Chemical Constituents, Antioxidant, and Antimicrobial Activities of Ethyl Acetate Fractionated Extract from Rhizomes of *Zingiber monophyllum* Gagnep.: In vitro and in silico Screenings

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Abstract

Objective/Background: *Zingiber monophyllum* Gagnep., a member of the Zingiberaceae family, is known for its significant biological activities. The current study aimed to determine the volatile components of the ethyl acetate (EtOAc) fractionated extract found in the rhizomes of this species. This is the first report on the chemical composition and bioactivities of *Z. monophyllum* rhizomes fractionated extract. **Methods:** The chemical constituents were analyzed and determined using gas chromatography-mass spectrometry (GC-MS). Antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays using ascorbic acid as a positive control. Antibacterial and antifungal properties of the EtOAc fractionated extract of *Z. monophyllum* rhizomes were assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans*. Density functional theory (DFT) and molecular docking were also employed to illustrate antioxidant and antimicrobial activities. **Results:** Nine components were identified by GC-MS analysis from the EtOAc fractionated extract of *Z. monophyllum* rhizomes. (*E*)-labda-8(17),12-diene-15,16-dial (**9**), spathulenol (**2**), and neointermedeol (**5**) were the major components (21.8%, 16.8%, and 11.9%, respectively). Moderate antioxidant activities of the EtOAc fractionated extract were observed *via* both the DPPH assay and the FRAP assay using ascorbic acid as the standard compound. The extract demonstrated remarkable antimicrobial activity against all examined microbial strains, except for *P. aeruginosa*. The DFT study analyzed the antioxidant potential of each component in the fractionated extract. Molecular docking study chose *E. faecalis* DNA gyrase B, *E. coli* DNA gyrase B, *S. aureus* biotin protein ligase, *E. faecalis* Alanine racemase, and *C. albicans* N-myristoyltransferase as potential target proteins for antimicrobial activity. **Conclusion:** In this study, the chemical composition of the EtOAc fractionated extract of *Z monophyllum* rhizomes was demonstrated through GC-MS analysis for the first time. Nine components, including alloaromadendrene, spathulenol, globulol, τ-cadinol, neointermedeol, aromadendrene oxide-(2), ambrial, *(E)*-15,16-dinorlabda-8(17),11-dien-13-one, and *(E)*-lambda-8 (17),12-diene-15,16-dial along with relative content were identified in this fractionated extract. The bioassays revealed that the fractionated extract showed moderate antioxidant activities and significant antimicrobial activities. The antioxidant and antimicrobial potential of each component was also theoretically examined by the DFT study and molecular docking study, respectively.

Introduction

Zingiber monophyllum Gagnep., belongs to the *Zingiber* genus (Zingiberaceae family), is a native plant from Vietnam and Laos.[1] *Z. monophyllum* is commonly found in Vietnam in several provinces, such as Thanh Hoa, Thua Thien-Hue, and Ha Nam.[2] The antibacterial activity of essential oils from the rhizomes and leaves of *Z. monophyllum* has been reported previously.[2] Additionally, some compounds, including β-pinene, borneol, α-pinene, and myrtenal found in the rhizomes and leaves of *Z. monophyllum* were proven to exhibit potent cytotoxicity against

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cancer cells (eg, breast cancer and leukemia), as well as anti-tumor, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, bronchodilatory, antiaggregative and antihemolytic, neuroprotective, and gastroprotective activities.[3–5]

In previous studies, the extracts from the rhizomes of *Zingiber* species also had antimicrobial properties. For example, several strains (eg, *Bacillus cereus*, *Pseudomonas aeruginosa*, etc) proved to be highly susceptible to the activity of the ethyl acetate extract of *Z. officinalis* rhizomes.[6] Similarly, the ethyl acetate extract from *Z. neesanum* rhizomes showed potential microbicidal effects.[7] Other extracts examined in previous reports also revealed interesting antibacterial activities.[8] However, there has been little information on the antimicrobial activity of the extracts from the rhizomes of *Z. monophyllum*, indicating the need for further study to diversify the source of promising antibacterial materials.

Previous studies have reported that antioxidant compounds have been widely used in medicine to treat various diseases and illnesses. They have the ability to interrupt, delay, or prevent oxidation.[9] As a result, antioxidants can protect the body by preventing aging, cell damage, and oxidative stress-related diseases.[10] Research on antioxidant compositions from plant-based extracts has received great interest for their potential as herbal drugs. Furthermore, antioxidants also exhibit various pharmacological effects including antibacterial, antiviral, anti-inflammatory, antiaging, and antitumor activities.[11] Because of these beneficial properties of antioxidants, the general aim of many studies has been carried out to find new natural sources of antioxidant properties. Up to now, the information about the phytochemical components and biological activities of the extract from *Z. monophyllum* rhizomes is still limited. In this work, we reported the volatile compositions of the EtOAc fractionated extract from *Z. monophyllum* rhizomes, *in vitro* antioxidant, antimicrobial activities combined with the density functional theory (DFT) approach, and molecular docking simulation.

