**8.63**	6.62
8.	β-bisabolene	6.01	4.31	5.32	3.61	5.06	3.74
9.	β-sesquiphelandrene	**11.28**	8.02	**10.16**	**6.94**	**10.24**	**7.28**

EO: essential oil

Bold value shows the top five major phytochemicals in each essential oil and hydrosol.

Chemical compound content, especially zingiberene in ginger essential oil, is used for quality determination [21]. This also applies to esters and oxygenated compounds found in essential oils, which contribute to a more fragrant aroma. Meanwhile, the lower the sesquiterpene compound content, the higher the essential oil quality. The comparison of compound groups in the ginger essential oil is illustrated in Figure 5.

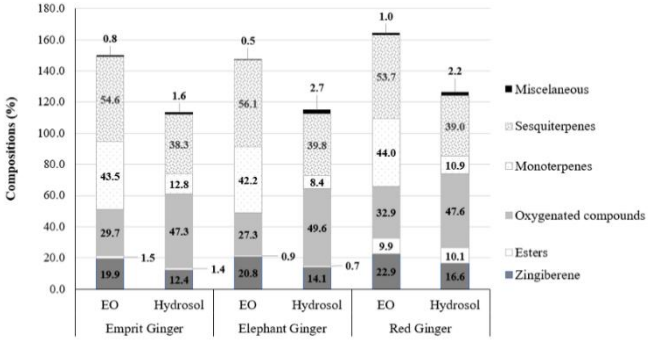

Figure 5. The group of chemical compounds determining the quality of the essential oils from three varieties of ginger. EO: essential oil.

According to Figure 5, it can be assumed that red ginger essential oil appears to have the best quality among the other essential oils, owing to the highest levels of zingiberene, oxygenated, and ester compounds, and the lowest sesquiterpene content. Next, emprit ginger essential oil showed better quality than elephant ginger, as it contains a higher amount of oxygenated and ester compounds and a lower amount of sesquiterpene content.

3.3 Antioxidant Activities of the Essential Oils and Hydrosol Extracts from Gingers

The antioxidant activities of the essential oils and hydrosol extracts of gingers were determined using the DPPH method. The results showed that the essential oil and hydrosol extracts of red ginger exhibited the highest antioxidant activity, whereas the emprit ginger extracts showed the lowest (Figure 6). The figure compares the antioxidant activity of the essential oils and hydrosol extracts of gingers, followed by the functional groups suspected to be responsible for their antioxidant activities.

Antioxidant activity commonly results from phytochemicals containing redox-active moieties, such as phenols of hydroxyl, including simple phenols, phenolic monoterpenes, flavonoids, enediol, thiol, amine, including imidazole, indole, pyrrole, and carbonyl, including quinone [22]. However, non-phenolic monoterpenes with pro-aromatic character, such as g-terpinene, may also exhibit antioxidant properties by undergoing aromatization to p-cymene via oxidation with the hydroperoxyl radical (HOO•) [23,24].

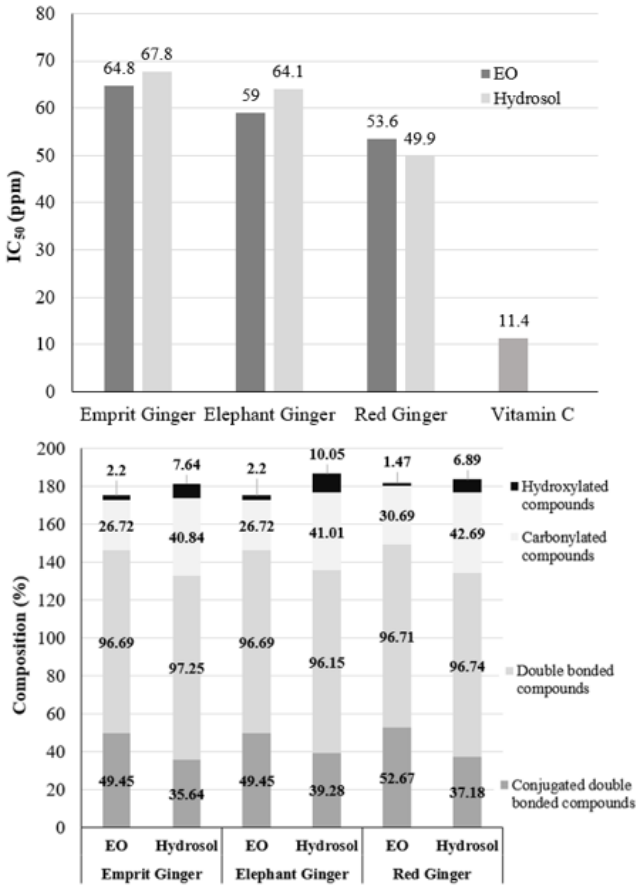

Figure 6. Antioxidant activities (top image) and functional groups (bottom image) of the essential oils and hydrosol extracts from three varieties of gingers and vitamin C. EO: essential oil.

