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Original Research Article

Chemical Composition, Biological Activities, and Docking Studies of Essential Oil from *Eupatorium odoratum* L. Collected in Dak Lak, Vietnam

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ABSTRACT

Eupatorium odoratum L. (Asteraceae), also known as Siam weed, is widely used to stop bleeding and treat diarrhea. However, studies on the chemical composition and biological properties of essential oil from individuals of this species collected in Dak Lak, Vietnam have not been investigated. The chemical composition of *E. odoratum* essential oil was analyzed using gas chromatography-mass spectrometry (GC–MS), while the antioxidant and antibacterial capacity of *E. odoratum* essential oil were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the agar disc diffusion methods, respectively. Molecular docking was used to study the binding energy between major constituents of the essential oil and *Escherichia coli* DNA (deoxyribonucleic) gyrase subunit B (GyrB). An essential oil yield of 0.61% was obtained, with 51 compounds identified. Among them, the major constituents were germacrene D (20.59%), caryophyllene oxide (12.10%), estragole (11.01%), caryophyllene (9.52%), geijerene (8.21%), cadinene (5.96%), guaia-6,9-diene (4.49%), 3-ethenylcyclopentene (3.54%), and copaen (3.08%). The antioxidant efficiency of the essential oil was determined, with an IC_{50} of 5.8 ± 0.3 µg/mL. In the antibacterial test, *E. odoratum* essential oil demonstrated strong antibacterial activity against *E. coli* (ATCC 25922), with an inhibition zone of 16.4 ± 0.4 mm and a lowest minimum inhibitory concentration (MIC) value of 4.2 ± 0.5 mg/mL. Molecular docking studies revealed binding energy between the essential oil's major constituents and GyrB ranges of −5.0 to −6.5 kcal/mol, predominantly involving hydrophobic interactions with active site residues. These findings indicate that *E. odoratum* collected in Dak Lak, Vietnam, possesses a high natural potential for pharmaceutical applications.

Keywords: *Eupatorium odoratum*, Essential oil, Antioxidant, Antibacterial, Molecular docking.

Introduction

The use of herbs in treatment is rising.[1] This includes the medicinal use of *Eupatorium odoratum* L. (synonyms: *Chromolaena odorata* King & Robinson), a member of the Asteraceae family. *E. odoratum* is an herbaceous plant that can grow up to 2 meters or taller. The branches usually grow horizontally; the leaves are opposite and ovate, with toothed edges, and the petioles are about 1 to 2 centimeters long. The plant flowers in late winter and early spring.[2,3] The achene is spindle-shaped, five-angled, and hairy. This plant adapts well to growing conditions, grows vigorously during the rainy season, and has strong regeneration ability.[4] In Vietnam, it is commonly found in mountainous, midland, and lowland areas, and is primarily used to stop bleeding and treat diarrhea.[5] The chemical composition of the plant includes protein (2.65%), potassium (2.48%), phosphorus (0.5%), essential oils, alkaloids, and tannins.[6] According to traditional medicine, *E. odoratum* has a slightly spicy taste, a mild odor, and a warm nature. It is used to treat bone pain, colitis, gingivitis, scabies, ulcers, boils, acute dysentery, and diarrhea in children.[7]

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It has antioxidant, anti-toxic, anti-purulent, antihemorrhagic, anti-inflammatory, anti-ulcer, and antibacterial effects.[1,8,9] *E. odoratum* essential oil shows great potential for medicinal use.[10] There have been several publications about the chemical composition of *E. odoratum* essential oil grown in Europe, Asia, and Africa, but mainly in Mexico, the West Indies, and tropical South America.[11,12] In Vietnam, the chemical composition of *E. odoratum* essential oil has also been studied.[3,5,7,13] However, no study has been conducted on the chemical composition and biological activity of the essential oil of *E. odoratum* collected in Buon Ma Thuot City, Dak Lak Province. Therefore, this study aims to determine the chemical composition and biological properties of *E. odoratum* essential oil collected in Buon Ma Thuot City, Dak Lak Province, Vietnam, to aid in its exploitation and effective utilization.

Materials and Methods

Chemicals

Butylated hydroxytoluene (BHT), DPPH, Tween 80, a homologous series of C7–C30 straight-chain hydrocarbons, and various reference chemicals for identification were procured from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals, including those of analytical grade, were acquired from Merck (Darmstadt, Germany). The culture media and standard antibiotic discs were obtained from Oxoid Ltd. (Basingstoke, Hampshire, UK).

Plant Material

Leaves and stems of *E. odoratum* were collected from Tan Tien commune (12°40′34″N 108°2′7″E), Buon Ma Thuot City, Dak Lak

Province, Vietnam in January 2023. To serve as a reference, a voucher specimen (No: CL-BMT-01) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City.

Essential Oil Extraction
Leaves and stems of *E. odoratum* were cleaned, cut into smaller pieces, and subjected to steam distillation using a Clevenger-type apparatus for 4 hours. The obtained essential oil was dehydrated with anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark before analysis.

