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Chemical Composition and Biological Activity of Vietnamese *Vernonia amygdalina* Essential Oil: *In Vitro* and *In Silico* Studies

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ABSTRACT

Vernonia amygdalina Del. (Asteraceae) is widely used to treat intestinal diseases, abdominal pain, helminth infections, dysentery, gastritis, and pinkeye. However, the chemical composition and biological properties of the essential oil of *V. amygdalina* collected in Dak Lak, Vietnam, have not been investigated. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical composition of *V. amygdalina* essential oil. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities of the essential oil, respectively. The binding energy between essential oil major constituents and *Escherichia coli* DNA gyrase subunit B (GyrB) was studied by molecular docking. The essential oil content was 0.67% by weight, and 40 constituents were identified, the major ones being 4-methylheptane (3.19%), 2-fluoromesitylene (26.61%), 4-(1,1-dimethylethyl) benzenethiol (10.99%), butylated hydroxytoluene (16.22%), 3-ethyl-4-heptanone (6.73%), and 3,5-dihydroxy-N,N-diethylbenzamide (13.91%). The strong antioxidant and antibacterial effects of *V. amygdalina* essential oil were demonstrated with an IC_{50} value of 6.4 ± 0.2 µg/mL and a lowest minimum inhibitory concentration (MIC) value of 4.6 ± 0.3 mg/mL, respectively. In molecular docking studies, the binding energy between the essential oil's major constituents and GyrB ranges from –6.0 to –4.5 kcal/mol. All major constituents were classified as "Caution" using the DL-AOT prediction server. Our results indicate that the essential oil of *V. amygdalina* collected in Dak Lak, Vietnam, has great potential in the pharmaceutical industry.

Keywords: *Vernonia amygdalina*, Essential oil, Antioxidant, Antibacterial, Molecular docking.

Introduction

Vernonia amygdalina Del. (synonyms *Gymnanthemum amygdalinum* or *Vernonia randii*) is a daisy family plant known in Vietnam as the "bear bile plant". *V. amygdalina* has a herbaceous form and develops in bushes. Depending on the quality of the soil and the amount of light, it will reach from 2 to 5 meters in height. Its leaves are oval-shaped with small serrated edges on both sides. They are moderately hard and are 6-10 centimeters in length and 2–4 centimeters in width. *V. amygdalina* flowers bloom from February to April, with pale yellow flower clusters each year. Each flower has 6 petals and is supported by many sepals. These leaf blades are arranged in 3 rings under the flowers, and the flower clusters will bloom at the top of the stem. After the flowers fade, green fruit will appear, which ripens from May to June to a green-brown color.[1] *V. amygdalina* can be found in Asian countries such as Vietnam, China, Nepal, and India.

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With its growth characteristics, it appears more in the northern mountainous areas of Vietnam, where the climate is cool and the terrain is high, though some also grow in the south, such as in Lam Dong Province.[1] *V. amygdalina* shows strong potential for medicinal use.[2] Many of its active constituents, such as ursolic acid, *β*-sitosterol, and glucoside, protect the liver.[3-5] It is also used to treat intestinal diseases, abdominal pain, helminth infections, dysentery, gastritis, and pink eyes.[4] Additionally, *V. amygdalina* helps reduce "bad" cholesterol and inhibits and prevents the growth and proliferation of cancer cells. At the same time, it prevents the spread of stomach cancer and breast cancer cells. Furthermore, it is antioxidant and anti-inflammatory and has been applied in treating cardiovascular diseases, mental illness, stress, and emotional disorders, and in the management of polycystic ovary syndrome.[6–8] *V. amygdalina* is also used as a diuretic tea and can treat constipation, diabetes, skin infections, and liver-related diseases.[6]

Several publications report the chemical composition of *V. Amygdalina*,[9,10] especially that of the essential oil.[11–16] However, studies have also shown that the raw material from different regions and different extraction methods also greatly affect the content of *V. Amygdalina*. Therefore, when testing its chemical activity, it is possible to discover different active constituents according to the above factors.[17] This study aims to analyze and characterize the chemical composition and biological properties of *V. amygdalina* essential oil collected in Dak Lak, Vietnam, in support of its potential exploitation and effective utilization. Determination of the chemical composition of *V. amygdalina* essential oil was conducted using gas chromatography-mass spectrometry (GC-MS). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate

the antioxidant and antibacterial activities, respectively.
AutoDock Vina 1.2.5, integrated with UCSF Chimera, was used for molecular docking. The binding sites of the protein *Escherichia coli* DNA gyrase subunit B (GyrB) were analyzed using PrankWeb. The SwissADME web tool was utilized to assess the pharmacokinetic properties of major constituents of the essential oil, and the DL-AOT prediction server was used to predict acute oral toxicity.