Results and Discussion

Chemical Composition of the EtOAc Fractionated Extract

From the EtOAc fractionated extract of *Z. monophyllum* rhizomes, nine components were identified by gas chromatography-mass spectrometry (GC-MS) analysis (Supplemental Figure S1-S10). These compounds belong to three terpenoid groups, including guaiane (**1-3** and **6**), cadinane (**4** and **5**), and labdane (**7-9**) types. Among them, (*E*)-labda-8(17),12-diene-15,16-dial (**9**), spathulenol (**2**), and neointermedeol (**5**) were the major compounds (21.8%, 16.8%, and 11.9%, respectively) confirmed by GC-MS analysis (Table 1 and Figure 1). The acetone extract of *Z. zerumbet* contained zerumbone (64.58%), diacetone alcohol (10.64%), α-humulene (8.93%), caryophyllene oxide (5.68%), humulene epoxide (3.18%), Eudesm-7(11)-en-4-ol (2.87%), aromadendrene oxide (1.98%), 14-hydroxy-α-Muurolene (0.91%), and octylcyclohexane (0.63%).[12] In another report, zingiberene (9%, 6%), β- bisabolene (4%, 5%), α-farnesne (11%, 7%), β-sesquiphellandrene (9%, 13%), α-curcumene (14%, 0%), gingerol (25%, 23%), and shogaol (18%, 25%) were identified in methanol and n-hexane extracts of *Z. officinale* rhizomes, respectively.[13]

Antioxidant Activity

The EtOAc fractionated extract of *Z. monophyllum* rhizomes was evaluated for its antioxidant activities *via* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays (Supplemental, Figures S11 and S12). As a result, the EtOAc fractionated extract showed moderate antioxidant effects, with an IC_{50} value of 1.862 ± 0.730 mg/mL in the DPPH assay using ascorbic acid as the reference drug (IC_{50} value of 0.015 ± 0.000 mg/mL) and an EC_{50} value of 1.519 mg/mL in the FRAP assay using ascorbic acid as the standard compound (IC_{50} value of 0.070 ± 0.000 mg/mL). Comparing to other ginger extracts, the EtOAc fractionated extract of *Z. monophyllum* rhizomes showed relatively smaller activities. Specifically, the high-pressurized CO_2 extract of ginger roots from Vietnam showed an IC_{50} of 0.64 μg/mL against DPPH free radicals.[14] In another study, the *n*-hexane and methanol extracts of ginger displayed the highest scavenging percentage of 82% to 88% against DPPH free radicals.[15] The smaller activities of the EtOAc fractionated extract of *Z. monophyllum* rhizomes than other ginger extracts could be

Table 1. Major Compounds Detected from the EtOAc Fractionated Extract of *Z. monophyllum* Rhizomes.

No	RT (min)	Compounds	RI (Cal.)	RI (Lit.)	Area (%)	Identification method
1	14.927	Alloaromadendrene	1453	1461	3.0	MS, RI
2	16.773	Spathulenol	1581	1576	16.8	MS, RI
3	17.174	Globulol	1617	-	6.5	MS, Co-GC
4	17.697	τ-Cadinol	1654	-	2.1	MS, Co-GC
5	17.907	Neointermedeol	1668	1660	11.9	MS, RI
6	18.199	Aromadendrene oxide-(2)	1688	1678	2.0	MS, RI
7	19.944	Ambrial	1817	1809	4.4	MS, RI
8	22.265	(*E*)-15,16-Dinorlabda-8(17),11-dien-13-one	2003	1994	1.2	MS, RI
9	26.644	(*E*)-Labda-8(17),12-diene-15,16-dial	2379	2383	21.8	MS, RI
		Total identified (%)			**69.7**	

Abbreviations: RT, retention time; RI(Cal.), retention indices on HP-5MS UI column; RI(Lit.): retention indices in the labrary; MS: mass spectrum; Co-GC: Co-injection with authentic compounds.
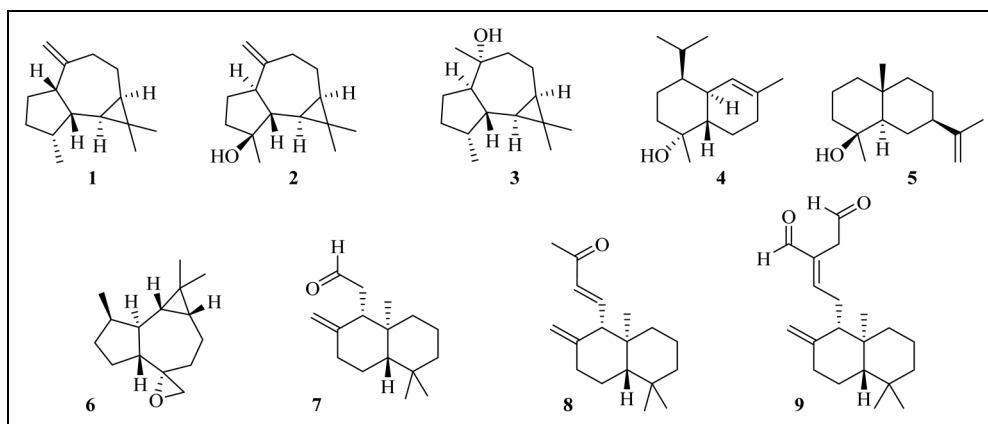
Figure 1. Chemical structures of nine main compounds identified from the EtOAc fractionated extract of *Z. monophyllum* rhizomes by GC-MS analysis.

explained based on the difference in extracted solvent and species. So far, there has been limited information on the antioxidant activity of *Z. monophyllum* rhizomes in the literature.

Antimicrobial Activity

The antimicrobial activity of the EtOAc fractionated extract of *Z. monophyllum* rhizomes was evaluated against pathogenic microorganisms, and the resulting IC_{50} values are presented in Table 2. This extract exhibited notable inhibitory activity against all examined microbial strains, except for *P. aeruginosa*. Notably, the EtOAc fractionated extract of *Z. monophyllum* rhizomes demonstrated significantly higher antibacterial and antiyeast activities when compared to the 2 drugs, streptomycin and cycloheximide, with IC_{50} values ranging from 4.89 to 5.34 μg/mL. Specifically, within the context of the antimicrobial assay, the EtOAc fractionated extract exhibited the most potent inhibition against *Staphylococcus aureus* ($IC_{50} = 4.89$ μg/mL), followed by *B. cereus* ($IC_{50} = 4.97$ μg/mL) and *C. albicans* ($IC_{50} = 4.98$ μg/mL). Regarding Gram-negative bacteria, *E. coli* and *Salmonella enterica* displayed similar sensitivity to the fractionated extract, with IC_{50} values of 5.23 and 5.18 μg/mL, respectively. However, no antibacterial activity against *P. aeruginosa* was observed. Kader et al[16] found that the crude ethanol extract of *Z. zerumbet* rhizomes and its fractions exhibited mild to moderate antimicrobial activity against 2 Gram-positive and 4 Gram-negative bacteria with MIC values of 128 to 256 μg/mL. In another study, various extracts of *Z. officinale* var. *officinale* and *Z. officinale* var. *rubrum* showed weak antimicrobial activity.[17] These findings may demonstrate *P. aeruginosa*'s relatively insensitive to the extract among the tested organisms, suggesting that Gram-positive bacteria tend to be more susceptible to the EtOAc fractionated extract than their Gram-negative counterparts.