Essential Oil Analysis
Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil from the leaves and stems of *E. odoratum* was conducted using a Thermo Trace GC Ultra - ITQ900 system (Thermo Fisher Scientific, MA, USA). Data interpretation was performed using MassFinder 4.0 software. Separation was achieved using a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

GC Operation Conditions
The GC operation conditions included an injector temperature of 250 °C, a detector temperature of 260 °C, and an oven temperature program from 60 to 260 °C at a heating rate of 4 °C/min. Helium served as the carrier gas at a flow rate of 1.0 mL/min. An oil sample (1 μL) was injected using the split mode with a split ratio of 1:10.

MS Operation Conditions
The mass spectrometer was operated in electron-impact (EI) mode, with an ionization energy of 70 eV, interface temperature of 280 °C, ion source temperature of 230 °C, MS quadrupole temperature of 200 °C, and scan range of 35–650 amu. The GC operation conditions were identical to those described above in "GC Operation Conditions".

Identification and Quantification of Essential Oil Constituents
The retention indices of the oil constituents were determined on an HP-5 MS column using standard C7–C30 straight-chain hydrocarbons (Sigma-Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparing their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09th Version) and/or with those reported in the literature. The relative percentages of the separated compounds were computed from GC data without the use of correction factors.

Antioxidant Activity
The antioxidant activity of the *E. odoratum* essential oil was assessed using the DPPH assay.[14] Different concentrations of the extract in methanol and positive control, BHT, were mixed with 200 μL of a methanolic solution containing DPPH radicals at a concentration of 150 μmol/L. The resulting mixtures were then vigorously shaken and allowed to stand for 30 minutes in the dark for the reactions to run to completion. Subsequently, the absorbance of the solutions was measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm against a blank (a control solution with no extract or BHT). Each test was performed in triplicate to maintain accuracy and reliability. The scavenging ability of the *E. odoratum* essential oil was calculated using the equation below:

$$Scavenging\ acbility\ (\%) = \frac{A_{517}\ of\ control - A_{517}\ of\ sample}{A_{517}\ of\ control}\ x\ 100 \quad (1)$$

Antimicrobial Activity
The antibacterial activity of the *E. odoratum* essential oil against a Gram-negative strain – *E. coli* (ATCC 25922) – obtained from laboratory stock cultures was evaluated using the agar disc diffusion method. A liquid culture of *E. coli* (at a concentration of 10^7 colony-forming units per milliliter, CFU/mL) was spread evenly on a solid medium in a Petri dish. Circular pieces of filter paper with a diameter of 6 mm were placed in the center of each dish. The essential oil from *E. odoratum* was extracted through steaming and then dissolved in 10% dimethyl sulfoxide (DMSO); 40 μL of the essential oil was then applied to each piece of filter paper, using 10% DMSO as a negative control. The Petri dishes were then sealed and incubated. The diameter of the inhibition zone formed around each filter paper was measured and used as an indicator of antimicrobial activity, and the entire assay was conducted in triplicate to maintain accuracy. The MIC was defined as the lowest concentration of the *E odoratum* essential oil that visibly inhibited the growth of the bacteria,[14] and thus served as a measure of the potency of the essential oil as an antimicrobial agent against *E. coli*. The essential oil was dissolved in ethanol, and two-fold serial dilutions were carried out in a 96-well plate to yield a concentration range of 1.0 to 10.0 mg/mL. A bacterial broth medium (20 μL) was added to each well to produce the different solution concentrations. The pH of the medium was adjusted to a value in the range of 7.4 to 7.6, and the microplates were incubated at 37 °C for 24 hours. Each assay was performed in triplicate to ensure the reliability and accuracy of the results.

Molecular Docking and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) Predictions of Studied Compounds
The crystal structure of *E. coli* DNA gyrase subunit B (GyrB; PDB ID: 6F86) is available on RSCB Protein Data Bank and was downloaded. All non-standard residues were removed using UCSF Chimera 1.17.3. Polar hydrogen atoms and Gasteiger charges were added to the proteins using the "Dock Prep" tool in UCSF Chimera. The PubChem CID of six studied compounds (geijerene, estragole, caryophyllene, germacrene D, cadinene, and caryophyllene oxide), along with that of the reference compound (ciprofloxacin), were pasted to the "Build Structure" tool in UCSF Chimera for ligand formation (ligands included geijerene, estragole, caryophyllene, germacrene D, cadinene, and caryophyllene oxide, and ligand REF, respectively). Ligand preparation was conducted using the "Dock Prep" tool in UCSF Chimera, where hydrogen atoms were added and Gasteiger charges were assigned to the ligands. The energy minimization of the ligands was executed with the "Minimize Structure" tool in UCSF Chimera. The prepared proteins and ligands were saved in PDB format.
In this study, AutoDock Vina 1.2.5, which had been integrated into UCSF Chimera, was used for the molecular docking process. DoGSiteScorer was employed to investigate the binding active sites of the proteins.[15] These binding pockets were ranked and chosen based on the criteria of highest score and probability. To cover the binding active sites, the grids were centered on an area that includes all the residues highlighted by the DoGSiteScorer. The best conformers were searched for using the Broyden-Fletcher-Goldfarb-Shanno algorithm. For each ligand, the number of conformers was set at a maximum of 10 during the molecular docking process. Default parameters of AutoDock Vina were selected for the docking performance. After the docking process, the conformers were ranked according to their binding energy with the proteins; the selection was performed on the lowest binding energy among all generated conformers. All the AutoDock Vina docking performances were run under Windows 10 Pro operating system, on the 2.53 GHz Intel Core i5 processor. All studied compounds were screened based on "Lipinski's rule of five".[16] The SwissADME web tool was used to calculate data relating to the pharmacokinetics of the compounds.[17] DL-AOT Prediction Server was used for acute oral toxicity prediction.[18]