Material and Methods

Chemicals
Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

Plant Material
Leaves and stems of *V. amygdalina* were collected from Khanh Xuan Commune (12°39'03.9"N, 107°59'05.7"E), Buon Ma Thuot City, Dak Lak Province, Vietnam, in January 2023. A voucher specimen (No. LĐ-BMT-01) was prepared and deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City, Dak Lak Province, Vietnam.

Extraction of Essential Oil
Collected leaves and stems were cleaned, cut into small pieces, and subjected to steam distillation for 4 hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10 ℃ in the dark prior to subsequent experiments.

Analysis of Essential Oil by GC-MS
To analyze the composition of the essential oil from the leaves and stems of *V. amygdalina*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC operational parameters included an injector temperature of 250 ℃, a detector temperature of 260 ℃, an oven temperature program of 60 to 260 ℃ at a heating rate of 4 ℃/min, carrier gas helium at a flow rate of 1.0 mL/min, and a sample injection volume of 1 µL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 ℃, ion source temperature 230 ℃, MS quadrupole temperature 200 ℃, and scan range 35–650 amu.[18] The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

Antioxidant Assay
The DPPH assay was used to evaluate the antioxidant activity of the essential oil from *V. amygdalina* leaves and stems.[18] The essential oil was dissolved in methanol to different concentrations (0.3125, 0.625, 1.25, 2.5, 5.0, and 10 µg/mL), and the positive control BHT was mixed with 200 µL of a methanolic solution (containing DPPH radicals at a concentration of 150 µmol/L). The mixture was then vigorously shaken and left in the dark for 30 minutes to complete the reaction. The absorbance solutions were measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm. A blank sample (a control solution without extract or BHT) was used for comparison. The scavenging ability of the essential oil was calculated as in Equation 1.

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

Antimicrobial Activity
The agar disc diffusion method was used to evaluate the antibacterial activity of the essential oil against the Gram-negative bacterium *E. coli* (ATCC 25922). A liquid culture of *E. coli* (10^7 CFU/mL) was evenly spread on solidified agar in Petri dishes. Circular filter paper discs (6 mm diameter) were placed at the center of each dish, and 40 µL of the essential oil (extracted by steam distillation and dissolved in 10% dimethyl sulfoxide, DMSO) was applied to the discs. DMSO (10%) served as a negative control. The plates were sealed and incubated at 37 ℃, and the diameters of the inhibition zones around the discs were measured to assess antibacterial activity. All experiments were conducted in triplicate for accuracy. The minimum inhibitory concentration (MIC) of the essential oil was determined by the broth microdilution method, as described by Hanh *et al.* (2023).[18] The essential oil was serially diluted two-fold with ethanol in a 96-well plate to achieve concentrations ranging from 1.0 to 10.0 mg/mL, and then 20 µL of bacterial suspension (pH 7.4–7.6) was added to each well. The plates were incubated at 37 ℃ for 24 h. MIC was defined as the lowest essential oil concentration that visibly inhibited bacterial growth. Each assay was performed in triplicate to ensure result reliability.