The hydrosol fraction and essential oil of red ginger showed the lowest IC_{50} value, indicating the highest antioxidant activity among all samples (Figure 6). The

IC_{50} value below 50 ppm indicated a powerful antioxidant, whereas an IC_{50} value between 50 and 100 ppm indicated strong antioxidant activity [25]. This research showed that all essential oils and hydrosol extracts of ginger exhibit potent antioxidant activity. The chemical constituents of the essential oils and hydrosol extracts of gingers may play an important role in antioxidant activity or in the ability to stop the chain reaction of free radicals. The hydroxyl group in the constituent of the essential oil and hydrosol extracts of ginger can donate a hydrogen atom to a free radical, forming a non-radical molecule. The conjugated double bond and the carbonyl group in the constituent components may also support the antioxidant activity. The methyl group in the ring, the number and position of the hydroxyl groups, and the antioxidant activity of a compound are influenced by the presence of more substituted hydroxyl groups, which confer a better ability to donate hydrogen atoms [26,27].

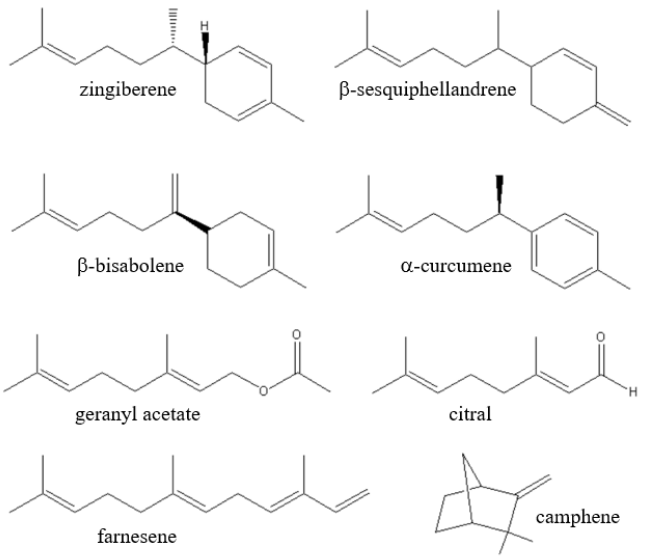

Figure 7. Chemical structures of phytochemicals in ginger essential oils.

In fact, as a part of major phytochemicals in ginger oil, zingiberene has one conjugated double bond in its structure, resulting in relatively low antioxidant activity due to the absence of a hydroxyl group as a hydrogen donor and the formation of a less-stabilized free radical. In this research, the major phytochemicals in ginger oil show a pattern similar to that of zingiberene (Figure 7). These phytochemicals have limited conjugated bonds and no hydroxylated groups. Therefore, the ability of hydrogen donors and radical stabilization from ginger phytochemicals is very limited, resulting in lower antioxidant activities compared to phenolic compounds such as ascorbic acid as a standard. However, the synergistic effect of all major components in ginger essential oil resulted in a low IC_{50} value, though it was not as low as that of ascorbic acid.

Table 3. Coefficient of determination (R^2) and *p*-values of Pearson correlation analysis of phytochemicals from three varieties of ginger toward antioxidant activities (IC_{50} values).