Previous studies have reported a range of biological effects associated with the EtOAc fraction of *Stachys schtschegleevii* leaves and stems, including antibacterial, antifungal, and

Table 2. Antimicrobial Activity of the EtOAc Fractionated Extract of *Z. monophyllum* Rhizomes.

		IC$_{50}$ (μg/mL)		
Microbial strains		EtOAc fractionated extract	Streptomycin	Cycloheximide
Gram (+)	*Enterococcus faecalis* ATCC299212	5.34 ± 0.49	50.34 ± 0.29	-
	Staphylococcus aureus ATCC25923	4.89 ± 0.50	25.23 ± 1.25	-
	Bacillus cereus ATCC14579	4.97 ± 0.49	20.45 ± 1.25	-
Gram (-)	*Escherichia coli* ATCC25922	5.23 ± 0.98	9.45 ± 0.21	-
	Pseudomonas aeruginosa ATCC27853	-	68.67 ± 2.23	-
	Salmonella enterica ATCC13076	5.18 ± 0.13	45.67 ± 1.87	-
Yeast	*Candida albicans* ATCC10231	4.98 ± 0.49	-	10.46 ± 0.84

Note: Mean ± SD, n = 3

antioxidant activities, among others. Therefore, several EtOAc fractions of various plant sources have been identified as potential reservoirs of antimicrobial agents. For example, the EtOAc fraction obtained from *Ruta officinalis* flowers was demonstrated to exhibit in vitro antibacterial, antioxidant, anti-inflammatory, and analgesic activities, likely attributed to their rich polyphenolic content.[18] In the present study, the EtOAc fractionated extract was found to contain a substantial quantity of bioactive compounds, including spathulenol (16.8%), which has previously been reported to possess antimicrobial effects against *Mycobacterium tuberculosis*. Additionally, neointermedeol (11.9%), a compound also found in *Artemisia argyi* essential oil, exhibited antibacterial effects against *Listeria monocytogenes*,

E. coli, Proteus vulgaris, Salmonella enteritidis, and *Aspergillus niger.*[19] Consequently, these results are consistent with the robust antibacterial activities observed in the EtOAc fractionated extract derived from *Z. monophyllum* rhizomes in this study. Given these findings, further comprehensive investigations of *Z. monophyllum* are warranted to illuminate and substantiate its potential applications in the pharmaceutical field.

DFT-based Optimized Structures and Quantum Chemical Properties

The DFT analysis was performed on the identified compounds in the EtOAc fractionated extract of *Z. monophyllum* rhizomes. These chemical constituents are responsible for the antioxidant potential of *Z. monophyllum*'s rhizomes. Through DFT, various characteristics were determined, including the energies of frontier orbitals (highest occupied molecular orbital [HOMO] and lowest unoccupied molecular orbital [LUMO]), as well as several reactivity parameters such as dipole moment, electron affinity, ionization energy, hardness, softness, electronegativity, chemical potential, electrophilicity, electron-accepting power, and electron-donor power, which are significant tools to describe the reactivity, stability, and binding capacity of molecules.[20] The M052X/def2-TZVPP method was employed for these calculations (Supplemental, Table S1 and Figure S13).

Pi-electron delocalization is a crucial factor in the stabilization of molecules and the reactivity of donor-acceptor sites, as per theoretical understanding. The electron transfer characteristics of the HOMO indicate the tendency for intermolecular electron donation, whereas the LUMO reflects electron-accepting capability. A greater E_{HOMO} (corresponding to a lower ionization potential, I_o) and a lower E_{LUMO} (corresponding to a higher electron affinity, A) contribute to enhanced electron-donating ability and increased sensitivity to receiving electrons, respectively. Meanwhile, lower gap energy, indicating easier electron transfer, signifies improved antioxidant reactivity. Figure 2 illustrates that the HOMO and LUMO profiles of several molecules, such as **1**, **2**, **3**, **4**, **5**, and **6**, exhibit a relatively even distribution across their molecular structures. This suggests that these molecules possess greater flexibility for intermolecular interactions concerning charge transfer. On the other hand, the remaining compounds concentrate the electron density within specific regions of their molecular structures. However, the overall stability cannot be definitively determined since it also depends on the chemical/physical affinity of the ligand target.