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) was obtained from the RCSB Protein Data Bank (Brookhaven National Laboratory, New York, USA). UCSF Chimera 1.17.3 (California, San Francisco, USA, 2023) was used to remove non-standard residues. Polar hydrogen atoms and Gasteiger charges were added to the protein using the Dock Prep tool in UCSF Chimera. The PubChem CIDs of the six studied compounds, including 4-methylheptane, 2-fluoromesitylene, 4-(1,1-dimethylethyl) benzenethiol, butylated hydroxytoluene, 3-ethyl-4-heptanone, and 3,5-dihydroxy-N,N-diethylbenzamide, along with the reference compound (ciprofloxacin), were used to generate ligand structures via the Build Structure tool in UCSF Chimera. Ligands were prepared by adding hydrogen atoms and assigning Gasteiger charges using the Dock Prep tool. Energy minimization of the ligands was performed with the Minimize Structure tool in UCSF Chimera. The prepared proteins and ligands were saved in PDB format. Molecular docking was carried out using AutoDock Vina 1.2.5 (Center for Computational Structural Biology, California, USA, 2021), integrated into UCSF Chimera. Binding active sites of the protein were identified using DoGSiteScorer (http://dogsite.zbh.uni-hamburg.de),[19] and the highest-scoring binding pockets were selected based on their ranking and probability. Grid boxes were centered on the binding sites to include all residues identified by the DoGSiteScorer. The Broyden-Fletcher-Goldfarb-Shanno algorithm was used to identify the best conformers, with a maximum of 10 conformers per ligand. Default parameters in AutoDock Vina were applied during the docking process. Conformers were ranked based on their binding energy, and the least binding energy among all generated conformers was used for further analysis. All docking simulations were performed on a Windows 10 Pro operating system running on a 2.53 GHz Intel Core i5 processor. The studied compounds were evaluated for drug-likeness using "Lipinski's rule of five".[20] Pharmacokinetics-related data, ADMET, were predicted using the SwissADME web tool (http://www.swissadme.ch/index.php).[21] Acute oral toxicity predictions were performed using the deep learning-based acute oral toxicity (DL-AOT) prediction server (Center of Quantitative Biology and Molecular Design Laboratory, Peking University, China, 2017).[22]

Statistical Analysis
All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean ± standard deviation (SD).

Results and Discussion

Chemical Composition of V. amygdalina Essential Oil

Essential oil was extracted from the leaves and stems of *V. amygdalina* via hydrodistillation, with a 0.67% yield (w/w, based on fresh weight). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 1, and the detailed composition is presented in Table 1. Forty compounds (accounting for 99.58% of the overall oil composition) were identified (Supplementary Material). Each compound was assigned a peak number in the chromatogram for easy identification and reference throughout this study.

Table 1: Chemical compositions of *V. amygdalina* essential oil.