Variables	*p*-values	R^2	Interpretation
Conjugated double-bonded compounds	0.711	0.038	Not significant
Double-bonded compounds	0.812	0.016	Not significant
Carbonylated compounds	0.934	0.002	Not significant
Hydroxylated compounds	0.600	0.075	Not significant

This deduction is supported by statistical data in Table 3, which shows *p*-values from a Pearson correlation analysis, using a standard threshold of 0.05 to determine statistical significance. The table indicates that the variables for carbonylated, conjugated double-bonded, and hydroxylated compounds are statistically significantly correlated with each other but not with the IC50 values of ginger essential oils. Moreover, Table 3 also shows that IC_{50} values exhibit a very weak correlation ($R^2 < 0.1$) with all tested structural features, indicating that the presence of conjugated double bonds, carbonyl groups, or hydroxyl groups did not significantly influence bioactivity. This suggests that other components, including non-volatile compounds, especially from phenolic compounds with conjugated and extended conjugated bonds such as flavonoids, and other molecular factors, may play a more dominant role in determining IC_{50}.

3.4 In Silico Antioxidant Activity Assessment

Based on the major phytochemical components of ginger oils and hydrosol listed in Table 1, an in silico assessment of antioxidant activity was performed using molecular docking against relevant protein receptors. The integration of experimental data and in silico computational simulations via molecular docking provides binding affinity values and the predicted positions and orientations of major phytochemicals in ginger oil that are responsible for antioxidant activity in the human body.

Two protein receptor targets used in this study are NOX, nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, and iNOS, inducible nitric oxide synthase. These proteins are produced by enzymes that inhibit antioxidant activity. NOX and iNOS also facilitate uncontrolled cell proliferation through intracellular signaling pathways, contributing a the development of various cancer cells [28–31]. Thus, inhibiting these two enzymes may improve immunity and treat diseases associated with oxidative stress.

Table 4. The binding energy (kcal/mol) of molecular interactions between protein targets and ginger oil's phytochemicals.

No.	Compounds	NOX (2CDU)	iNOS (4NOS)
1.	Zingiberene	- 7.30	- 8.87
2.	Trans-citral	- 6.77	- 7.24
3.	Cis-citral	- 6.02	- 6.80
4.	a-farnesene	- 6.97	- 7.93
5.	b-sesquiphellandrene	- 7.09	- 8.56
6.	Ar-curcumene	- 6.80	- 7.97
7.	Camphene	- 5.33	- 6.86
8.	Ascorbic acid	- 8.55	- 9.28
9.	Native ligand (ADP)	- 14.99	-
10.	Native ligand (H2B)	-	- 10.66

The inhibition of NOX (PDB ID: 2CDU) and iNOS (PDB ID: 4NOS) by ginger oil's major phytochemicals results in zingiberene as the best chemical component with the highest binding energies (Table 4). However, this number remains lower than the interaction between ascorbic acid and NOX or iNOS. It is consistent with the experimental data showing that the antioxidant activity of ascorbic acid is higher than that of ginger essential oils and hydrosols.

Native ligands, ADP (adenosine-5'-diphosphate) and H2B (2-amino-6-(1,2-dihydroxy propyl)-7,8-dihydro-6H-pteridin-4-one), are used as standards for the redocking process toward NOX and iNOS, respectively. The molecular interactions between native ligands and current proteins are also becoming molecular references for comparing ginger phytochemicals, with the protein moiety serving as the active site. The two- and three-dimensional interactions between zingiberene and 2CDU and 4NOS, as shown in Figure 8, showed some hydrophobic interactions but no hydrogen bond formation, as observed with the native ligands, ADP and H2B, respectively. It may lead to a lower antioxidant activity of zingiberene compared to native ligands. However, the amino acid residues in protein targets that interact with native ligands and zingiberene are pretty similar. Tyr188 and Val214 are two amino acid residues from 2CDU (NOX) that are responsible for the interaction with native ligand ADP and also with zingiberene. Meanwhile, Met120, Arg381, Trp463, Phe476, and Glu479 are amino acid residues of 4NOS that interact with the native ligand H2B and zingiberene (Table 5).

Interestingly, ascorbic acid, the standard compound for antioxidant assessment, showed similar interactions with the native ligands. Many hydrogen bonds and additional interactions, such as stacking and salt bridges, are also formed between the protein receptors and ascorbic acid. This kind of interaction is thought to account for the observed high antioxidant activity of ascorbic acid.