Upon analyzing the detailed parameters, all structures (**1-9**) exhibit significant electronic stability due to their low E_{HOMO} values, which are below −7 eV (a commonly accepted threshold of below −5 eV).[21] Additionally, a higher energy gap (E_{gap}) indicates greater hardness and lower reactivity, resulting in increased molecule stability. The energy gaps of these components from the ethyl acetate fraction range from 6.5 to 9.8 eV (Figure 2), consistent with the findings of the EtOAc fraction of Black Ginger rhizomes studied using the same method.[21] Based on the energy gap results, the reactivity order of the major compounds is as follows: Alloaromadendrene > (*E*)-Labda-8(17),12-diene-15,16-dial > *(E)*-15,16-Dinorlabda-8(17),11-dien-13-one > Ambrial > τ-cadinol >
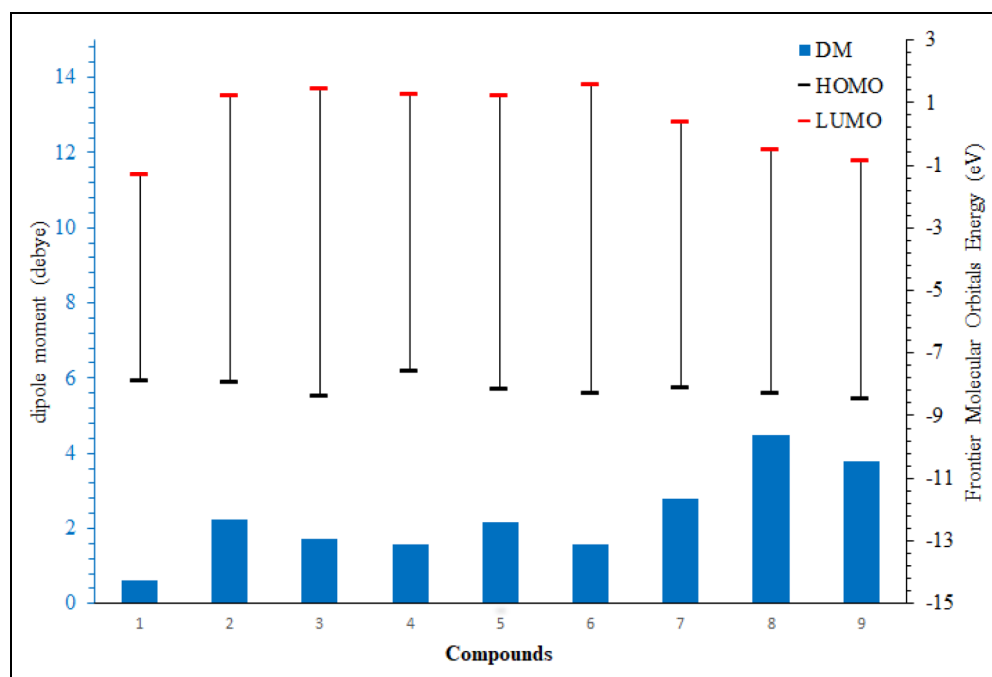

Figure 2. $E_{gap} = E_{LUMO} - E_{HOMO}$ and dipole moment of the structures 1–9. Abbreviations: LUMO, lowest unoccupied molecular orbital; HOMO, highest occupied molecular orbital.

Spathulenol > Neointermedeol > Globulol > Aromadendrene oxide-(2). Among the major compounds, Alloaromadendrene, *(E)*-Labda-8(17),12-diene-15,16-dial, *(E)*-15,16-Dinorlabda-8(17),11-dien-13-one, and Ambrial have higher chemical potentials compared to the other 5 phytochemicals. Alloaromadendrene exhibits the highest electron-donor power (ω^-) value, while τ-cadinol has the lowest among the molecules. In terms of dipole moment, the reactivity order of the main compounds is as follows: *(E)*-15,16-Dinorlabda-8(17),11-dien-13-one > *(E)*-Labda-8(17),12-diene-15,16-dial > Ambrial > Spathulenol > Neointermedeol > Globulol > τ-cadinol > Aromadendrene oxide-(2) > Alloaromadendrene.

Molecular Docking

Docking studies have been conducted in an endeavor to predict the antimicrobial mechanisms of action of test molecules. *Enterococcus faecalis* DNA gyrase B (PDB ID: 4GEE), *E. coli* DNA gyrase B (PDB ID: 4DUH), *S. aureus* biotin protein ligase (PDB ID: 3V7R), *E. faecalis* Alanine racemase (PDB ID: 3E6E), and *C. albicans* N-myristoyltransferase (PDB ID: 1IYK) have been selected as potential target proteins for antimicrobial activity. Based on available information in the RCSB Protein Data Bank, proteins 4GEE and 4DUH are essential enzymes that regulate the structural state of DNA binding during replication, while the binding strategy of the biotin ligase enzyme of *S aureus* as depicted by protein 3V7R suggests a potential avenue for selective inhibition.[22-24] As a constituent of the Alanine racemase family, 3E6E serves as a prevalent bacterial enzyme facilitating the interconversion between L- and D-alanine, critical precursors for cell wall peptidoglycan synthesis.[25] Myristoyl-CoA:protein N-myristoyltransferase (PDB ID: 1IYK) emerges as a promising target for antifungal drugs, recognized as a monomeric enzyme catalyzing the transfer of myristate from myristoyl-CoA to the N-terminal glycine residue of various viral and eukaryotic proteins.[26]

Verification prior to screening is executed to ensure the reliability and precision of the AutoDock Vina v1.2.3 program. Consequently, validation is established through the redocking of the inhibitory compound in the complex with the crystal structure of *E coli* DNA gyrase B, yielding a reasonable low root-mean-square deviation (RMSD) value (< 2 Å) of 0.461546 Å.[27] The co-crystallized structures of 4-[[4′-methyl-2′-(propanoylamino)-4,5′-bi-1,3-thiazol-2-yl]amino]benzoic are depicted in Figure S14 (Supplemental information). In this study, molecular docking analysis has been conducted to assess the interactions and binding affinities of the main compounds of the EtOAc fractionated extract from *Z. monophyllum* rhizomes against target proteins. Table 3 demonstrates binding energies, and Figures S15 to S19 (Supplemental information) depict the amino acid residues within the receptor with which the ligands interact.

As evident from Table 3, binding affinities range from −5.772 to −7.617 kcal/mol for the 4GEE protein, −4.511 to −6.85 kcal/mol for protein 4DUH, −5.04 to −7.293 kcal/

Table 3. The Binding Affinity of Main Compounds in the EtOAc Fractionated Extract with *Enterococcus faecalis* DNA gyrase B, *Escherichia coli* DNA gyrase B, *Staphylococcus aureus* Biotin Protein Ligase, *E. faecalis* Alanine Racemase, and *C. albicans* N-myristoyltransferase.

