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Chemical Composition and Multiple Bioactivities of *Ageratum conyzoides* Essential Oil Collected in Dak Lak, Vietnam

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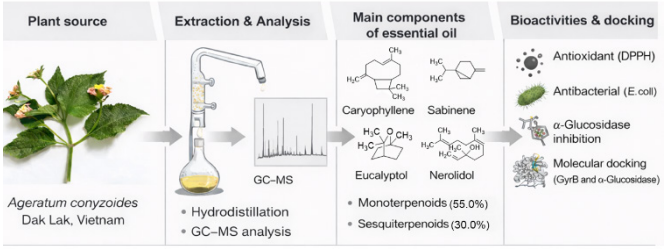
Abstract: The essential oil of *Ageratum conyzoides* collected from Dak Lak Province, Vietnam, was investigated for its chemical composition and biological activities. Gas chromatography-mass spectrometry (GC-MS) analysis identified 26 volatile constituents, accounting for 78.8 % of the total oil content, predominantly composed of monoterpenoids (60.6 %) and sesquiterpenoids (18.2 %). The major components included caryophyllene (15.57 %), sabinene (14.78 %), isomyocorene (3.62 %), eucalyptol (12.46 %), humulene (8.91 %), nerolidol (5.80 %), α-pinene (3.09 %), and 3-carene (3.05 %). The essential oil exhibited notable antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with an IC_{50} value of 6.03 ± 0.4 mg/mL. In addition, moderate antibacterial activity against *Escherichia coli* was observed, with an inhibition zone diameter of 27.0 mm at 3.00 mg/mL and a minimum inhibitory concentration of 100 µg/mL. The oil also demonstrated moderate α-glucosidase inhibitory activity with an IC_{50} value of 415.4 µg/mL. Molecular docking analysis provided only preliminary and hypothesis-generating insights, suggesting that major constituents such as caryophyllene and nerolidol may be associated with the observed activities. However, these *in silico* results do not provide direct evidence of DNA GyrB or α-glucosidase inhibition by individual compounds. These findings indicate that *A. conyzoides* essential oil represents a potential natural source of multifunctional bioactive compounds for further pharmacological investigation.



Keywords: *Ageratum conyzoides,* essential oil, antibacterial, antioxidant, α-glucosidase, molecular docking

1 Introduction

Medicinal plants are important sources of bioactive compounds with diverse pharmacological potential[1, 2]. Increasing antimicrobial resistance and concerns regarding the safety of synthetic agents have stimulated growing interest in plant-

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derived natural products[3, 4]. Among these, essential oils, which are complex mixtures of mono- and sesquiterpenoids, have attracted considerable attention due to their broad spectrum of biological activities, including antimicrobial, antioxidant, and enzyme-modulating effects[5, 6].

Ageratum conyzoides Linn. (Asteraceae), commonly known as billygoat weed, is a medicinal plant widely distributed throughout tropical and subtropical regions, including many ecological zones of Vietnam[7–10]. The plant has long been used in traditional medicine for the treatment of respiratory infections, sinusitis, inflammation, and various infectious diseases[7, 8]. Previous phytochemical studies have revealed that *A. conyzoides* contains a variety of biologically active secondary metabolites, including flavonoids, alkaloids, coumarins, and essential oils[9–13]. Extracts and isolated constituents from this species have been reported to exhibit diverse biological activities, such as antioxidant, antibacterial, anti-inflammatory, and wound-healing effects, demonstrating its pharmacological relevance[14–17].

The essential oil of *A. conyzoides* has been investigated in different geographic regions and is characterized by a chemically diverse composition dominated by terpenoid constituents[18, 19]. Gas chromatography-mass spectrometry (GC-MS) analyses have identified numerous volatile compounds, including precocene derivatives, caryophyllene, germacrene D, and α-humulene, which are considered to contribute to its biological properties[16, 17]. However, considerable variations in chemical composition have been reported depending on geographic origin, environmental conditions, and extraction methods. Essential oils from West African populations are typically dominated by chromene derivatives such as precocene I, whereas samples from Asian regions often exhibit more terpene-rich profiles[17, 20–22]. Such chemotypic variability is widely attributed to environmental factors, including climate, soil composition, altitude, and seasonal variation, which significantly influence the biosynthesis of volatile secondary metabolites in plants[23].