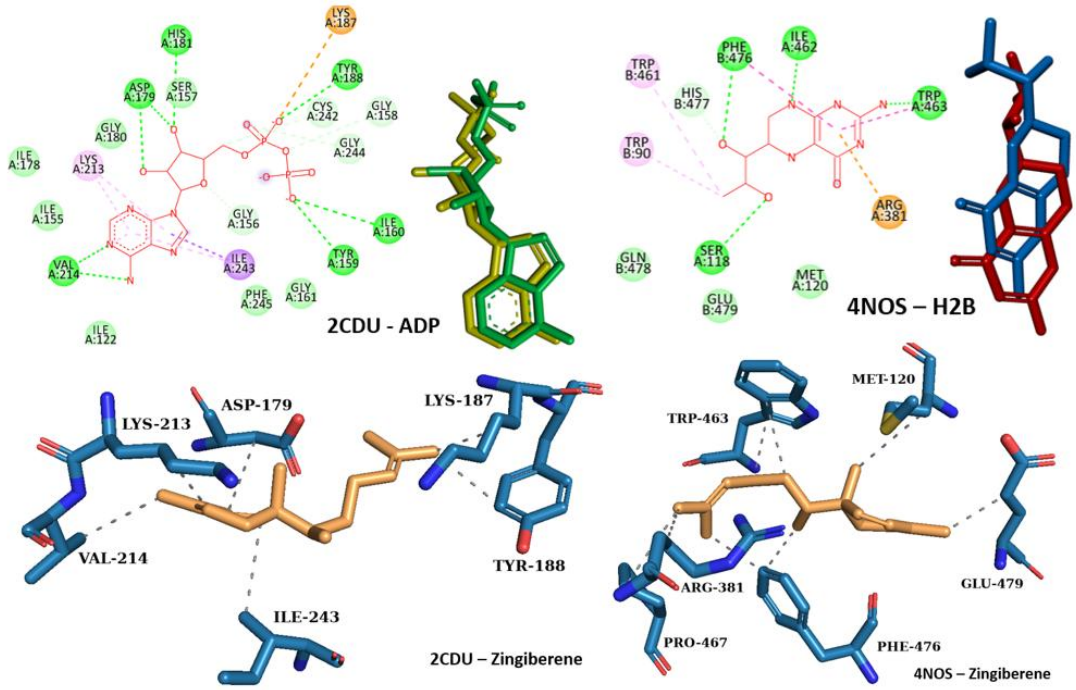

Figure 8. 2D and 3D interactions between zingiberene and both protein targets, NOX (2CDU) and iNOS (4NOS).

Table 5. Chemical interactions between amino acid residues of protein targets and native ligands, or zingiberene.

Compounds	Interactions
NOX (2CDU)	
Native ligand (ADP)	Hydrogen bonds: Gly156 (3.48 Å), **Ser157 (3.34 Å)**, **Gly158 (2.78 Å)**, Tyr159 (2.23 Å), Ile160 (1.88 Å), Gly161 (2.08 Å), Gly180 (2.47 Å), His181 (2.27 dan 3.18 Å), **Tyr188 (2.19 Å)**, **Val214 (2.64 and 2.07 Å)**, Cys242 (2.23 Å), Gly244 (3.18 Å).
Zingiberene	Hydrophobic interactions: Asp179 (4.00 Å), Lys187 (3.76 Å), **Tyr188 (3.46 Å)**, Lys213 (3.36 Å), **Val214 (3.73 Å)**, Ile243 (3.86 Å).
Ascorbic acid	Hydrogen bonds: **Ser157 (2.58 and 2.33 Å)**, **Gly158 (2.36 Å)**, Asp179 (2.10 and 1.88 Å), **His181 (2.01 Å)**, Tyr186 (2.17 Å), Lys187 (2.09 Å), **Tyr188 (2.94, 2.89 and 3.42 Å). Salt bridges: His181 (5.47 Å)**, Lys187 (5.20 Å).
iNOS (4NOS)	
Native ligand (H2B)	Hydrogen bonds: **Arg381 (2.77 and 2.11 Å), Trp461 (2.46 Å), Ile462 (2.72 Å), Phe476 (3.04 and 2.64 Å)**, His477 (1.88 Å). Hydrophobic interactions: **Met120 (3.23 Å)**, **Glu479 (3.13 Å)**. π-stacking: **Trp463 (4.36 Å)**. π-cation interactions: **Trp463 (3.89 and 3.86 Å)**.
Zingiberene	Hydrophobic interaction: **Met120 (3.96 Å)**, **Arg381 (3.99 Å)**, **Trp463 (3.85 and 3.56 Å)**, Pro467 (3.84 Å), **Phe476 (3.22 and 3.29 Å)**, **Glu479 (3.09 Å)**.
Ascorbic acid	Hydrogen bonds: Trp90 (2.68 Å), Ser118 (2.00 and 1.77 Å), Ile119 (3.02 Å), **Trp463 (1.74 Å), Glu479 (2.50 Å)**. π-stacking: **Trp461 (4.97 Å). Salt bridges: Arg199 (5.28 Å).**

Amino acid residues in **bold** are found to interact with native ligands, zingiberene and ascorbic acid.