Peak	Retention time (min)	Compounds	Molecular fomular	Relative amount (%)
1	5.226	2-ethyl-4,5-dimethyl-1,2-oxaborolane	$C_7H_{15}BO$	0.31
2	5.296	2-methyl-1-butanol	$C_5H_{12}O$	0.40
3	7.877	3-hexen-1-ol	$C_6H_{12}O$	0.14
4	7.928	2,3,4-trimethylpentane	C_8H_{18}	0.44
5	8.108	4-heptanol	$C_7H_{16}O$	1.21
6	9.585	1-(2-methyl-1-butenyl)pyrrolidine	C_9H_7N	0.35
7	9.675	2-pyrrolidinylmethylamine	$C_5H_{12}N_2$	1.77
8	9.796	4,5-dimethyl-2-isopropyloxazole	$C_8H_{13}NO$	0.64
9	9.986	3,4-dimethyldihydrofuran-2,5-dione	$C_6H_8O_3$	0.74
10	10.338	1,7-dihydro-6*H*-purin-6-one	$C_5H_4N_4O$	0.55
11	10.438	Benzyl alcohol	C_7H_8O	0.58
12	10.659	4-methylheptane	C_8H_{18}	3.19
13	11.001	Linalool	$C_{10}H_{18}O$	0.55
14	11.192	Phenylethyl alcohol	$C_{10}H_{18}O$	0.71
15	11.242	2,6-dimethylcyclohexanol	$C_8H_{16}O$	0.64
16	11.372	Benzyl methyl ketone	$C_9H_{10}O$	1.21
17	11.513	2,6,6-trimethyl-2-cyclohexen-1,4-dione	$C_9H_{12}O_2$	0.19
18	11.734	Terpinen-4-ol	$C_{10}H_{18}O$	0.20
19	12.075	2-fluoromesitylene	$C_9H_{11}F$	26.61
20	12.367	4-(1,1-dimethylethyl)benzenethiol	$C_{10}H_{14}S$	10.99
21	12.557	Indole	C_8H_7N	0.38
22	12.778	4-ethoxy-3-anisaldehyde	$C_{10}H_{12}O$	2.01
23	12.909	2,5-dimethoxybenzaldehyde	$C_9H_{10}O_3$	0.24
24	12.989	2,5-dimethyl-1,3benzenediol	$C_8H_{10}O_2$	0.98
25	13.080	2,4,6-trihydroxytoluene	$C_7H_8O_3$	1.09
26	13.321	2,5-dimethoxybenzaldehyde	$C_9H_{10}O_3$	0.74
27	13.602	3-ethyl-4-heptanone	$C_9H_{18}O$	1.52
28	13.692	4-methoxy-3,5-dimethylbenzenamine	$C_9H_{13}NO$	0.84
29	13.742	Butylated hydroxytoluene	$C_{15}H_{24}O$	16.22
30	13.813	3-ethyl-4-heptanone	$C_9H_{18}O$	6.73
31	13.903	3-butoxycyclooctene	$C_{12}H_{22}O$	0.19
32	13.973	Ethyldigermane	$C_2H_5Ge_2$	0.29
33	14.054	6-methyl-2-propyl-4(1*H*)pyrimidinone	$C_8H_{12}N_2O$	0.97
34	14.325	1-methoxy-2-methyl-1-propene	$C_5H_{10}O$	0.19
35	14.385	3,7-dimethyloct-6-en-1-yloctadeca-9,12,15-trienoate	$C_{28}H_{48}O_2$	0.19
36	14.546	3-amino-3-(4-isopropylphenyl)propionic acid	$C_{12}H_{17}NO_2$	0.35
37	14.626	τ-muurolol	$C_{15}H_{26}O$	0.15
38	15.329	3,5-dihydroxy-N,N-diethylbenzamide	$C_{11}H_{15}NO_3$	13.91

39	15.701	Unknown		0.42
40	15.902	N-isobutyltetradeca-2,4-dienamide	$C_{18}H_{33}NO$	0.56
41	17.478	4-phenylpyrido[2,3-d]pyrimidine	$C_{13}H_9N_3$	0.63
Total number of constituents			41	
Total number of constituents identified (%)			40 (99.58%)	
Total number of monoterpenoids (%)			3 (7.30%)	
Total number of hemiterpenoids (%)			2 (4.90%)	
Total number of sesquiterpenes (%)			2 (4.90%)	
Total number of nitrogen compounds (%)			11 (28.88%)	
Total number of different compounds (%)			22 (53.60%)	

The essential oil predominantly consists of terpenes, including 3 monoterpenoids (7.30% of the total oil) and 2 sesquiterpenoids (4.90% of the total oil). Additionally, nitrogen-containing compounds and other diverse compounds make up 22.88% and 53.60% of the oil, respectively. The compounds 4-methylheptane (3.19%), 2-fluoromesitylene (26.61%), 4-(1,1-dimethylethyl)benzenethiol (10.99%), butylated hydroxytoluene (16.22%), 3-ethyl-4-heptanone (6.73%), and 3,5-dihydroxy-N,N-diethylbenzamide (13.91%) are the major components of *V. amygdalina* essential oil. These significant constituents contribute to the unique chemical and bioactive profiles of *V. amygdalina* essential oil.

Antioxidant Activity of V. amygdalina Essential Oil

The antioxidant activity was evaluated by the DPPH radical scavenging assay, with the results summarized in Figure 2. The IC_{50} value of *V. amygdalina* essential oil was determined to be 6.4 ± 0.2 µg/mL, compared to 6.1 ± 0.1 µg/mL for BHT, a well-known synthetic antioxidant. These results indicate that the essential oil of *V. amygdalina* collected in Dak Lak has strong antioxidant activity comparable to BHT, highlighting its potential as a natural antioxidant agent.