Despite previous reports on the chemical composition and biological activities of *A. conyzoides*, studies integrating comprehensive chemical profiling with multiple bioactivity evaluations and mechanistic insights remain limited, particularly for samples collected from the Central Highlands of Vietnam[16–19]. In addition, molecular-level investigations linking major volatile constituents to specific biological targets are still scarce[24]. Samples from the Central Highlands of Vietnam, particularly Dak Lak Province, remain underexplored in terms of essential oil composition and associated biological activities. Accordingly, this study provides new data on the chemical profile and bioactivity of *A. conyzoides* from this geographically distinct region.

Therefore, the present study aims to (i) characterize the chemical composition of *A. conyzoides* essential oil from Dak Lak Province using GC-MS, (ii) evaluate its antioxidant, antibacterial, and α-glucosidase inhibitory activities, and (iii) provide mechanistic insights through molecular docking and *in silico* ADMET (absorption, distribution, metabolism, excretion, toxicity) analysis. This study employs an integrated experimental-computational approach, combining GC-MS analysis, biological assays, molecular docking, and ADMET prediction to provide a comprehensive evaluation of the essential oil and its structure-activity relationships (SAR).

2 Materials and methods

2.1 Chemicals

Reference compounds, including butylated hydroxytoluene (BHT) and a homologous series of *n*-alkanes (C_7–C_{30}), were obtained from Sigma-Aldrich (St. Louis, MO, USA) and employed to support chromatographic peak identification. All remaining analytical-grade reagents, microbiological media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK).

2.2 Plant material

Plant material consisting of leaves and flowers of *A. conyzoides* was harvested in January 2025 from Khanh Xuan Commune (12°39′03.9″ N, 107°59′05.7″ E), located in Buon Ma Thuot City, Dak Lak Province, Vietnam. The collected material was taxonomically documented, and a representative voucher specimen (No. NS-BMT-01) has been archived at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City, Vietnam.

2.3 Essential oil isolation

After collection, the leaves and flowers of *A. conyzoides* were sorted to remove impurities, rinsed with distilled water, and allowed to drain at room temperature for approximately 2.5 h, followed by air-drying for an additional 2 h. A representative portion (100 g) of the chopped plant material was then transferred to a Clevenger-type apparatus and subjected to hydrodistillation with 1000 mL of distilled water for 3 h under electric heating. The volatile constituents were co-distilled with steam and subsequently condensed, yielding a distillate that separated into aqueous and essential oil phases. The essential oil layer was recovered by extraction with diethyl ether, dried over anhydrous sodium sulfate

(Na_2SO_4), and concentrated by solvent removal under reduced pressure to obtain the final essential oil sample.

2.4 Chemical profiling of the essential oil by GC-MS

The volatile profile of *A. conyzoides* essential oil was determined by GC-MS employing a Trace GC Ultra gas chromatograph coupled with an ITQ900 mass selective detector (Thermo Fisher Scientific, MA, USA). Data acquisition and spectral processing were conducted using MassFinder software (version 4.0). Chromatographic separation was achieved on a TG-SQC (DB-5/HP-5 type) fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was set at 250 °C, and the oven temperature was programmed from 60 to 260 °C at a ramp rate of 4 °C/min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Samples (1 µL) were injected in split mode with a split ratio of 1:10.

Mass spectrometric detection was performed under electron ionization (EI) conditions at 70 eV. The temperatures of the transfer line, ion source, and quadrupole were maintained at 280, 230, and 200 °C, respectively, and mass spectra were collected over an m/z range of 35–650[25]. Compound identification was carried out by matching mass spectra with the NIST 20 library and by comparing retention behavior with literature retention indices reported under similar chromatographic conditions. In the present study, a homologous series of *n*-alkanes was not analyzed under the same experimental conditions; therefore, experimental retention indices could not be determined. Accordingly, only literature retention indices are reported to support compound identification. Quantitative composition was estimated based on normalized peak area percentages obtained from the total ion chromatogram, without the application of correction factors.

2.5 Antioxidant activity assay

The antioxidant potential of *A. conyzoides* essential oil was assessed by measuring its ability to quench 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, following a modified procedure adapted from Nguyen and Eun (2013)[26]. For the assay, essential oil solutions were prepared at concentrations of 25.0, 12.5, 6.25, 3.125, and 1.5625 mg/mL using methanol as a solvent to ensure adequate dispersion of the essential oil. An aliquot (1 mL) of each solution was combined with 5 mL of DPPH solution in a test tube, and the mixture was vigorously shaken to ensure homogeneity. The reaction mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Janway 6305, UK). A control solution containing methanol instead of the essential oil was prepared in parallel.