Compound	Antimicrobial protein target (kcal/mol)				
	4GEE	4DUH	3V7R	3E6E	1IYK
1	−5.772	−4.903	−6.505	−6.024	−6.995
2	−6.1	−5.278	−5.04	−6.602	−6.215
3	−6.374	−5.286	−5.127	−6.228	−6.726
4	−7.617	−4.69	−5.646	−6.717	−6.03
5	−7.168	−6.775	−6.164	−7.062	−6.744
6	−6.471	−6.85	−6.376	−7.216	−6.635
7	−6.187	−4.511	−6.221	−6.333	−6.996
8	−7.397	−5.516	−7.293	−8.194	−7.67
9	−7.324	−5.509	−5.76	−7.974	−7.441
Streptomycin	−7.102	−4.811	−5.363	−5.046	-
Cycloheximide	-	-	-	-	−8.094

mol for protein 3V7R, −6.024 to −8.194 kcal/mol for protein 3E6E, and −6.03 to −7.67 kcal/mol for protein 1IYK. Intriguingly, compound **8** exhibits the lowest binding affinity across 3 target proteins, namely *S. aureus* biotin protein ligase, *E. faecalis* Alanine racemase, and *C. albicans* N-myristoyltransferase, with respective values of −7.293, −8.194, and −7.670 kcal/mol. On the other hand, compounds **4** and **6** display strong affinities towards the target proteins DNA gyrase B from *E. faecalis* and *E. coli*, respectively. In comparison with the positive control compounds, streptomycin and cycloheximide, it is noteworthy that compounds **5**, **8**, and **9** all exhibit greater binding affinities than the standard streptomycin across all studied target proteins. This observation suggests that these compounds could potentially serve as key contributors to the potent antibacterial activity of the sample. Furthermore, interaction analysis within the ligand-receptor complex for *E. faecalis* DNA gyrase B has revealed that compound **9** forms 2 hydrogen bonds with Asn48 (2.55 Å) and Ser122 (2.62 Å), while compounds **2** and **3** establish a hydrogen bond at residues Glu52 (1.90 Å) and Asn48 (2.65 Å), respectively. Hydrogen bonding with residues Asn48 (2.14 Å) and Glu52 (2.04 Å) is observed in the streptomycin-protein 4GEE complex (Supplemental Figure S15). Furthermore, the pi-alkyl interactions of the studied compounds predominantly occur with amino acid residues Val96, Ile80, Pro81, Val169, Phe97, and Ile45. In the case of the bacterial DNA gyrase B from *E. coli*, compounds **6**, **7**, and **9** all form hydrogen bonds with Arg76 (3.08 Å), Gly117 (2.82 Å), and Asn46 (2.30 Å), respectively, whereas streptomycin displays multiple hydrogen bonds with Arg136 (2.35 Å), Gly101 (2.86 Å), Thr80 (2.49 Å), Gly81 (1.91 Å), and His83 (2.27 Å) (Supplemental Figure S16). Concerning the *S. aureus* biotin protein ligase 3V7R, hydrogen bonds are observed to form between compounds **2** and **7** with residue Lys187, compound **5** with residue His126 (2.19 Å), compound **8** with residue Arg125 (2.82 Å), and compound **9** with residue Arg227 (2.62 Å), whereas, in the case of

streptomycin, interactions are observed with Asp322, Arg227, and His126 (Supplemental, Figure S17). For *E. faecalis* Alanine racemase 3E6E, compounds **2**, **4**, **5**, and **9** form hydrogen bonds with the corresponding amino acid residue positions including Lys236 (2.55 Å), Tyr44 (2.00 Å), Asp174 (2.21 Å), and Tyr267 (3.03 Å) (Supplemental Figure S18). The interaction with residue Tyr267 forming a hydrogen bond with the molecule is also observed in the positive control streptomycin. In the context of *C. albicans* N-myristoyltransferase 1IYK, hydrogen bonds are observed with Thr211 (2.11 Å), Glu109 (3.06 Å), Asn392 (2.52 Å), and Asp412 (2.42 Å), corresponding to compounds **3**, **5**, **7**, and **9**, while the positive control cycloheximide establishes a hydrogen bond with Phe240 (Supplemental, Figure S19).

Conclusions

The current research marks the first concentrated effort to establish the volatile compositions of the EtOAc fractionated extract of *Z. monophyllum* through the GC-MS analysis. The research also demonstrated the antioxidant and antibacterial potential of the EtOAc fractionated extract of *Z. monophyllum* rhizomes. Nine compounds along with their relative content were identified, including alloaromadendrene, spathulenol, globulol, τ-cadinol, neointermedeol, aromadendrene oxide-(2), ambrial, *(E)*-15,16-dinorlabda-8(17),11-dien-13-one, and *(E)*-lambda-8 (17),12-diene-15,16-dial. The antioxidant screening revealed that the extract showed moderate antioxidant activity and this was supported by the DFT study. Furthermore, the extract exhibited remarkable antimicrobial activity except for *P. aeruginosa*. Molecular docking studies were also performed to display binding affinities of identified compounds with selected antibacterial target proteins. The isolation of these bioactive compounds and more in-depth studies to analyze their biological activities and conduct clinical trials for exploring and developing novel drug formulations will be carried out and reported in the future.

Materials and Methods

Chemicals and Reagents

Ascorbic acid (99%), Folin–Ciocalteu, 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), 24,6-Tris(2-pyridyl)-s-triazine, dimethyl sulfoxide (DMSO), n-hexane, ethyl acetate, methanol, streptomycin and cycloheximide were purchased from Sigma-Aldrich. All microorganisms used in this work were purchased from the National Institute for Food Control (Hanoi, Vietnam). All other chemicals were of the highest grade available.

Plant Material

Rhizomes of *Z. monophyllum* were collected from Kon Ka Kinh National Park, Gia Lai Province, Vietnam in December 2022. Identification of the plant material was performed by Assoc.