4. Conclusions

Three varieties of ginger showed a similar pattern of chemical components for their essential oils and hydrosols. Mainly, they contained camphene, cis-citral, trans-citral, geranyl acetate, α-curcumene, zingiberene, α-farnesene, β-bisabolene, and β-sesquiphellandrene in a different percentages. Chemical markers in red ginger oil are geranyl acetate and citronellyl acetate, while in elephant ginger oil are β-fellandrene and citronella. Emprit ginger oil does not show any significantly different chemical components compared to the other two ginger oils. Thus, it affected the antioxidant properties, resulting in red ginger essential oil and hydrosol having the highest antioxidant activities among other oils and hydrosols. However, Pearson correlation analysis showed that conjugated double bond, carbonyl, and hydroxyl groups did not influence the antioxidant activity in ginger oils. Zingiberene is predicted to be the chemical marker in ginger oils and hydrosols, resulting in high antioxidant activity. Molecular docking of zingiberene against NADPH oxidase (2CDU) and inducible nitric oxide synthase (4NOS) showed some crucial hydrophobic interactions in amino acid residues of Tyr88, Val 124, and Met120, Arg381, Trp463, Phe476, Glu479, respectively.

Declarations

Author Contributions

Conceptualization, IO and EYR; methodology, IO and EYR; software, DTF; validation, DTF, AAR and WH; formal analysis, AAR; investigation, EYR; resources, WH; data curation, IO and EYR.; writing—original draft preparation, IO, EYR, WH; writing—review and editing, SAC; visualization, AAR and SAC; supervision, IO; project administration, IO; funding acquisition, IO. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Institutional Review Board Statement

Not Applicable

Informed Consent Statement

Not Applicable.

Conflicts of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

Acknowledgements

The authors gratefully acknowledge the support of Universitas Jember for this study through the Hibah Penelitian Pasca Doktor 2025 scheme, under contract number 2829/UN25.3.1/LT/2025.

References

[1] Elpawati, Wiranthi PE, Aisyah SN. Forecasting Indonesia's ginger export with major competing countries in the international market. *Anjoro: International Journal of Agriculture and Business*. 2022;3:73–80. https://doi.org/10.31605/anjoro.v3i2.2061.

[2] Saputro WA, Anggrasari H. The Role, Development and Opportunities of Spice Commodities for International Relations between Indonesia and Other Countries in the International Market. *Journal of Advance in Social Sciences and Policy*. 2021;1:154–66. https://doi.org/10.23960/jassp.v1i2.34.

[3] Oktavianawati I, Kurniati HI, Maghfiroh K, Hanifah N, Handayani W, Winata INA. Essential oils from rhizomes of five Zingiberaceae species in Meru Betiri National Park. *Jurnal Ilmu Dasar*. 2018;2026:1–8. https://doi.org/10.1063/1.5065034.

[4] Oktavianawati I, Winata INA, Aji JMM. The Application of Distillation Technology of Rhizomes Essential Oils in TOGA Community of Sari Hutani in the Border Area of Meru Betiri National Park. *Jurnal Inovasi Sains Dan Teknologi Untuk Masyarakat*. 2023;2:110–8. https://doi.org/10.19184/instem.v2i2.1510.

[5] Yanti CF, Usman AN, Ahmad M, Ilhammudin, Ariyandi A, Budiaman. Analysis of Ginger Varieties (Zingiber Officinale) in Indonesia, Nutritional Content and Potential for Health. *BIO Web of Conferences*. 2024;96:1–9. https://doi.org/10.1051/bioconf/20249601021.

[6] Yunita O, Fadhilah NURA, Pramadiyanti S, Jonatan S. Molecular characterization of red ginger varieties (Zingiber officinale Roxb. var. rubrum) by DNA markers. *Biodiversitas Journal of Biological Diversity*. 2023;24:6905–13. https://doi.org/10.13057/biodiv/d241252.