The percentage of antioxidant activity was calculated using Equation (1):

$$Antioxidant\ (\%) = \frac{AB-AA}{AB}\ x\ 100\ \% \qquad (1)$$

In this equation, *AB* denotes the absorbance recorded for the control solution containing methanol only, whereas *AA* refers to the absorbance obtained from the reaction mixture in the presence of the essential oil. Antioxidant potency was subsequently expressed as the half maximal inhibitory concentration (IC_{50}), which was derived from the corresponding dose–response curve.

2.6 Antimicrobial activity assay

The antibacterial potential of *A. conyzoides* essential oil was examined by means of the disc diffusion assay, with slight procedural adaptations based on Clinical and Laboratory Standards Institute guidelines (2020)[27]. Essential oil solutions were prepared at concentrations of 3.00, 2.50, 2.00, 1.50, 1.00, and 0.50 mg/mL using dimethyl sulfoxide (DMSO) as a solvent to facilitate dispersion of the hydrophobic oil. The final concentration of DMSO did not exceed 1 % (v/v), and a solvent control containing DMSO alone was included to confirm that it had no effect on microbial growth. Sterile paper discs (6 mm diameter) were individually loaded with 50 µL of each solution, air-dried, and subsequently employed for antibacterial evaluation.

Bacterial inocula of *Escherichia coli* (ATCC 25922, approximately 10^6 CFU/mL) were uniformly distributed onto tryptic soy agar (TSA) plates supplemented with 2 % NaCl using a sterile triangular spreader, followed by a drying period of 3–5 min. The impregnated discs were carefully positioned on the inoculated agar surfaces, and the plates were incubated at 28 °C for 24 h. The incubation temperature (28 °C) was selected to maintain consistent experimental conditions across all tested microorganisms. It is noted that this temperature is lower than the optimal growth temperature for *E. coli* (37 °C) and may influence growth kinetics. Discs containing standard antibiotics at a concentration of 1 mg/mL served as positive controls, with ciprofloxacin used as the reference compound against *E. coli*.

All experiments were performed in triplicate. Antibacterial activity was assessed by measuring the diameters of the inhibition zones (IZD) surrounding the discs after 24 h of incubation at 28 °C.

2.7 Determination of minimum inhibitory concentration (MIC)

The antimicrobial activity of the essential oil was further evaluated by determining the MIC using a broth microdilution method in 96-well microplates, following previously reported procedures with minor modifications[28]. The tested microorganisms included Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 25923), Gram-positive bacteria (*Bacillus subtilis* ATCC 11774 and *Staphylococcus aureus* subsp. *aureus* ATCC 11632), filamentous fungi (*Aspergillus niger* and *Fusarium oxysporum*), and yeasts (*Candida albicans* ATCC 7754 and *Saccharomyces cerevisiae*). Microbial strains were cultured in appropriate media and adjusted to a turbidity equivalent to the 0.5 McFarland standard prior to testing. Serial dilutions of the essential oil were prepared in suitable growth media using DMSO as a solvent (final concentration ≤ 1 %, v/v) to ensure adequate dispersion of the hydrophobic oil. A solvent control containing DMSO alone was included to confirm that it had no effect on microbial growth. The prepared solutions were then dispensed into microtiter plates containing the microbial inocula. The plates were incubated at 37 °C for 24 h for bacterial strains and at 30 °C for 48 h for fungi and yeasts. The MIC was defined as the lowest concentration of the sample at which no visible microbial growth was observed. Reference antibiotics were included as positive controls to validate the assay, while negative controls consisted of inoculated media without test samples.

2.8 α-Glucosidase inhibitory activity assay

The inhibitory effect of *A. conyzoides* essential oil on α-glucosidase activity was assessed using a protocol adapted from Sihvonen et al. (1999)[29]. Essential oil solutions were prepared in 0.1 M phosphate buffer (pH 6.8) at concentrations of 1500, 750.0, 375.0, 187.5, 93.75, and 46.875 µg/mL. For each assay, an aliquot of 50 µL of the essential oil solution was combined with 30 µL of α-glucosidase solution (2 U/mL) and incubated at 37 °C for 10 min.

Following the pre-incubation step, 50 µL of *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG, 2.5 mM), prepared in the same phosphate buffer, was added to initiate the enzymatic reaction. The mixture was then incubated at 37 °C for an additional 30 min. Absorbance was recorded at 405 nm using a BIO-RAD iMark microplate reader. A parallel control assay was conducted under identical conditions, substituting the essential oil with solvent only.

The percentage of α-glucosidase inhibition was calculated according to Equation (2):

$$Inhibition\ \% = \frac{A0 - A1}{A0}\ x\ 100\ \% \qquad (2)$$

where A_0 represents the absorbance of the control reaction containing solvent alone, and A_1 corresponds to the absorbance measured for the reaction mixture containing the essential oil[29].