Statistical Analysis
All treatments were carried out in triplicate, and the data were subjected to statistical analysis using analysis of variance (ANOVA) in Statistica 5.5 software (Stat Soft Inc., Tulsa, OK, USA). The results are presented as the mean ± standard deviation (SD).

Results and Discussion

Chemical Composition of E. odoratum Essential Oil
The essential oil from the leaves and stems of *E. odoratum* was obtained in a yield of 0.61% (w/w, fresh weight) via hydrodistillation. The total ion chromatogram was obtained from the GC-MS analysis of the oil (Figure 1); the detailed composition is provided in Table 1. A total of 51 compounds were identified in the essential oil (accounting for 100% of the overall oil composition) by comparing their mass spectra and retention indices with reference data from GC-MS libraries (Supplementay Information).

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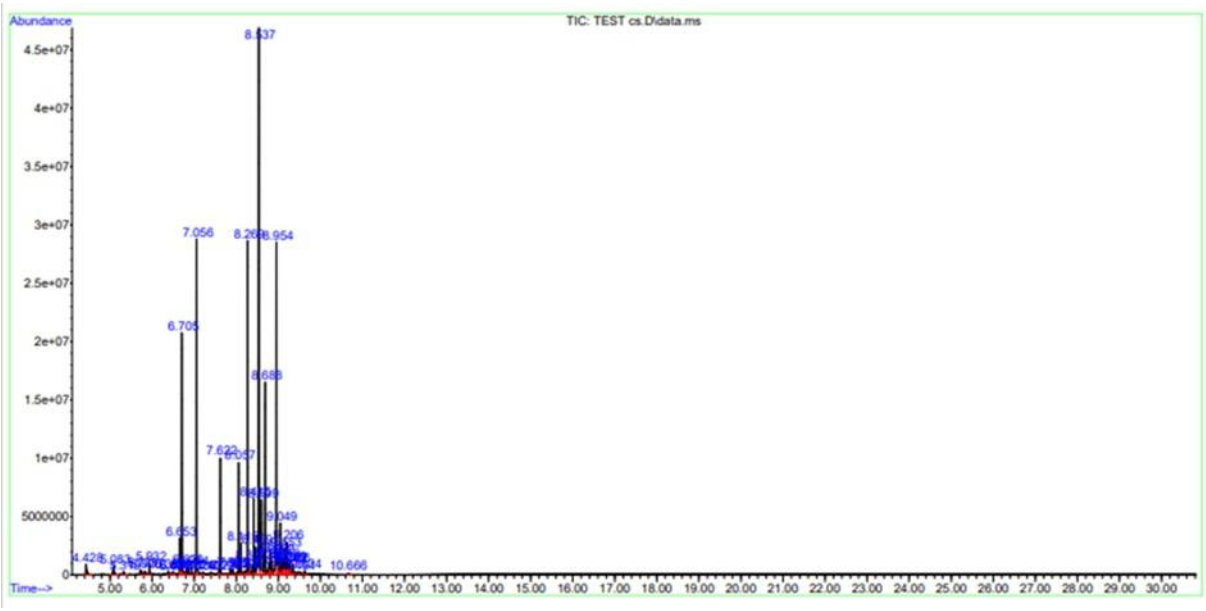

Figure 1: GC-MS total ion chromatogram of *E. odoratum* essential oil

Table 1: Chemical compositions of the essential oil of *E. odoratum* leaves and stems