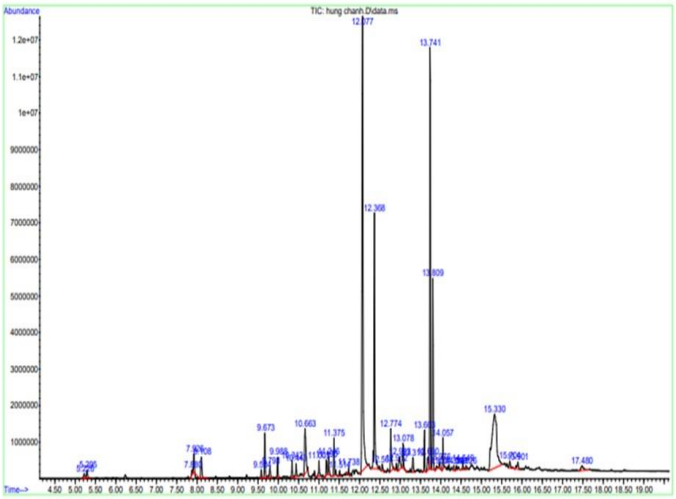

Figure 1: GC-MS total ion chromatogram of *V. amygdalina* essential oil.

Antibacterial Activity of V. amygdalina Essential Oil

The antimicrobial activity of *V. amygdalina* essential oil against *E. coli* is summarized in Table 2. The essential oil demonstrated strong activity, with an inhibition zone of 19.5 ± 0.7 mm and an MIC of 4.6 ± 0.3 mg/mL. Ciprofloxacin used as a positive control, exhibited an inhibition zone of 36.1 ± 1.4 mm and a MIC of 0.09 ± 0.01 mg/mL. These findings highlight the significant antibacterial potential of *V. amygdalina* essential oil, though its activity is less potent than that of ciprofloxacin. This suggests that *V. amygdalina* essential oil could serve as a natural antimicrobial agent, with promising applications in combating bacterial infections.

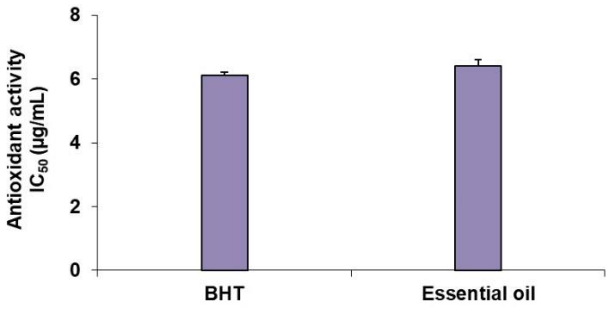

Figure 2: Antioxidant activity of *V. amygdalina* essential oil; The values are mean values ± SD (n = 3); BHT: positive control for antioxidant activity.

Table 2: Antibacterial activity of *V. amygdalina* essential oil against *E. coli.*

Sample	Antibacterial activity	
	IZD (mm)	MIC (mg/mL)
DMSO	6.0 ± 0.1	-
Essential oil	19.5 ± 0.7	4.6 ± 0.3
Ciprofloxacin	36.1 ± 1.4	0.09 ± 0.1

(-): not tested; Ciprofloxacin: positive control for antibacterial activity; IZD: in-hibition zone diameters.

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

Docking simulations of the GyrB protein with the studied ligands, including 4-methylheptane, 2-fluoromesitylene, 4-(1,1-dimethylethyl) benzenethiol, butylated hydroxytoluene, 3-ethyl-4-heptanone, and 3,5-dihydroxy-N,N-diethylbenzamide revealed their potential as GyrB inhibitors. Several interactions were identified between the ligands and residues in the receptor's active site. Figure 3 illustrates the best-docked poses of the ligands, highlighting their binding interactions with the active site of the GyrB receptor. Binding affinities, quantified as free binding energies, were calculated using AutoDock Vina and are presented in Table 3. These results provide insights into the affinity of each ligand for the GyrB target, supporting their potential role as effective inhibitors.

The free binding energies of all studied constituents towards the GyrB enzyme were ranked from –6.0 to –4.5 kcal/mol, while that of ciprofloxacin was –7.2 kcal/mol. Among the six studied compounds, 3,5-dihydroxy-N,N-diethylbenzamide appears to be the strongest

inhibitor of the GyrB enzyme, with a free binding energy value of –6.0 kcal/mol. This can be explained by three hydrogen bond formations with residues in the active site of the GyrB enzyme, specifically Asn46, Glu50, and Thr165. Notably, Ile78 participated in hydrophobic interactions with all studied compounds.