Prof. Dr Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam). A voucher specimen (No. G1LE) was deposited in the Herbarium of the Department of Chemistry, Vinh University, Vinh City, Nghean, Vietnam.

Preparation of Ethyl Acetate Fractionated Extract

Dried rhizomes of *Z. monophyllum* (4.5 kg) were crushed into coarse powder, and extracted with methanol (5 times, 6.0 L each) at room temperature. The combined methanol extracts were concentrated under reduced pressure to give a residue that was suspended in water and partitioned successively with hexane and ethyl acetate (EtOAc). The crude extracts were stored in a refrigerator (4 °C) to future analyses.

GC-MS Analysis

The GC-MS analysis was conducted with an Agilent 7890B GC coupled with an Agilent 7890B GC system, equipped with HP-5MS UI column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium was the carrier gas, at a flow rate of 1.5 mL/min. A volume of 1.0 μL of diluted samples (1000 ppm) was injected with a split ratio was 10:1. The oven temperature rose from 80 °C (1 min kept) up to 300 °C at the rate of 20 °C/min, then kept constant at 300 °C for 15 min. Mass spectra were recorded at 70 eV. The mass range was from 50 to 550 m/z (2.0 scan/s). The chemical components of the EtOAc fractionated extract were identified by comparison of their MS fragmentation patterns, retention indices with those in the literature (NIST17) and by co-injection with authentic compounds. The percentage of each component was calculated by comparing the average area of its peak to the total area of all the peaks.[28,29]

In Vitro Antioxidant Activity

The antioxidant activity of the EtOAc fractionated extract was determined using the DPPH method,[30] with ascorbic acid used as a positive control. The samples (0.1 mL) or negative control (ionized water) were mixed with the 3 mM DPPH solution (0.1 mL) and incubated for 30 min. The absorbance of the mixture was measured at 517 nm. The DPPH scavenging activity (%) was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)}$$
$$= [(A_{NC} - A_t) / A_{NC}] \times 100$$

where A_{NC} denoted the absorbance of the negative control and A_t represented the absorbance of the tested samples.

The ferric-reducing power was determined using the FRAP method in previous studies.[31,32] The working FRAP (240 mL) solution was mixed with the fractionated extract (10 mL) and incubated for 15 min. The absorbance was then spectroscopically measured at 593 nm. The reducing power was expressed

as an absorbance. The concentration of fractionated extract with an absorbance of 0.5 is known as EC_{50}.

In Vitro Antimicrobial Activity

E. coli ATCC 25922, *P. aeruginosa* ATCC 27853, *S. enterica* ATCC 13076, *E. faecalis* ATCC 299212, *S. aureus* ATCC 25923, *B. cereus* ATCC 14579, and *C. albicans* ATCC 10231 were purchased from the National Institute for Food Control (Hanoi, Vietnam). To assess the antibacterial and antifungal properties of the EtOAc fractionated extract of *Z. monophyllum* rhizomes, we followed the methodology outlined by Hadacek and Greger.[33]

Stock solutions of the EtOAc fractionated extract were prepared in 1% DMSO. In brief, the bacterial strains and yeast were cultured to achieve an approximate concentration of 2×10^5 CFU/mL. Subsequently, 50 μL of the bacterial or yeast culture was introduced into Luria-Bertani medium supplemented with various concentrations of the EtOAc fractionated extract, along with DMSO as a control. The mixtures were then incubated at 37 °C for a duration of 24 h. After the 24 h incubation period, we measured the optical density of the culture wells using a spectrophotometer (BioTeK Instruments, Inc., Highland Park) equipped with Rawdata software. The antimicrobial properties of the EtOAc fractionated extract were quantified in terms of Half-Maximal Inhibitory Concentration (IC_{50}), which represents the concentration of the EtOAc fractionated extract required to reduce cell growth by half after 24 h of incubation. As a positive control, bacterial and yeast cells were subjected to exposure to streptomycin and cycloheximide, respectively. All experimental procedures were performed in triplicate.

Statistical Analysis

All the experiments were conducted in triplicate. The results were represented as mean ± standard deviation. The statistical comparison was performed using *F*-test 2-sample (Microsoft Excel, Microsoft, 2018). *P* values less than .5 were considered significant.

Quantum Chemical Calculation

Using theoretical chemistry simulations, the bioactivity of compounds was connected with their molecular electronic structures in order to reveal probable interaction pathways with active sites in biological molecules.[34] The chosen compounds were subjected to geometric optimization using the Gaussian 09 software in the gas phase.[35] The frozen-core approximation for nonvalence-shell electrons with a greater basis set def2-TZVPP obtained single-point energies at M052X/6-311++G(d,p)-level optimized geometries. Frontier orbital analysis was carried out using NBO 5.1 at the level of theory M052X/def2-TZVPP.[36] Following that, the conformational analysis of the HOMO, LUMO, and molecular electrostatic potential surface were used to assess the local reactivity. The following relationships were used to determine molecular characteristics, including the HOMO energy (E_{HOMO}), LUMO energy (E_{LUMO}), band gap energy (E_{gap}), electron chemical potential (μ), softness (σ), hardness (η), electrophilicity (ω), and electronegativity (χ).[37,38]