Peak	Retention time (min)	Compounds	Molecular fomular	Relative amount (%)
1	4.429	*α*-Pinene	$C_{10}H_{16}$	0.61
2	5.082	*β*-Pinene	$C_{10}H_{16}$	0.52
3	5.319	*β*-Myrcene	$C_{10}H_{16}$	0.13
4	5.729	D-Limonene	$C_{10}H_{16}$	0.38
5	5.818	Benzeneacetaldehyde	C_8H_8O	0.25
6	5.933	*β*-Ocimene	$C_{10}H_{16}$	0.42
7	6.387	3-Carene	$C_{10}H_{16}$	0.17
8	6.494	Fenchol	$C_{10}H_{18}O$	0.14
9	6.514	2-methyl-6-methylene-1,7-octadien-3-one	$C_{10}H_{14}O$	0.07
10	6.654	Tricyclo[2.2.1.0(2,6)]heptane	$C_{12}H_{18}$	1.08
11	6.704	Geijerene	$C_{12}H_{18}$	8.21
12	6.754	1-ethenyl-4-methoxybenzene	$C_9H_{10}O$	0.12
13	6.819	Pinocarvone	$C_{10}H_{14}O$	0.32
14	6.874	2-methyl-1,3,5-hexatriene	C_7H_{10}	0.22
15	6.943	Terpinen-4-ol	$C_{10}H_{18}O$	0.30
16	7.056	Estragole	$C_{10}H_{12}O$	11.01
17	7.136	Bicyclo[3.1.1]hept-3-en-2-one	$C_{10}H_{14}O$	0.16
18	7.210	9-(oxabicyclo[3.3.1]non-6-en-3-yl)methanol	$C_9H_{12}O_2$	0.08
19	7.402	Tricyclo[4.1.0.0(2,7)]heptane	C_7H_{10}	0.11
20	7.622	3-ethenylcyclopentene	C_6H_{10}	3.54
21	7.727	1,2,3,4-tetrahydro-1,4-dimethylnaphthalene	$C_{12}H_{16}$	0.08
22	7.857	4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)cyclohexene	$C_{15}H_{24}$	0.18
23	7.919	Eugenol	$C_{10}H_{12}O_7$	0.22
24	8.059	Copaene	$C_{15}H_{24}$	3.08
25	8.116	1-ethenyl-1-methyl-2,4-*bis*(1-methylethenyl)cyclohexane	$C_{15}H_{24}$	0.99

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26	8.191	1a,2,6,7,7a,7b-hexahydro-1,1,7,7a-tetramethyl-1*H*-cyclopropa[a]naphthalene	$C_{15}H_{22}$	0.11
27	8.236	1,7-dimethyl-1,3,7-Cyclodecatriene	$C_{12}H_{18}$	0.19
28	8.268	Caryophyllene	$C_{15}H_{24}$	9.52
29	8.306	*α*-Farnesene	$C_{15}H_{24}$	0.55
30	8.415	Humulene	$C_{15}H_{24}$	2.51
31	8.458	4-(2-butenyl)-1,2-dimethylbenzene	$C_{12}H_{16}$	0.92
32	8.538	Germacrene D	$C_{15}H_{24}$	20.59
33	8.600	Guaia-6,9-diene	$C_{15}H_{24}$	4.49
34	8.687	Cadinene	$C_{15}H_{24}$	5.96
35	8.732	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene	$C_{15}H_{24}$	0.14
36	8.785	4-ethenyl-4-trimethyl-3-(1-methylethenyl)cyclohexanemethanol	$C_{15}H_{26}O$	0.67
37	8.820	3-methyl-6-(1-methylethenyl)cyclohexene	$C_{10}H_{16}$	0.85
38	8.852	1,5-dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene	$C_{15}H_{24}$	0.44
39	8.954	Caryophyllene oxide	$C_{15}H_{24}O$	12.10
40	8.989	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)naphthalene	$C_{15}H_{24}$	0.79
41	9.049	1-nitro-bicyclo[6.1.0]nonan-2-one	$C_9H_{13}NO_3$	2.02
42	9.111	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene	$C_{15}H_{24}$	0.63
43	9.154	2-isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene	$C_{15}H_{24}$	1.50
44	9.206	τ-Muurolol	$C_{15}H_{26}O$	1.34
45	9.244	4-isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene	$C_{15}H_{20}$	0.46
46	9.269	Aromandendrene	$C_{15}H_{24}$	0.45
47	9.291	4-ethenyl-4,8,8-trimethyl-2-methylenebicyclo[5.2.0]nonane	$C_{15}H_{24}$	0.54
48	9.356	Tetradecanal	$C_{14}H_{28}O$	0.42
49	9.491	2-pentadecen-4-yne	$C_{14}H_{22}O$	0.14
50	9.623	4-methylene-2,8,8-trimethyl-2-vinylbicyclo[5.2.0]nonane	$C_{15}H_{24}O$	0.22
51	10.666	Phytol	$C_{20}H_{40}O$	0.06
Total number of constituents			51	
Number (%) of constituents identified			51 (100.00%)	
Number (%) of monoterpenoids			14 (15.30%)	
Number (%) of sesquiterpene			22 (67.26%)	
Number (%) of diterpenoids			1 (0.06%)	
Number (%) of nitrogen compounds			1 (2.02%)	
Number (%) of different compounds			13 (15.36 %)	

The peak numbers in the chromatogram were used as a notation system to identify each compound and facilitate further discussions within this report. The essential oil was predominantly composed of terpenes, including 14 monoterpenoids (accounting for 15.30% of the total oil) and 22 sesquiterpenoid compounds (the most abundant group, constituting 67.26% of the total oil). A further 13 compounds were identified, accounting for 15.36% of the total oil. The major components of the essential oil, along with their respective percentages, include germacrene D (20.59%), caryophyllene oxide (12.10%), estragole (11.01%), caryophyllene (9.52%), geijerene (8.21%),

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cadinene (5.96%), guaia-6,9-diene (4.49%), 3-ethenylcyclopentene (3.54%), and copaen (3.08%). These represent the main constituents of *E. odoratum* essential oil and provide valuable insights into its potential applications and properties.