2.9 *In silico* molecular docking and ADMET (absorption, distribution, metabolism, excretion, toxicity) profiling of selected compounds

The three-dimensional crystal structures of the target proteins were obtained from the RCSB Protein Data Bank. Specifically, the structure of DNA GyrB from *E. coli* (PDB ID: 6F86) and α-glucosidase from *Saccharomyces cerevisiae* (PDB ID: 3AJ7) were selected for subsequent molecular docking and *in silico* pharmacokinetic evaluation. Due to the limited availability of high-resolution crystal structures of human α-glucosidase, a homologous enzyme model from yeast was employed as a surrogate for docking analysis. Protein preparation was performed using UCSF Chimera version 1.19, including removal of non-standard residues. Polar hydrogen atoms were added and Gasteiger charges were assigned using the Dock Prep tool.

Three-dimensional structures of the eight selected compounds, including *α*-pinene, sabinene, 3-carene, eucalyptol, caryophyllene, humulene, nerolidol, and isomyocorene, along with the reference ligands ciprofloxacin and acarbose, were generated from their corresponding PubChem compound identifiers using the Build Structure tool implemented in UCSF Chimera. Ligand preparation included the addition of polar hydrogen atoms and the assignment of Gasteiger partial charges via the Dock Prep module, followed by energy minimization using the Minimize Structure function. The prepared ligand and protein structures were subsequently saved in PDB format for docking calculations.

Molecular docking was performed using AutoDock Vina (version 1.2.5) integrated within the UCSF Chimera platform. Potential ligand-binding sites of the target proteins were identified using the DoGSite Scorer server, and the most probable binding pockets were selected based on their pocket scores and probability rankings[30]. For each selected site, grid boxes were defined to encompass all key residues predicted by DoGSite Scorer. Docking simulations were conducted using the Broyden-Fletcher-Goldfarb-Shanno (BFGS) optimization algorithm, generating up to ten binding poses per ligand, while all other parameters were kept at their default values. The resulting docking poses were ranked according to predicted binding affinities, and the conformation with the lowest binding energy was selected for further analysis.

All docking calculations were carried out on a Windows 10 Pro workstation equipped with an Intel Core i5 processor operating at 2.53 GHz. The drug-likeness of the investigated compounds was evaluated based on Lipinski's rule of five[31],

while pharmacokinetic behavior and ADMET-related parameters were estimated using the SwissADME web-based platform[32]. *In silico* toxicity prediction was performed using the ProTox 3.0 web server (DL-AOT-based), which integrates deep learning algorithms and molecular similarity analyses to estimate acute oral toxicity (LD_{50}), toxicity class, and potential toxicological endpoints. The Simplified Molecular Input Line Entry System (SMILES) of each compound was submitted to the server, and the predicted toxicity classes were assigned according to the Globally Harmonized System (GHS) of classification[33].

2.10 Statistical analysis

All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD) based on three independent measurements.

3 Results

3.1 Chemical profile of *A. conyzoides* essential oil

The essential oil obtained from *A. conyzoides* by steam distillation showed a yield of 0.38 % (w/w, calculated on a fresh weight basis). The volatile composition of the oil was subsequently profiled by GC-MS, with the chromatographic features presented in **Fig. 1** and the identified constituents listed in **Table 1**.

Twenty-six constituents were tentatively characterized, collectively representing 78.8 % of the total essential oil content, while seven unknown components accounted for the remaining 21.2 %. Compound identification was achieved through comparison of mass spectral data with the NIST library, together with comparison to literature retention indices reported under similar chromatographic conditions. All identified constituents, along with their corresponding GC-MS peak numbers assigned for clarity in chromatographic interpretation, are provided in the Supplementary Material.

The essential oil was predominantly composed of terpenoid compounds, including 20 monoterpenoids 60.6 %) and 6 sesquiterpenoids (18.2 %) of the total oil content. Unknown constituents contributed an additional 21.2 %.

The major components of the *A. conyzoides* essential oil were α-pinene (3.09 %), sabinene (14.78 %), 3-carene (3.05 %), isomyocorene (3.62 %), eucalyptol (12.46 %), caryophyllene (15.57 %), humulene (8.91 %), and nerolidol (5.80 %). These predominant constituents are likely responsible for the distinctive chemical profile and contribute significantly to the biological activities of the essential oil.