ADMET Predictions of Studied Compounds

The classic "Lipinski's rule of five" serves as a guideline for evaluating a compound's drug-like properties. None of the studied constituents analyzed in this study exceed a molecular mass of 500 Daltons. All studied compounds have fewer than five hydrogen bond donors and fewer than ten hydrogen bond acceptors, with log P values below 5, except for BHT. These findings suggest that most of the studied compounds comply with Lipinski's rule of five, indicating favorable drug-likeness. Additionally, other physicochemical parameters, including the number of rotatable bonds, topological polar surface area (TPSA), and aqueous solubility (log S), were evaluated. To support good oral bioavailability and intestinal absorption, the number of rotatable bonds should not exceed 10, and the TPSA should remain below 140 Å².[23] Table 4 presents a comprehensive summary of these parameters, confirming that the studied compounds generally exhibit good physicochemical properties suitable for drug development.

Table 3: Docking results toward GyrB.

Compound	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic interaction
4-methylheptane	–4.5		Val43, Val71, Ile78, Val120, Val167
2-fluoromesitylene	–5.6		Val43, Ala47, Val71, Ile78, Met95, Val120, Val167
4-(1,1-dimethylethyl)benzenethiol	–4.6	Asp73	Asn46, Gly77, Ile78
Butylated hydroxytoluene	–4.9		Glu50, Ile78, Pro79
3-ethyl-4-heptanone	–4.9	Asn46	Val43, Ala47, Ile78, Val120, Val167
3,5-dihydroxy-N,N-diethylbenzamide	–6.0	Asn46, Glu50, Thr165	Ala47, Ile78
Ciprofloxacin	–7.2	Asn46, Asp73, Gly77, Thr165	Ala47, Ile78

Ciprofloxacin: positive control compound.

Table 4: Physicochemical properties of *V. amygdalina* essential oil major components analyzed with SwissADME.

Compound	MW (g/mol)	Log P	nHBD	nHBA	TPSA	MR	Lipinski violation	Log S	nRotB
4-methylheptane	114.23	3.39	0	0	0.00	40.57	0	–2.97	4
2-fluoromesitylene	138.18	3.21	0	1	0.00	41.30	0	–3.11	0
4-(1,1-dimethylethyl)benzenethiol	166.28	3.29	0	0	38.80	52.96	0	–3.43	1
Butylated hydroxytoluene	220.36	5.27	1	1	20.20	72.34	1	–4.38	2
3-ethyl-4-heptanone	124.24	2.56	0	1	17.07	45.58	0	–1.99	5
3,5-dihydroxy-N,N-diethylbenzamide	209.24	1.22	2	3	60.77	58.00	0	–1.51	4
Ciprofloxacin	331.34	1.10	2	5	74.57	95.25	0	–1.32	3

Ciprofloxacin: positive control compound; 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 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 ciprofloxacin. Several cytochrome P enzymes play a crucial role in drug biotransformation, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The studied compounds were predicted not to inhibit CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The log(LD_{50}) values were calculated using the DL-AOT prediction server and found to be between 2.77 and 3.44 (Table 6). Based on these predicted results, all studied compounds were classified as "Caution". There have been several studies on the chemical composition of *V. amygdalina* essential oil. Compared with the *V. amygdalina* stems collected from Cao Bang Province,[11] the main compounds, including 9,12-octadecadienoic acid, 9,17-octadecadienal, *n*-hexadecanoic acid, *β*-sitosterol, *cis*-13-octadecenoic acid, campesterol, stigmasterol, stigmast-4-en-3-one, and vitamin E, were not present in *V. amygdalina* collected from Dak Lak. In contrast, the main compounds 4-methylheptane, 2-fluoromesitylene, 4-(1,1-dimethylethyl)benzenethiol, BHT, 3-ethyl-4-heptanone, and 3,5-dihydroxy-N,N-diethylbenzamide were detected in high amounts in *V. amygdalina* collected from Dak Lak but not in those from Cao Bang Province.[11] Hieu *et al*. studied the chemical composition of the leaves and stems of *V. amygdalina* collected from Thai Nguyen, findfing the main substances to be flavonoids, saponins, tannins, and reducing sugars.[12] However, these components were not found in *V. amygdalina* collected in Dak Lak. Asawalam and Hassanali studied the chemical composition of the essential oil of *V. amygdalina* collected from

Table 5: ADME predictions computed by SwissADME.