Antioxidant Activity of E. odoratum Essential Oil

The antioxidant activity of *E. odoratum* essential oil was evaluated using the DPPH radical scavenging assay, as presented in Table 2. The IC_{50} value of *E. odoratum* essential oil was 5.8 ± 0.3 µg/mL, while that of BHT was 6.0 ± 0.2 µg/mL. This implies that *E. odoratum* essential oil collected in Dak Lak exhibits greater antioxidant activity compared to BHT, which is a well-known antioxidant.

Antibacterial Activity of E. odoratum Essential Oil

Table 2 presents the antimicrobial activity of *E. odoratum* essential oil against *E. coli* bacteria. The essential oil of *E. odoratum* exhibited strong antimicrobial activity against *E. coli* under the inhibition zone (16.4 ± 0.4 mm) and lowest MIC value (4.2 ± 0.5 mg/mL) testing. In this study, ciprofloxacin, used as a positive control, had an inhibition zone of 35.4 ± 0.2 mm and the lowest MIC value (0.09 ± 0.1 mg/mL) (Table 2). Overall, *E. odoratum* essential oil showed great activity against the *E. coli* bacterial strain.

Table 2: Antioxidant and antibacterial activities of *E. odoratum* essential oil

Sample	Antioxidant activity (IC_{50}, µg/mL)	Antibacterial activity	
		IZD (mm)	MIC (mg/mL)
DMSO		6.2 ± 0.1	-
Essential oil	5.8 ± 0.3	16.4 ± 0.4	4.2 ± 0.5
BHT	6.0 ± 0.2		
Ciprofloxacin		35.4 ± 0.2	0.09 ± 0.1

The values were expressed as mean values ± S.D of three parallel measurements; (-): Not test; BHT: Positive control for antioxidant activity; Ciprofloxacin: Positive control for antibacterial activity; IZD: inhibition zone diameters.

Interaction and Binding Affinity Between the Studied Compounds and the Binding Active Ste of GyrB Enzyme

E. coli DNA gyrase subunit B (GyrB), a subunit of DNA gyrase, is an essential enzyme in bacteria and a type II topoisomerase that introduces negative supercoils into DNA. This process is crucial for DNA replication, transcription, and recombination. DNA gyrase is a heterotetramer composed of two GyrA and two GyrB subunits.[19,20] The GyrB subunit is responsible for *adenosine triphosphate* (ATP) binding and hydrolysis, providing the energy required for the supercoiling process. In *E. coli*, the interaction between the GyrB enzyme and the bacterium is fundamental to its ability to replicate and maintain its DNA, as DNA supercoiling is essential for compacting the bacterial chromosome to fit inside the cell and for various DNA metabolic processes. Negative supercoiling also helps unwind the DNA helix, facilitating the progression of the replication fork and the transcription machinery. Without the activity of DNA gyrase, including the GyrB subunit, *E. coli* would struggle to replicate its DNA and transcribe genes efficiently. The GyrB subunit is the target of antibiotics such as aminocoumarins and quinolone.[21] These antibiotics inhibit the enzyme's activity, preventing bacterial DNA replication and cell death.[17] Although gyrase inhibitors currently exist, their use is impeded by high levels of antibiotic resistance and instances of drug toxicity. To investigate the ability of *E. odoratum* essential oils to kill *E. coli*, GyrB was chosen as a target protein for the *in silico* docking of compounds found in this oil. This target may reveal the mechanism of action of these substances against the growth and development of *E. coli*. The best-docked poses of the ligands are displayed in Figure 2, illustrating their interactions with the binding active site of the protein. The free binding energies of the ligands were also calculated in AutoDock Vina, providing the basis for assessing the affinity of the ligands toward the GyrB target (Table 3). All studied compounds showed potential as moderate inhibitors since these ligands formed some hydrophobic interactions with residues in the active binding site of the GyrB enzyme.

Table 3: Docking results toward GyrB

Ligand	Compound	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic interaction
1	Geijerene	–5.0		Arg76, Ile78
2	Estragole	–5.1	Glu50	Glu50, Arg76, Ile78, Val167
3	Caryophyllene	–5.5		Ala53, Ile78, Pro79, Ile94
4	Germacrene D	–6.5		Ile78, Ile94
5	Cadinene	–5.6		Ile78, Ile94
6	Caryophyllene oxide	–5.2	Asn46	Ala53, Ile78, Pro79, Ile94
REF	Ciprofloxacin	–7.2	Asn46, Asp73, Arg76, Thr165	Ile78

REF: reference compound.