3.2 Antioxidant capacity of *A. conyzoides* essential oil

The radical scavenging performance of *A. conyzoides* essential oil was assessed by the DPPH assay, and the corresponding data are presented in **Table 2**. The oil demonstrated a pronounced scavenging response that increased progressively with concentration. An IC_{50} value of 6.03 ± 0.4 mg/mL was obtained for the essential oil, which was comparable to that of the synthetic antioxidant BHT (IC_{50} = 6.10 ± 0.10 mg/mL). These results suggest that the essential oil derived from *A. conyzoides* collected in Dak Lak exhibits substantial antioxidant capacity and may serve as a promising natural substitute for conventional synthetic antioxidants.

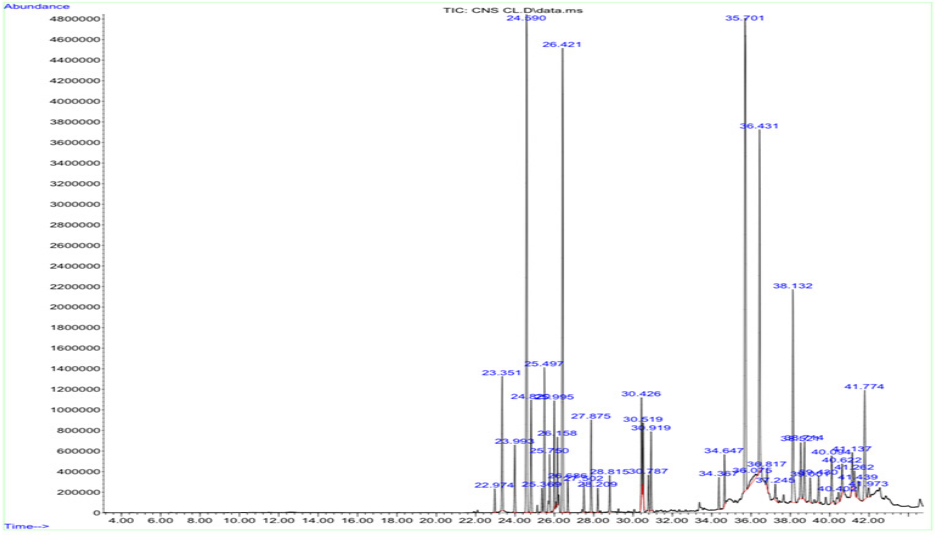

Fig. 1 GC-MS total ion chromatogram of *A. conyzoides* essential oil.

Table 1 Chemical profile of *A. conyzoides* essential oil.

Peak	Retention time (min)	Compounds	Molecular formula	Literature retention index	Relative amount (%)
1	22.974	*α*-Thujene	$C_{10}H_{16}$	924	0.50
2	23.351	*α*-Pinene	$C_{10}H_{16}$	939	3.09
3	23.993	Camphene	$C_{10}H_{16}$	953	1.54
4	24.590	Sabinene	$C_{10}H_{16}$	976	14.78
5	24.819	*β*-Pinene	$C_{10}H_{16}$	980	2.57
6	25.369	2-Carene	$C_{10}H_{16}$	1001	0.58
7	25.497	3-Carene	$C_{10}H_{16}$	1012	3.05
8	25.995	*α*-Terpinene	$C_{10}H_{16}$	1014	0.76
9	26.158	Isomyocorene	$C_{10}H_{16}$	1017	3.62
10	26.421	Eucalyptol	$C_{10}H_{18}O$	1026	12.46
11	27.502	*cis*-*β*-Ocimene	$C_{10}H_{16}$	1032	1.18
12	27.875	*trans*-*β*-Ocimene	$C_{10}H_{16}$	1049	2.11
13	28.209	*γ*-Terpinene	$C_{10}H_{16}$	1056	0.66
14	28.815	Linalool	$C_{10}H_{18}O$	1099	0.49
15	30.426	*trans*-Sabinol	$C_{10}H_{18}O$	1140	1.94
16	30.519	2-Bornanone	$C_{10}H_{16}O$	1141	1.96
17	30.787	*cis*-Sabinol	$C_{10}H_{18}O$	1143	0.77
18	30.919	endo-Borneol	$C_{10}H_{16}O$	1165	0.77
19	34.367	Terpinen-4-ol	$C_{10}H_{18}O$	1174	1.24
20	34.647	*α*-Terpineol	$C_{10}H_{18}O$	1186	1.62
21	35.701	Caryophyllene	$C_{15}H_{24}$	1419	15.57
22	36.431	Humulene	$C_{15}H_{24}$	1452	8.91
23	37.245	*γ*-Muurolene	$C_{15}H_{24}$	1477	0.52
24	38.132	Nerolidol	$C_{15}H_{26}O$	1535	5.80
25	39.430	Unknown		1541	1.46
26	40.094	Unknown		1544	1.50
27	40.402	Unknown		1552	0.65
28	40.622	Unknown		1561	0.72
29	41.137	Spathulenol	$C_{15}H_{24}O$	1577	1.32
30	41.262	Caryophyllene oxide	$C_{15}H_{24}O$	1581	0.88
31	41.439	Unknown		1612	1.07
32	41.774	Unknown		1635	2.58
33	41.973	Unknown		1652	0.33
Total number of constituents (%)				33 (100 %)	
Number (%) of constituents identified				26 (78.8 %)	
Number (%) of monoterpenoids				20 (60.6 %)	
Number (%) of sesquiterpenoids				6 (18.2 %)	
Number (%) of unknown constituents				7 (21.2 %)	