$$E_{HOMO} = IE \tag{1}$$

$$E_{LUMO} = EA \tag{2}$$

$$\Delta E_{gap} = E_{LUMO} - E_{HOMO} \tag{3}$$

$$\mu = -\chi = \frac{E_{HOMO} + E_{LUMO}}{2} \tag{4}$$

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2} \tag{5}$$

$$\sigma = \frac{1}{\eta} \tag{6}$$

$$\omega = \frac{\chi^2}{2\eta} \tag{7}$$

Molecular Docking Simulation. To identify the manner of contact and binding energy via ligand-enzyme interaction that supports the biological activity (antimicrobial activity) of the main compounds in the EtOAc fractionated extract, a molecular docking study was employed. The PDB format files of *E. faecalis* DNA gyrase B, *E. coli* DNA gyrase B, *S. aureus* biotin protein ligase, *E. faecalis* Alanine racemase, and *C. albicans* N-myristoyltransferase target proteins were downloaded from the RCSB protein data bank (https://www.rcsb.org/), with PDB entries including 4GEE, 4DUH, 3V7R, 3E6E, and 1IYK, respectively.[20–24] The target proteins were then thoroughly prepared for established docking by adding missing hydrogen, Kollman charge, and energy minimization using the software AutoDockTools and Swiss-PdbViewer.[39] The generic docking technique was used in accordance with the default parameters, and the AutoDock Vina 1.2.3 program carried out the docking operation for the chosen proteins and compounds.[40–42] The docked pose with the highest binding affinity and most pronounced amino acid residue interactions for each docked ligand was chosen and visualized by the Discovery Studio Visualizer software.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

1. POWO. 2023. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet: http://www.plantsoftheworldonline.org/ Retrieved December 2023.

2. Tuan DQ, Dinh D, Tran TN, et al. Chemical composition of essential oil from the *Zingiber monophyllum* (Zingiberaceae) from Vietnam. *J Essent Oil-Bear Plants*. 2022;25(5):987-993. doi: 10.1080/0972060X.2022.2132835

3. Salehi B, Upadhyay S, Erdogan Orhan I, et al. Therapeutic potential of α- and β-Pinene: A miracle gift of nature. *Biomolecules*. 2019;9(11). doi: 10.3390/biom9110738

4. Liu S, Long Y, Yu S, et al. Borneol in cardio-cerebrovascular diseases: pharmacological actions, mechanisms, and therapeutics. *Pharmacol Res*. 2021;169:105627. doi: 10.1016/j.phrs.2021.105627

5. Dragomanova S, Tancheva L, Georgieva M. A review: Biological activity of myrtenal and some myrtenal-containing medicinal plant essential oils. *Scr Sci Pharm*. 2018;5(2). doi: 10.14748/ssp.v5i2.5614

6. Yousfi F, Abrigach F, Petrovic J, Sokovic M, Ramdani M. Phytochemical screening and evaluation of the antioxidant and antibacterial potential of *Zingiber officinale* extracts. *S Afr J Bot*. 2021;142:433-440. doi: 10.1016/j.sajb.2021.07.010

7. Aswati Nair R, Ganapathy G, Harshitha K. Polyphenolic characteristics, antimicrobial and antioxidant activity of *Zingiber neesanum* (graham) ramamoorthy rhizomes and identification of volatile metabolites by GC-MS analysis. *Nat Prod Res*. 2021;35(17):2947-2951. doi: 10.1080/14786419.2019.1677654

8. Deng M, Yun X, Ren S, Qing Z, Luo F. Plants of the genus Zingiber: a review of their ethnomedicine, phytochemistry and pharmacology. *Molecules*. 2022;27(9):2826. doi: 10.3390/molecules27092826

9. Viet TD, Xuan TD, Van TM, Andriana Y, Rayee R, Tran HD. Comprehensive fractionation of antioxidants and GC-MS and ESI-MS fingerprints of *Celastrus hindsii* leaves. *Medicines*. 2019;6(2). doi: 10.3390/medicines6020064

10. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int J Biol Sci*. 2015;11(8):982-991. doi: 10.7150/ijbs.12096

11. Xu DP, Li Y, Meng X, et al. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int J Mol Sci*. 2017;18(1). doi: 10.3390/ijms18010096

12. Das B, Sahoo A, Ray A, Jena S, Nayak S. Identification of chemical constituents of *Zingiber zerumbet* rhizome extract using GC/MS. *J Biol Act Prod Nat*. 2020;10(5):411-417. doi: 10.1080/22311866.2020.1821775

13. Hassan HA, Raauf AMS, Razik BM, Hassan BAR. Chemical composition and antimicrobial activity of the crude extracts isolated from *Zingiber officinale* by different solvents. *Pharm Anal Acta*. 2012;3(9):81000184. doi: 10.4172/2153-2435.1000184

14. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chem*. 2007;102(3):764-770. doi: 10.1016/j.foodchem.2006.06.023

15. El-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (Cuminum cyminum). *J Agric Food Chem*. 2010;58(14):8231-8237. doi: 10.1021/jf101202x

16. Kader G, Nikkon F, Rasshid MA, Yeasmin T. Antimicrobial activities of the rhizome extract of *Zingiber zerumbet* Linn. *Asian Pac J Trop Biomed*. 2011;1(5):409-412. doi: 10.1016/S2221-1691(11)60090-7

17. Philip K, Malek SNA, Sani W, et al. Antimicrobial activity of some medicinal plants from Malaysia. *Am J Appl Sci*. 2009;6(8):1613-1617. doi: 10.3844/ajassp.2009.1613.1617

18. Karadağ AE, Demirci B, Çaşkurlu A, et al. In vitro antibacterial, antioxidant, anti-inflammatory and analgesic evaluation of *Rosmarinus officinalis* L. flower extract fractions. *S Afr J Bot*. 2019;125:214-220. doi: 10.1016/j.sajb.2019.07.039

19. Guan X, Ge D, Li S, Huang K, Liu J, Li F. Chemical composition and antimicrobial activities of *Artemisia argyi* Lévl. et Vant essential oils extracted by simultaneous distillation-extraction, subcritical extraction and hydrodistillation. *Molecules*. 2019;24(3):483. doi: 10.3390/molecules24030483

20. Patial PK, Cannoo DS. Phytochemical profile, antioxidant potential and DFT study of *Araucaria columnaris* (G. Forst.) hook. Branch extracts. *Nat Prod Res*. 2021;35(22):4611-4615. doi: 10.1080/14786419.2019.1696330