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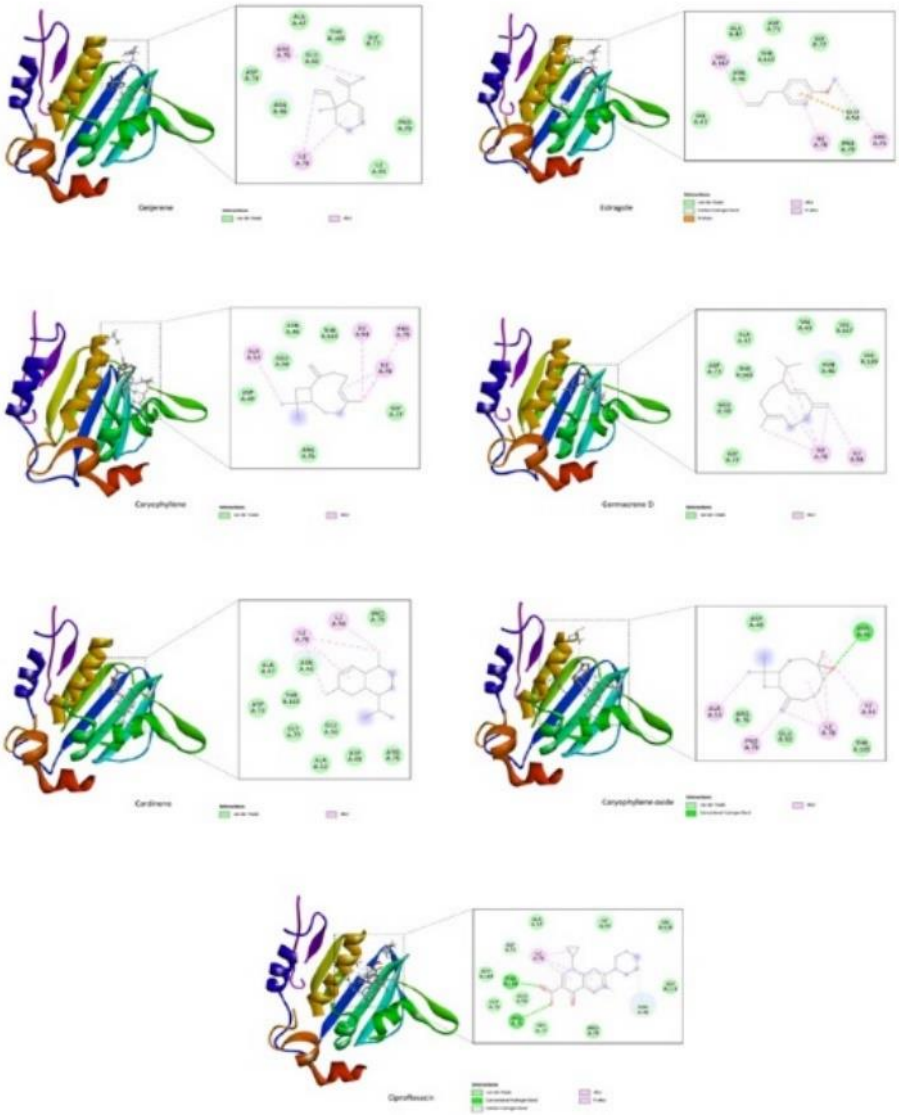

Figure 2: Compounds and GyrB interactions

All studied essential oil compounds' free binding energies towards the GyrB enzyme were ranked from –6.5 to –5.0 kcal/mol, while that of ciprofloxacin was –7.2 kcal/mol. Among the six studied compounds, germacrene D appeared to be the strongest inhibitor of the GyrB enzyme, with a free binding energy value of –6.5 kcal/mol. Notably, Ile78 formed hydrophobic interactions with all studied compounds.

ADMET Predictions of the Studied Compounds

The classic "Lipinski's Rule of Five" has traditionally served as a criterion for assessing a compound's druggability. In this study, no major components in the essential oil of *E. odoratum* have a mass exceeding 500 Daltons. All major components also exhibit fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and log P values smaller than 5, meaning they do not violate the classic Lipinski's Rule of Five. Additionally, we evaluated the number of rotatable bonds, topological polar surface area (TPSA), and aqueous solubility (log S) as physicochemical parameters. To ensure good oral bioavailability and intestinal absorption, the number of rotatable bonds should not exceed 10, and the TPSA value should stay below 140 $Å^2$.[22] Comprehensive data on these compounds are provided in Table 4, illustrating good physicochemical properties.

Furthermore, Table 5 presents *in silico* predictions of the ADME properties of the studied compounds. These compounds were predicted not to be P-gp substrates, except for the reference compound of ciprofloxacin. Several cytochrome P enzymes play a crucial role in drug biotransformation, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. All six major components were predicted not to inhibit CYP2D6 and CYP3A4.

The Log(LD_{50}) values were calculated using the DL-AOT Prediction Server and found to be between 3.18 and 3.75 (Table 6). Based on these predicted results, all studied compounds were classified as "Caution".