Compounds were tentatively identified based on MS library matching and comparison with literature retention indices. Experimental retention indices are not available due to the absence of retained *n*-alkane retention time data.

3.3 Antibacterial and antimicrobial activities of *A. conyzoides* essential oil

The antibacterial performance of *A. conyzoides* essential oil against *E. coli* was assessed by a disc diffusion assay[27], with the corresponding data presented in **Table 3** and **Fig. 2**. The oil displayed a dose-responsive inhibitory behavior, as evidenced by progressively enlarged inhibition zones at increasing concentrations. Notably, at 3.00 mg/mL, a distinct inhibition zone measuring 27.0 mm was observed, corresponding to an antibacterial effectiveness of 79.41 %.

In comparison, ciprofloxacin, used as the positive control at a concentration of 1.00 mg/mL, showed a larger inhibition zone of 34.0 mm and relative activity of 100 %, indicating stronger antibacterial activity under the same experimental conditions. Nevertheless, the inhibition zones observed for the essential oil suggest that *A. conyzoides* essential oil exhibits moderate antibacterial activity against *E. coli*. The antibacterial activity was initially assessed using a disc

diffusion assay and further evaluated using MIC determination. While IZDs provide a preliminary indication of antimicrobial effects, MIC values offer a more reliable quantitative assessment. It should be noted that IZDs obtained from disc diffusion assays may not directly correlate with intrinsic antimicrobial potency, particularly for hydrophobic essential oils.

Overall, these findings demonstrate that *A. conyzoides* essential oil exhibits moderate antibacterial activity and may represent a potential source of bioactive compounds with selective antibacterial activity.

The antimicrobial activity of *A. conyzoides* essential oil was further evaluated using the microdilution method[28], and the results are summarized in **Table 4**. The essential oil exhibited selective inhibitory effects depending on the tested microorganisms. Among Gram-negative bacteria, moderate activity was observed against *E. coli* with an MIC of 100 μg/mL, whereas no inhibition was detected against *P. aeruginosa* at the highest tested concentration (> 200 μg/mL). For Gram-positive bacteria, the essential oil showed no observable activity against both *B. subtilis* and *S. aureus* (MIC > 200 μg/mL). In contrast, a relatively higher sensitivity was noted for filamentous fungi and yeast strains. The essential oil inhibited the growth of *A. niger* and *S. cerevisiae* with MIC values of 200 μg/mL, while no activity was detected against *F. oxysporum* and *C. albicans* (MIC > 200 μg/mL). Overall, these results indicate that *A. conyzoides* essential oil exhibits moderate and selective antimicrobial activity, with relatively higher activity against *E. coli* compared to other tested strains. The observed selective activity against *E. coli* may be attributed to the ability of lipophilic terpene constituents to interact with the outer membrane of Gram-negative bacteria, leading to increased membrane permeability. However, the lack of activity against *P. aeruginosa* and Gram-positive strains suggests that the antibacterial spectrum of the essential oil is limited and may depend on strain-specific membrane characteristics and intrinsic resistance mechanisms. This selective and moderate activity is consistent with previous reports indicating that essential oils generally exhibit variable efficacy depending on microbial species and structural composition. Further studies, including determination of minimum bactericidal concentration (MBC), would provide deeper insight into the bactericidal potential of the essential oil.

Table 2 DPPH radical scavenging capacity of *A. conyzoides* essential oil.

Essential oil (mg/mL)	% Inhibition				IC_{50} (mg/mL)
	1	2	3	Mean ± SD	
25	95.37	96.79	97.12	96.43 ± 0.93	
12.5	65.86	65.38	66.27	65.84 ± 0.45	
6.25	51.38	51.15	52.02	51.52 ± 0.45	6.03 ± 0.4
3.125	43.61	44.05	43.28	43.65 ± 0.39	
1.5625	37.28	37.76	37.55	37.53 ± 0.24	
BHT[a]					6.10 ± 0.1

[a] Positive control for antioxidant activity. Values are expressed as mean ± SD (n = 3). IC_{50}values were calculated from dose-response curves.