[7] Singh M, Khan MM., Naeem M. Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in Zingiber officinale Rosc. *Journal of the Saudi Society of Agricultural Sciences*. 2016;15:171–8. https://doi.org/10.1016/j.jssas.2014.11.002.

[8] Mohammed HH, Laftah WA, Ibrahim AN, Yunus MAC. Extraction of essential oil from Zingiber officinale and statistical optimization of process parameters. *RSC Advances*. 2022;12:4843–51. https://doi.org/10.1039/D1RA06711G.

[9] Kamal GM, Nazi N, Sabir A, Saqib M, Zhang X, Jiang B, et al. Yield and Chemical Composition of Ginger Essential Oils as Affected by Inter-Varietal Variation and Drying Treatments of Rhizome. *Separations*. 2023;10:1–20. https://doi.org/10.3390/separations10030186.

[10] D'Amato S, Serio A, Lopez CC, Paparella A. Hydrosols: Biological activity and potential as antimicrobials for food applications. *Food Control*. 2018;86:126–37. https://doi.org/10.1016/j.foodcont.2017.10.030.

[11] Hassiotis CN, Vlachonasios KE. How Biological and Environmental Factors Affect the Quality of Lavender Essential Oils. *Physiologia*. 2025;5:1–11. https://doi.org/10.3390/physiologia5010011.

[12] Susihono W. Indonesian ginger yield quality as the basis for saleability of ginger oil on the international market. *Widyariset*. 2011;14:579–88.

[13] Irfan S, Ranjha MMAN, Mahmood S, Mueen-ud-Din G, Rehman S, Saeed W, et al. A Critical Review on Pharmaceutical and Medicinal Importance of Ginger. *Acta Scientific Nutritional Health*. 2019;3:78–82.

[14] Hamad A, Wahyuningrum R, Pusparini DH, Faridaeni D. Pharmacognostic authentication and bioactivity of polyherbal essential oils from red ginger, turmeric, and aromatic ginger. *Biodiversitas Journal of Biological Diversity*. 2025;26:5132–44. https://doi.org/10.13057/biodiv/d261028.

[15] Harun NH, Mohamad MF. Zingiber officinale (ginger): A systematic review and meta-analysis on antimicrobial activities. *Journal of Applied Pharmaceutical Science*. 2023;13:001–11. https://doi.org/10.7324/JAPS.2023.8330.

[16] Randlkofer B, Obermaier E, Hilker M, Meiners T. Vegetation complexity — The influence of plant species diversity and plant structures on plant chemical complexity and arthropods. *Basic and Applied Ecology*. 2010;11:383–95. https://doi.org/10.1016/j.baae.2010.03.003.

[17] Meier CL, Bowman WD. Links between plant litter chemistry, speciesdiversity, and below-ground ecosystem function.pdf. *Proceedings of the National Academy of Sciences*. 2008;105:19780–5. https://doi.org/10.1073/pnas.0805600105.

[18] Farhan AM, Setyati D, Gilani NA, Su'udi M, Ulum FB. Phytochemistry Profile and Antioxidant Activity of Dumortiera hirsuta (Sw.) Nees from Gumitir, East Java. *Hayati Journal of Biosciences*. 2025;32:1643–53. https://doi.org/10.4308/hjb.32.6.1643-1653.

[19] Elsharkawy ER, Abdallah EM, Markb AA. Potential Cytotoxic , Antifungal , and Antioxidant Activity of Dithymoquinone and Thymoquinone. *Journal of Hunan University (Natural Sciences)*. 2021;48:90–9.

[20] Arpiwi NL, Made N, Suarni R. Essential Oil from Fresh and Dry Leaves of Rosemary (Rosmarinus Officinalis L .): Antioxidant Activity and Microscopic Structure. *Journal of Hunan University (Natural Sciences)*. 2023;50:84–93. https://doi.org/10.55463/issn.1674-2974.50.3.9.

[21] Akshitha HJ, Umesha K, Leela NK, Shivakumar MS, Prasath D. Quality attributes and essential oil profiling of ginger (Zingiber officinale Rosc.) genotypes from India. *Journal of Essential Oil Research*. 2020;32:456–63. https://doi.org/10.1080/10412905.2020.1789000.

[22] Ruseva N, Bakalova A, Cherneva E. Funtional Groups and Structural Features of Antioxidants: A Review. *Journal of Chemical Technology and Metallurgy*. 2025;60:881–912. https://doi.org/10.59957/jctm.v60.i6.2025.1.