Compound	Log K_p (cm/s)	GI Abs	BBB per	Inhibitor Interaction					
				P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
4-methylheptane	–3.97	Low	Yes	No	No	No	No	No	No
2-fluoromesitylene	–4.93	Low	Yes	No	No	No	No	No	No
4-(1,1-dimethylethyl)benzenethiol	–4.81	High	Yes	No	Yes	No	No	No	No
Butylated hydroxytoluene	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3-ethyl-4-heptanone	–5.37	High	Yes	No	No	No	No	No	No
3,5-dihydroxy-N,N-diethylbenzamide	–7.19	High	Yes	No	No	No	No	No	No
Ciprofloxacin	–9.09	High	No	Yes	No	No	No	No	No

Ciprofloxacin: positive control compound; Log K_p: log of skin permeability; GI Abs: gastrointestinal absorption; BBB: per blood-brain barrier permeability; P-gp: p-glycoprotein; CYP: cytochrome-P; (-): not test.

Umudike, Nigeria, identifying 17 main compounds.[13] Another study showed that the main components in the essential oil of *V. amygdalina* collected at the Botanical Garden, University of Ibadan, Nigeria, include thymol, (*E*)-phytol, ocymene, *β*-selinene, *γ*-terpinene, *β*-caryophyllene, and apiole.[14] Similarly, a study by Ogunbinu *et al.* also showed that the main compounds in the essential oil of *V. amygdalina* harvested from Iwo, Ibadan, Nigeria, were *α*-muurolol, terpinen-4-ol, *γ*-muurolene, and isophorone.[15] Notably, these main compounds identified in the above research were different from those in the essential oil of *V. amygdalina* collected in Dak Lak. It can be concluded that varying climatic conditions, terrain, and soil fertility lead to different compositions of essential oil.[16]

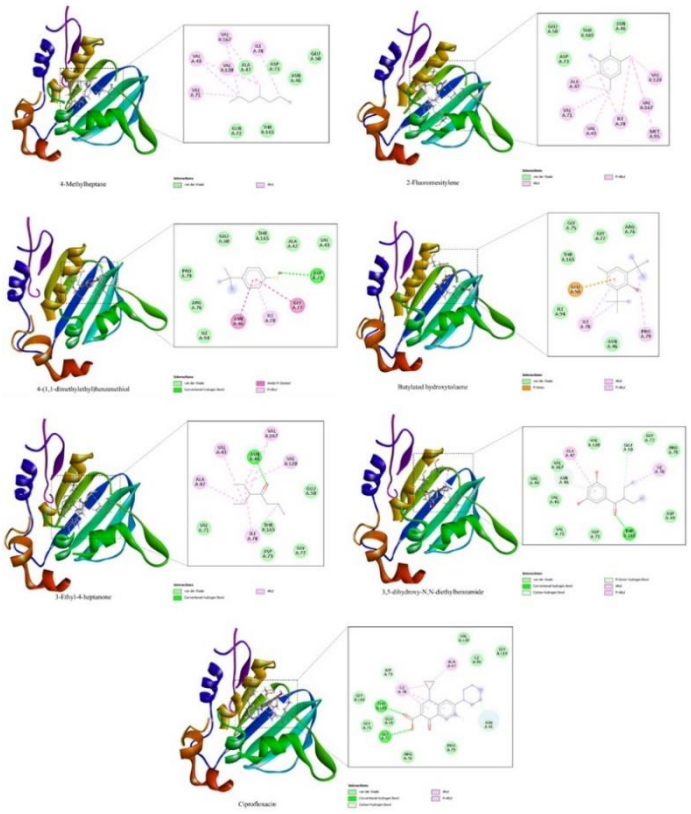

Figure 3: Compounds and gyrase subunit B (GyrB) interactions.