Table 3 Inhibition zones of *A. conyzoides* essential oil against *E. coli*.

Bacterial density	Essential oil (mg/mL)	IZD (mm)				Relative activity (%) compared with ciprofloxacin
		1	2	3	Mean ± SD	
10^6 CFU/mL	3.00	27.0	27.0	27.0	27.00 ± 0.00	79.41
10^6 CFU/mL	2.50	21.0	21.0	21.0	21.00 ± 0.00	61.76
10^6 CFU/mL	2.00	15.0	15.0	15.5	15.17 ± 0.29	44.61
10^6 CFU/mL	1.50	9.0	9.0	9.5	9.17 ± 0.29	26.96
10^6 CFU/mL	1.00	3.5	4.0	4.0	3.83 ± 0.29	11.27
10^6 CFU/mL	0.50	0	0	0	0.00 ± 0.00	0
Ciprofloxacin[a]	1.00	34.0	34.0	34.0	34.00 ± 0.00	100

[a] Reference antibiotic used as the positive control. IZD: inhibition zone diameter. Values are expressed as mean ± SD (n = 3). Relative activity (%) was calculated based on inhibition zone diameter relative to ciprofloxacin.

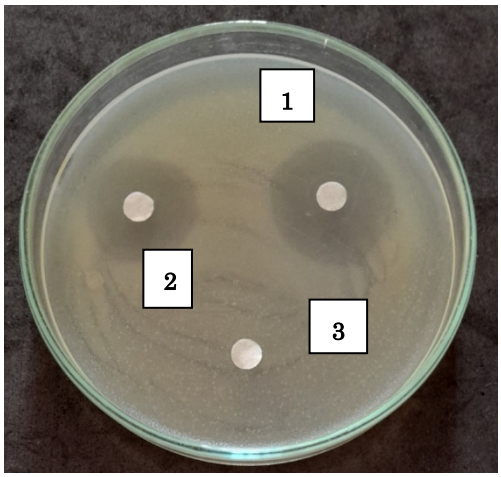

Fig. 2 Disc diffusion assay illustrating the antibacterial effect of *A. conyzoides* essential oil in comparison with the reference antibiotic against *E. coli*. (1): Ciprofloxacin (1 mg/mL, positive control), (2): essential oil at 3.00 mg/mL, and (3): essential oil at 0.50 mg/mL.

From a safety perspective, the potential application of essential oils for nutraceutical or oral use requires careful consideration. Essential oils often exhibit non-selective antimicrobial effects, which may not only target pathogenic microorganisms but also affect beneficial gut microbiota, as reported in previous studies[34, 35]. In the present study, the antimicrobial evaluation was limited to a selected panel of microorganisms and did not include representative commensal strains. Therefore, the potential impact of *A. conyzoides* essential oil on beneficial microbiota remains unclear. Further studies involving a broader range of clinically relevant and commensal microorganisms are required to better assess its safety and suitability for oral applications.

3.4 α-Glucosidase inhibition by *A. conyzoides* essential oil

The inhibitory response of *A. conyzoides* essential oil toward α-glucosidase was quantified by a spectrophotometric method[29], with the corresponding data presented in **Table 5**. The oil demonstrated a pronounced dose-dependent effect, as inhibition values increased from 14.58 % at 46.875 μg/mL to 85.84 % at 1500 μg/mL. Based on interpolation of the concentration–response data, the IC_{50} value was estimated to be 415.4 μg/mL. Although this value is higher than that of the reference inhibitor acarbose (IC_{50} = 18.1 μg/mL), it indicates a moderate α-glucosidase inhibitory potency for a complex natural essential-oil system. The observed activity may be partially associated with the presence of monoterpenes that have previously been reported to interact with carbohydrate-hydrolyzing enzymes, supporting the potential antidiabetic relevance of *A. conyzoides* essential oil.

3.5 Molecular docking of *A. conyzoides* essential oil compounds

Molecular docking simulations were conducted to investigate the possible interaction mechanisms of major constituents from *A. conyzoides* essential oil, including α-pinene, sabinene, 3-carene, eucalyptol, caryophyllene, humulene, nerolidol, and isomyocorene, with two biologically relevant targets: DNA GyrB (6F86) and α-glucosidase (3AJ7). Docking was conducted using AutoDock Vina, and ligand-protein interactions were analyzed with BIOVIA Discovery Studio Visualizer 2024. These terpenoid constituents represent the predominant bioactive constituents of *A. conyzoides* essential oil, while the selected proteins correspond to pathways underlying their reported antibacterial (GyrB)[36], and antidiabetic (α-glucosidase)[37].