21. Van Chen T, Cuong TD, Quy PT, et al. Antioxidant activity and α-glucosidase inhibitability of *Distichochlamys citrea* MF Newman rhizome fractionated extracts: *in vitro* and *in silico* screenings. *Chem Pap*. 2022;76(9):5655-5675. doi: 10.1007/s11696-022-02273-2

22. Tari LW, Trzoss M, Bensen DC, et al. Pyrrolopyrimidine inhibitors of DNA gyrase B (GyrB) and topoisomerase IV (ParE). part I: structure guided discovery and optimization of dual targeting agents with potent, broad-spectrum enzymatic activity. *Bioorganic Med Chem Lett*. 2013;23(5):1529-1536. doi: 10.1016/j.bmcl.2012.11.032

23. Brvar M, Perdih A, Renko M, Anderluh G, Da T, Solmajer T. Structure-based discovery of substituted 4, 5′-bithiazoles as novel DNA gyrase inhibitors. *J Med Chem*. 2012;55(14):6413-6426. doi: 10.1021/jm300395d

24. Da Costa TPS, Tieu W, Yap MY, et al. Selective inhibition of biotin protein ligase from *Staphylococcus aureus*. *J Biol Chem*. 2012;287(21):17823-17832. doi: 10.1074/jbc.M112.356576

25. Priyadarshi A, Lee EH, Sung MW, et al. Structural insights into the alanine racemase from *Enterococcus faecalis*. *Biochim Biophys Acta Bioenerg*. 2009;1794(7):1030-1040. doi: 10.1016/j.bbapap.2009.03.006

26. Sogabe S, Masubuchi M, Sakata K, et al. Crystal structures of *Candida albicans* N-myristoyltransferase with two distinct inhibitors. *Chem Biol*. 2002;9(10):1119-1128. doi: 10.1016/s1074-5521(02)00240-5

27. Tuan NA, Khanh PN, Ha NX, Binh TC, Khanh ND, Oanh TT. Compounds isolated from *Lawsonia inermis* L. Collected in Vietnam and evaluation of their potential activity against the main protease of SARS-CoV-2 using in silico molecular docking and molecular dynamic simulation. *Nat Prod Commun*. 2022;17(10): 1934578X221125161. doi: 10.1177/1934578X221125161

28. Tran-Trung H, Thang TD, Nguyen THD, et al. Essential oils from the trunks and leaves of *Paramignya scandens* (griff.) craib from Vietnam: phytochemical composition, *In vitro* α-amylase and tyrosinase inhibitory activities and in silico molecular docking studies. *Nat Prod Commun*. 2023;18(12):1934578X231222383. doi: 10.1177/1934578X231222383

29. Tran-Trung H, Dau XD, Nguyen TC, et al. Phytochemical analysis of the essential oils from the rhizomes of three Vietnamese *Curcuma* Species and their antimicrobial activity. *Nat Prod Commun*. 2023;18(4):1934578X231167229. doi: 10.1177/1934578X231167229

30. Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol*. 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5

31. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239(1):70-76. doi: 10.1006/abio.1996.0292

32. Nguyen Hoang T, Tran-Trung H, Giang LD, et al. *Alpinia nelumboides* Nob. Tanaka, TTK Van & V. Hoang: phytochemical analysis and antioxidant activities of pseudo-stem and rhizome essential oils. 2023:1-7. doi: 10.1080/14786419.2023.2256021

33. Hadacek F, Greger H. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochem Anal*. 2000;11(3):137-147. doi: 10.1002/(SICI)1099-1565(200005/06)11:3 < 137::AID-PCA514 > 3.0.CO;2-I

34. Marković ZS, Dimitrić Marković JM, Milenković D, Filipović N. Mechanistic study of the structure–activity relationship for the free radical scavenging activity of baicalein. *J Mol Model*. 2011;17:2575-2584. doi: 10.1007/s00894-010-0942-y

35. Frisch MJ. Trucks GW, Schlegel HB, et al. *Gaussian 09, Revision B.01, Gaussian 09, Revis. B.01*. Gaussian, Inc; 2009.

36. Weigend F, Ahlrichs R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to rn: design and assessment of accuracy. *Phys Chem Chem Phys*. 2005;7(18):3297-3305. doi: 10.1039/B508541A

37. Van Hue N, Cuong TD, Quy PT, et al. Antimicrobial properties of *Distichochlamys citrea* MF newman rhizome n-hexane extract against *Streptococcus pyogenes*: experimental evidences and computational screening. *ChemistrySelect*. 2022;7(17):e202200680. doi: 10.1002/slct.202200680

38. Oucheikh L, Ou-Ani O, Moujane S, Ansari A, Oubair A, Znini M. Chemical composition, in vitro antifungal activity, DFT, molecular docking and molecular dynamics simulation studies of the essential oil from *Anvillea gracinii* subsp. *radiata* (Coss. & Durieu) Anderb. *J Essent Oil Res*. 2023;35(1):35-50. doi: 10.1080/10412905.2022.2109767

39. Johansson MU, Zoete V, Michielin O, Guex N. Defining and searching for structural motifs using DeepView/Swiss-PdbViewer. *BMC Bioinform*. 2012;13:1-11. doi: 10.1186/1471-2105-13-173

40. Trott O, Olson A. Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455-461. doi: 10.1002/jcc.21334

41. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. Autodock Vina 1.2. 0: new docking methods, expanded force field, and python bindings. *J Chem Inf*. 2021;61(8):3891-3898. doi: 10.1021/acs.jcim.1c00203

42. Pham TV, Ha NX, Luyen ND, et al. Chemical composition, mosquito Larvicidal and antimicrobial activities, and molecular docking study of essential oils of *Cinnamomum melastomaceum*, *Neolitsea buisanensis* and *Uvaria microcarpa* from Vietnam. *Chem Biodivers*. 2023;20(9):e202300652. doi: 10.1002/cbdv.202300652