E. odoratum essential oil is known to have great potential in treating diseases.[12] Therefore, prior research on this species has been conducted worldwide, although the results obtained are very different in terms of the contents of, and main components in the essential oil. [3,5,7,11-13] Hac *et al.*[5] found higher overall quantities of the main components germacrene D (20.5%) and geijerene (20.7%) in the *E. odoratum* essential oil collected in Nghe An than were found in the samples collected in the present study in Dak Lak (20.59% and 8.21%, respectively). Conversely, components like estragole (11.01%), caryophyllene oxide (12.10%), and caryophyllene (9.52%) were abundant in the *E. odoratum* essential oil collected from the Dak Lak species.[5] Another study, by Huynh Thi Ngoc Ni, showed that the main component in the essential oil of *E. odoratum* collected from Tuy Hoa, Phu Yen, Vietnam, was caryophyllene (12.083%), which was detected at higher levels than were found in the essential oil of *E. odoratum* collected in Dak Lak in the present study (9.52%). Other major components in the essential oil of *E. odoratum* collected from Tuy Hoa species included 5,6-diethenyl-1-methylcyclohexene, (8.093%), 1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene (17.626%),

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Table 4: Physicochemical properties of *E. odoratum* essential oil major components analyzed with SwissADME

Compound	MW (g/mol)	Log P	nHBD	nHBA	TPSA	MR	Lipinski violation	Log S	nRotB
Geijerene	162.27	3.66	0	0	0.00	56.00	0	–3.61	2
Estragole	148.20	2.78	0	1	9.23	47.04	0	–3.09	3
Caryophyllene	204.35	4.24	0	0	0.00	68.78	0	–3.87	0
Germacrene D	204.35	4.30	0	0	0.00	70.68	0	–4.03	1
Cadinene	206.37	4.48	0	0	0.00	69.52	0	–4.54	1
Caryophyllene oxide	220.35	3.68	0	1	12.53	68.27	0	–3.45	0
Ciprofloxacin	331.34	1.10	2	5	74.57	95.25	0	–1.32	3

MW: molecular weight; log P: logarithmic octanol/water partition coefficient; nHBD: number of hydrogen bond donor(s); nHBA: number of hydrogen bond acceptor(s); TPSA: topological polar surface area; MR: molar refractivity; log S: log of solubility; nRotB: number of rotatable bond(s).

Table 5: ADME predictions computed by SwissADME.

Compound	Log K_p (cm/s)	GI Abs	Inhibitor Interaction						
			BBB per	P-gp substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
Geijerene	–4.03	Low	Yes	No	No	No	Yes	No	No
Estragole	–4.81	High	Yes	No	Yes	No	No	No	No
Caryophyllene	–4.44	Low	No	No	No	Yes	Yes	No	No
Germacrene D	–4.18	Low	No	No	No	No	Yes	No	No
Cadinene	–3.63	Low	No	No	No	Yes	Yes	No	No
Caryophyllene oxide	–5.12	High	Yes	No	No	Yes	Yes	No	No
Ciprofloxacin	–9.09	High	No	Yes	No	No	No	No	No

Log K_P: logarithmic skin permeation coefficient; GI Abs: gastrointestinal absorption; BBB per: blood-brain barrier permeation; P-gp: P-glycoprotein.

1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene (6.363%), which were not present in the essential oil of *E. odoratum* collected in Dak Lak in the present study.[13] In contrast, germacrene D (20.59%), caryophyllene oxide (12.10%), estragole (11.01%), and geijerene (8.21%) were only found in the essential oil of *E. odoratum* collected in Dak Lak. Luan *et al.*[7] studied the chemical composition of essential oil from *E. odoratum* collected in Dong Hoa and Tuy Hoa, Phu Yen. The results showed the four main components in the essential oil of *E. odoratum* collected in Dong Hoa were 5,6-diethyl-1-methylcyclohexene (23.1%), *β*-cubebene (14.3%), 4,4-dimethyltetracyclo[5.2.1.0(2,6).0(3,5)]decane (12.5%), and *β*-caryophyllene (10.1%), while the main components in the essential oil from *E. odoratum* collected in Tuy Hoa were 1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene (17.626%) and 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene (6.363%).[7] Thus, the main components in the essential oil of *E. odoratum* collected in Dong Hoa and Tuy Hoa, Phu Yen are different to those in the oil collected in Dak Lak. Nguyen Thi Thuy Trang identified 52 compounds in the essential oil of *E. odoratum* collected in Binh Dinh, with germacrene D (18.67%) found in lower amounts compared to the present study (20.59%). Other major compounds in the essential oil of *E. odoratum* collected in Binh Dinh included isocaryophyllene (19.22%), 8-methylenedispiro[2.0.2.5]undecane (14.15%), α,α-dimethyl-1-vinyl-o-menth-8-ene-4-methanol (6.15%), cadina-3,9-diene (6.00%), and *α*-caryophyllene (4.12%),[3] which were absent in the essential oil of *E. odoratum* collected in Dak Lak in the present study. Conversely, caryophyllene oxide (12.10%), estragole (11.01%), caryophyllene (9.52%), and geijerene (8.21%) were only found in the essential oil of *E. odoratum* collected in Dak Lak. The chemical composition of *E. odoratum* essential oil collected in the Ivory Coast has also been reported; the main components included *α*-Pinene (18.8%), pregeijerene (14.3%), *β*-Pinene (10.5%), and germacrene D (8.2%).[23] While the contents of *α*-Pinene and *β*-Pinene were higher than those in the present study, the content of germacrene D (8.2%) in the essential oil of *E. odoratum* collected in the Ivory Coast was lower than that in the essential oil of *E. odoratum* collected in Dak Lak (20.59%). Other major components were not found in the essential oil of *E. odoratum* collected in the Ivory Coast. Conversely, caryophyllene oxide (12.10%), estragole (11.01%), caryophyllene (9.52%), and geijerene (8.21%) were found in the essential oil of *E. odoratum* collected in Dak Lak. In another study, the major components in the leaf extract of *E. odoratum* from India included *cis*-muurola-4(14),5-diene (10.79%) and isocaryophyllene (5.39%).[24] However, the Indian report did not find the major components present in the essential oil of *E. odoratum* collected in Dak Lak in the present study, such as germacrene D (20.59%), estragole (11.01%), and caryophyllene oxide (12.10%). These variations in chemical composition may be due to the influence of the weather, soil, and geographical factors on the chemical profile of *E. odoratum* essential oil. Such differences have implications for the potential uses and properties of the essential oil from different locations. Regarding antioxidant activity, previous research showed that the essential oil of *E. odoratum* collected in Phu Yen had a weaker antioxidant effect (IC_{50} = 22.57 µg/mL) than that of the essential oil of *E. odoratum* collected in Dak Lak (5.8 ± 0.3 µg/mL).[13] In addition, the strong antioxidant activity of the essential oil of *E. odoratum* collected in India, with an IC_{50} value of 10.58 µg/mL, was still weaker than that of the essential oil of *E. odoratum* collected in Dak Lak (5.8 ± 0.3 µg/mL).[24] The result of another research group also showed that the