[23] Mollica F, Gelabert I, Amorati R. Synergic Antioxidant E ff ects of the Essential Oil Component γ - Terpinene on High-Temperature Oil Oxidation. *Food Science and Technology*. 2022;2:180–6. https://doi.org/10.1021/acsfoodscitech.1c00399.
[24] Amorati R, Foti MC, Valgimigli L. Antioxidant Activity of Essential Oils. *Journal of Agricultural and Food Chemistry*. 2013;61:10835–47. https://doi.org/10.1021/jf403496k.
[25] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*. 2004;26:211–9.
[26] Yamauchi M, Kitamura Y, Nagano H, Kawatsu J, Gotoh H. DPPH Measurements and Structure — Activity Relationship Studies on the Antioxidant Capacity of Phenols. *Antioxidants*. 2024;13:1–16. https://doi.org/10.3390/antiox13030309.
[27] Gulcin İ. Antioxidants : a comprehensive review. *Archives of Toxicology*. 2025;99:1893–997. https://doi.org/10.1007/s00204-025-03997-2.
[28] Al Shammari BR. Antioxidant, antimicrobial and in silico NADHP oxidase inhibition of chemically-analyzed essential oils derived from Ballota deserti (Noe) Jury. *Molecules*. 2022;27:1–14. https://doi.org/10.3390/molecules27196636.
[29] Mhya DH, Ph D, Jakwa AG, Sc M, Agbo J, Ph D. In Silico Analysis of Antioxidant Phytochemicals with Potential. *Journal of Health Science and Medical Research*. 2023;41:1–16. https://doi.org/10.31584/jhsmr.2022912.
[30] Herrera-calderon O, Chacaltana-ramos LJ, Carmen I, Algarni MA, Alqarni M, Batiha GE. Chemical Constituents , In Vitro Antioxidant Activity and In Silico Study on NADPH Oxidase of Allium sativum L. (Garlic) Essential Oil. *Antioxidants*. 2021;10:1–16. https://doi.org/10.3390/antiox10111844.
[31] Poleboyina PK, Rampogu S, Doneti R, Pasha A, Poleboyina SM, Bhanothu S, et al. Screening and Identification of Potential iNOS Inhibitors to Curtail Cervical Cancer Progression : an In Silico Drug Repurposing Approach. *Applied Biochemistry and Biotechnology*. 2022;194:570–86. https://doi.org/10.1007/s12010-021-03718-2.

参考文:

[1] Elpawati, Wiranthi PE, Aisyah SN. 印度尼西亚生姜出口预测及其在国际市场中的主要竞争国家。《Anjoro：国际农业与商业期刊》，2022年；3：73–80。https://doi.org/10.31605/anjoro.v3i2.2061。
[2] Saputro WA, Anggrasari H. 香料作物在印度尼西亚与其他国家国际关系中的作用、发展与机遇。《社会科学与政策进展期刊》，2021年；1：154–166。https://doi.org/10.23960/jassp.v1i2.34。
[3] Oktavianawati I, Kurniati HI, Maghfiroh K, Hanifah N, Handayani W, Winata INA. 默鲁贝蒂里国家公园五种姜科植物根茎精油研究。《基础科学期刊》，2018年；2026：1–8。https://doi.org/10.1063/1.5065034。
[4] Oktavianawati I, Winata INA, Aji JMM. 默鲁贝蒂里国家公园边境社区根茎精油蒸馏技术应用。《科技创新服务期刊》，2023年；2：110–118。https://doi.org/10.19184/instem.v2i2.1510。
[5] Yanti CF, Usman AN, Ahmad M, Ilhammudin, Ariyandi A, Budiaman. 印度尼西亚生姜品种分析、营养成分及健康潜力。《BIO Web of Conferences》，2024年；96：1–9。https://doi.org/10.1051/bioconf/20249601021。
[6] Yunita O, Fadhilah NURA, Pramadiyanti S, Jonatan S. 红姜分子特征研究。《Biodiversitas》，2023年；24：6905–6913。https://doi.org/10.13057/biodiv/d241252。
[7] Singh M, Khan MM., Naeem M. 氮对生姜生长及品质的影响。《沙特农业科学学会期刊》，2016年；15：171–178。https://doi.org/10.1016/j.jssas.2014.11.002。
[8] Mohammed HH, Laftah WA, Ibrahim AN, Yunus MAC. 生姜精油提取及工艺优化。《RSC Advances》，2022年；12：4843–4851。https://doi.org/10.1039/D1RA06711G。
[9] Kamal GM 等 生姜精油产量及成分研究。《Separations》，2023年；10：1–20。https://doi.org/10.3390/separations10030186。
[10] D'Amato S 等 水提液的抗菌潜力。《Food Control》，2018年；86：126–137。https://doi.org/10.1016/j.foodcont.2017.10.030。
[11] Hassiotis CN, Vlachonasios KE. 薰衣草精油质量影响因素。《Physiologia》，2025年；5：1–11。https://doi.org/10.3390/physiologia5010011。
[12] Susihono W. 印尼生姜品质与市场价值。《Widyariset》，2011年；14：579–588。
[13] Irfan S 等 生姜药用价值综述。《Acta Scientific Nutritional Health》，2019年；3：78–82。
[14] Hamad A 等 红姜等精油药理研究。《Biodiversitas》，2025年；26：5132–5144。https://doi.org/10.13057/biodiv/d261028。
[15] Harun NH, Mohamad MF. 生姜抗菌活性综述。《应用药学期刊》，2023年；13：001–011。https://doi.org/10.7324/JAPS.2023.8330。
[16] Randlkofer B 等 植物多样性与生态功能。《基础与应用生态学》，2010年；11：383–395。https://doi.org/10.1016/j.baae.2010.03.003。
[17] Meier CL, Bowman WD. 植物化学与生态系统关系。《美国科学院院刊》，2008年；105：19780–19785。https://doi.org/10.1073/pnas.0805600105。