Regarding antioxidant activity, Karlina *et al.* determined that the essential oil of *V. amygdalina* collected in Cirebon had antioxidant activity with an IC_{50} value of 13 ± 0.10 µg/mL,[24] which was 2 times higher than that of the essential oil collected in Dak Lak (6.4 ± 0.2 µg/mL). Other research also determined that the essential oil of *V. amygdalina* grown in South Africa had antioxidant activity with an IC_{50} value of 0.15 mg/mL, which was higher than that of the essential oil collected in Dak Lak (6.4 ± 0.2 µg/mL).[16] In addition, Rumengan et al. also showed that the essential oil of *V. amygdalina* grown in North Sulawesi had an antioxidant activity with IC_{50} values ranging from 8.98 to 22.25 µg/mL,[25] which was relatively high compared to that collected in Dak Lak (6.4 ± 0.2 µg/mL). The above results show that the antioxidant capacity of the essential oil of *V. amygdalina* collected in Dak Lak is stronger than that of samples collected or grown in other regions. This may be due to the difference in chemical composition of the essential oils.

Table 6: Toxicity predicted by DL-AOT prediction server.

Compound	Log (LD_{50}) (mg/kg)	Toxicity
4-methylheptane	3.32	Caution
2-fluoromesitylene	3.31	Caution
4-(1,1-dimethylethyl)benzenethiol	2.77	Caution
Butylated hydroxytoluene	3.44	Caution
3-ethyl-4-heptanone	3.30	Caution
3,5-dihydroxy-N,N-diethylbenzamide	3.19	Caution
Ciprofloxacin	3.42	Caution

Ciprofloxacin: positive control compound; LD_{50}: lethal dose 50.

Regarding antibacterial activity, Nhan and Linh determined that the essential oil of *V. amygdalina* collected in Can Tho, Soc Trang, and Tien Giang could not inhibit *E. coli* in the 5–80 mg/mL concentration range.[26] Olusola-Makinde *et al.* also showed that the inhibition zone (10.333 ± 0.882 mm) of *V. amygdalina* essential oil collected in Nigeria was much lower than that collected in Dak Lak (19.5 ± 0.7 mm).[27] In addition, the anti-*E. coli* activity (14.3 mm inhibition zone) of the essential oil collected in Kano, Nigari, Nigeria, by Ali *et al.*[28] was lower than that of the samples collected in Dak Lak (19.5 ± 0.7 mm). The results show that the antibacterial activity of the essential oil of *V. amygdalina* collected in Dak Lak is stronger than that of samples collected in other regions. Overall, the strong antibacterial activity of *V. amygdalina* essential oil collected in Dak Lak makes it a promising candidate for the essential oil and fragrance industry.

Conclusion

This study is the first to investigate the essential oil from the leaves and stems of *V. amygdalina* collected in Dak Lak, Vietnam. GC-MS analysis identified a diverse chemical composition comprising 40 natural components. The major constituents were 4-methylheptane (3.19%), 2-fluoromesitylene (26.61%), 4-(1,1-dimethylethyl)benzenethiol (10.99%), BHT (16.22%), 3-ethyl-4-

heptanone (6.73%), and 3,5-dihydroxy-N,N-diethylbenzamide (13.91%). The essential oil exhibited notable biological activities, demonstrating strong antioxidant activity with an IC_{50} value of 6.4 ± 0.2 μg/mL, surpassing the reference compound, BHT (6.1 ± 0.1 μg/mL). Additionally, the essential oil showed effective antibacterial activity against *E. coli*, with an MIC of 4.6 ± 0.3 mg/mL. Molecular docking studies revealed significant interactions between the major oil constituents and the active site of the GyrB enzyme, with binding energies ranging from −6.0 to −4.5 kcal/mol. Among the components, 3,5-dihydroxy-N,N-diethylbenzamide demonstrated the strongest binding affinity. All studied compounds were classified as "Caution". These findings underscore the potential of *V. amygdalina* essential oil as a natural resource for pharmaceutical applications, particularly in antioxidant and antibacterial therapies.

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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