antioxidant activity of the essential oil of *E. odoratum* was weaker than that of the essential oil of *E. odoratum* collected in Dak Lak in the present study.[25] These findings highlight the resistance to oxidation of *E. odoratum* essential oil collected in Dak Lak as a natural antioxidant.

Table 6: Toxicity predicted by DL-AOT Prediction Server

Compound	Log (LD_{50}) (mg/kg)	Toxicity
Geijerene	3.18	Caution
Estragole	3.36	Caution
Caryophyllene	3.61	Caution
Germacrene D	3.32	Caution
Cadinene	3.75	Caution
Caryophyllene oxide	3.61	Caution
Ciprofloxacin	3.42	Caution

In terms of antibacterial activity, the research results of Luan *et al.*[7] showed that the essential oil of *E. odoratum* collected in Phu Yen did not demonstrate antibacterial activity against *E. coli*,[7] while the essential oil of *E. odoratum* collected in Dak Lak in the present study had quite high antibacterial activity against *E. coli* (16.4 ± 0.4 mm). In contrast, the antibacterial activity of the essential oil of *E. odoratum* collected in Dak Lak (16.4 ± 0.4 mm) was lower than that of previous reports (19.00 and 32.3 mm).[24,26] *E. coli* was selected as the target in this study due to its importance as a member of the normal intestinal microflora in humans and other mammals. Additionally, *E. coli* is known to be a versatile and potentially dangerous pathogen that can cause a wide range of intestinal and extraintestinal diseases.[27]

In *in silico* study, several compounds from *E. odoratum* essential oil showed potential as moderate inhibitors, forming hydrophobic interactions with residues in the active binding site of the GyrB enzyme (Figure 2). The free binding energies of all studied essential oils towards the GyrB enzyme ranged from –6.5 to –5.0 kcal/mol, compared to –7.2 kcal/mol for ciprofloxacin. Among them, the top six compounds with the lowest binding energies were identified as geijerene, estragole, caryophyllene, germacrene D, cadinene, and caryophyllene oxide. Interestingly, germacrene D was suggested as the strongest inhibitor of the GyrB enzyme, with a free binding energy value of –6.5 kcal/mol. Notably, Ile78 formed hydrophobic interactions with all the studied compounds (Table 3). Overall, the experimental findings provide strong evidence for the antimicrobial activity of *E. odoratum* essential oil against *E. coli*, making the essential oil a promising candidate as a natural antibacterial agent.

Conclusion

In conclusion, this study is the first to report on the essential oil extracted from the leaves and stems of *E. odoratum* collected in Dak Lak, Vietnam. GC-MS analysis revealed a diverse chemical composition, with 51 identified natural components. The major components of the essential oil were germacrene D (20.59%), caryophyllene oxide (12.10%), estragole (11.01%), caryophyllene (9.52%), geijerene (8.21%), cadinene (5.96%), guaia-6,9-diene (4.49%), 3-ethenylcyclopentene (3.54%), and copaen (3.08%). The essential oil demonstrated promising biological activity. It exhibited normal resistance to oxidation, with a significant ability to inhibit DPPH free radicals, demonstrated by an IC_{50} value of 5.8 ± 0.3 µg/mL. Additionally, the essential oil demonstrated effective inhibition against *E. coli* bacteria, with a MIC of 4.2 ± 0.5 mg/mL. Molecular docking analysis revealed binding energy between essential oil's major constituents and GyrB ranging from –5.0 to –6.5 kcal/mol. Germacrene D exhibited superior binding energy profiles with GyrB compared to other components. These results indicate *E. odoratum* essential oil is a potential therapeutic agent and can be used in medicinal and pharmaceutical applications.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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