[18] Farhan AM 等 植物抗氧化活性研究。《Hayati》，2025年；32：1643–1653。https://doi.org/10.4308/hjb.32.6.1643-1653。
[19] Elsharkawy ER 等 生物活性研究。《湖南大学学报（自然科学版）》，2021年；48：90–99。
[20] Arpiwi NL 等 迷迭香精油抗氧化研究。《湖南大学学报（自然科学版）》，2023年；50：84–93。https://doi.org/10.55463/issn.1674-2974.50.3.9。
[21] Akshitha HJ 等 生姜精油品质研究。《Journal of Essential Oil Research》，2020年；32：456–463。https://doi.org/10.1080/10412905.2020.1789000。
[22] Ruseva N 等 抗氧化剂结构综述。《Journal of Chemical Technology and Metallurgy》，2025年；60：881–912。https://doi.org/10.59957/jctm.v60.i6.2025.1。
[23] Mollica F 等 精油抗氧化协同效应。《Food Science and Technology》，2022年；2：180–186。https://doi.org/10.1021/acsfoodscitech.1c00399。
[24] Amorati R 等 精油抗氧化活性。《Journal of Agricultural and Food Chemistry》，2013年；61：10835–10847。https://doi.org/10.1021/jf403496k。
[25] Molyneux P. DPPH抗氧化测定方法。《Songklanakarin Journal》，2004年；26：211–219。
[26] Yamauchi M 等 酚类抗氧化能力研究。《Antioxidants》，2024年；13：1–16。https://doi.org/10.3390/antiox13030309。
[27] Gulcin İ. 抗氧化剂综述。《Archives of Toxicology》，2025年；99：1893–1997。https://doi.org/10.1007/s00204-025-03997-2。
[28] Al Shammari BR. 精油抗氧化抗菌研究。《Molecules》，2022年；27：1–14。https://doi.org/10.3390/molecules27196636。
[29] Mhya DH 等 植物抗氧化成分分析。《Journal of Health Science》，2023年；41：1–16。https://doi.org/10.31584/jhsmr.2022912。
[30] Herrera-calderon O 等 大蒜精油抗氧化研究。《Antioxidants》，2021年；10：1–16。https://doi.org/10.3390/antiox10111844。
[31] Poleboyina PK 等 抗癌药物筛选研究。《Applied Biochemistry and Biotechnology》，2022年；194：570–586。https://doi.org/10.1007/s12010-021-03718-2。

Manuscript Information
Word count: 7,619 words (excluding references).
Peer-Review Record

Fast-track status: Not fast-tracked.
First-round reviews received: 3 reports.
Revision cycles completed: 3 rounds.
Final version submitted: March 25, 2026