Docking analysis indicated that the major constituents of the essential oil interacted with DNA GyrB with moderate binding strength, as reflected by predicted binding energies between −4.83 and −6.04 kcal/mol. In comparison, ciprofloxacin and the co-crystallized native ligand (CWW) exhibited stronger interactions, with binding energies of −6.16 and −7.32 kcal/mol, respectively (**Table 6**). Among the evaluated compounds, nerolidol displayed the most favorable binding profile, which can be attributed to the presence of an oxygenated sesquiterpene framework that enhances protein–ligand interactions. The binding mode of nerolidol was mainly stabilized by hydrophobic interactions with key residues such as Ile78, Val71, and Ala47, which are located within the ATP-binding pocket of the enzyme (**Fig. 3**), together with limited hydrogen bonding contributions.

Table 4 Antimicrobial activity of *A. conyzoides* essential oil expressed as MIC values (μg/mL).

Sample	Max. concentration (μg/mL)	Gram-negative bacteria		Gram-positive bacteria		Filamentous fungi		Yeasts	
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
A. conyzoides essential oil	200	100	> 200	> 200	> 200	200	> 200	200	> 200

Values > 200 μg/mL indicate no observable inhibitory activity at the highest tested concentration. Values represent MIC values determined from triplicate experiments.

Table 5 α-Glucosidase inhibitory activity of *A. conyzoides* essential oil.

Essential oil (μg/mL)	% Inhibition	IC_{50} (μg/mL)
1500	85.84	415.4
750.0	61.06	
375.0	46.82	
187.5	32.14	
93.75	21.12	
46.875	14.58	
Acarbose[a]		18.1

[a] Positive control for anti-α-glucosidase. Values represent mean values of the measurements.
IC_{50} values were determined from dose–response curves.

Table 6 Docking interactions of selected compounds with DNA GyrB (PDB ID: 6F86).

Compounds	Docking score (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	Other interactions
α-Pinene	–5.36	-[a]	ILE78	-
Sabinene	–5.10	-	VAL43, ALA47, VAL71, VAL167	-
3-Carene	–5.47	-	ILE78	-
Eucalyptol	–4.83	THR165	ALA47, ILE78	-
Caryophyllene	–6.04	-	ILE78	-
Humulene	–5.86	-	ILE78	-
Nerolidol	–5.58	GLU50	VAL43, ALA47, VAL71, ILE78, VAL167	-
Isomyocorene	–4.88	-	VAL43, ALA47, VAL71, VAL120, VAL167	-
Ciprofloxacin	–6.16	GLU50, ASP73, GLU77	ILE78, PRO79	ARG76
CWW (Native ligand)	–7.32	ASN46, GLU50, ASP73, GLU77, THR165	ALA47, VAL71, ILE78, PRO79, ILE94, VAL167	ARG76

[a] Not forming hydrogen bonds and other interactions.

In contrast, monoterpene hydrocarbons, including α-pinene, 3-carene, and isomyocorene, exhibited weaker binding energies (approximately –4.88 to –5.47 kcal/mol), reflecting their limited functional groups and a predominance of non-specific hydrophobic interactions (**Table 6**). Ciprofloxacin, used as the reference antibacterial agent, showed a significantly stronger binding affinity (approximately –6.16 kcal/mol), forming multiple hydrogen bonds and hydrophobic interactions with key active-site residues (**Fig. 3**), which is consistent with its well-established inhibitory activity against DNA GyrB. Taken together, the docking results provide a possible computational rationale for the observed antibacterial effect of the whole essential oil. However, these results should not be interpreted as direct evidence that the individual compounds inhibit DNA GyrB, and further enzyme-based validation using isolated compounds is required.

Overall, the docking results indicate that hydrophobic interactions play a dominant role in stabilizing the binding of *A. conyzoides* essential oil constituents within the DNA GyrB active site, thereby potentially contributing to the observed antibacterial activity of the whole essential oil.

For α-glucosidase, the docking scores of the essential oil constituents ranged from –4.52 to –6.62 kcal/mol, while acarbose exhibited a markedly stronger binding affinity (–11.39 kcal/mol), indicating moderate binding affinities toward the enzyme (**Table 7**). The binding modes were predominantly governed by hydrophobic interactions with residues lining the catalytic pocket, whereas hydrogen bonding interactions were limited due to the largely nonpolar nature of the terpene compounds.

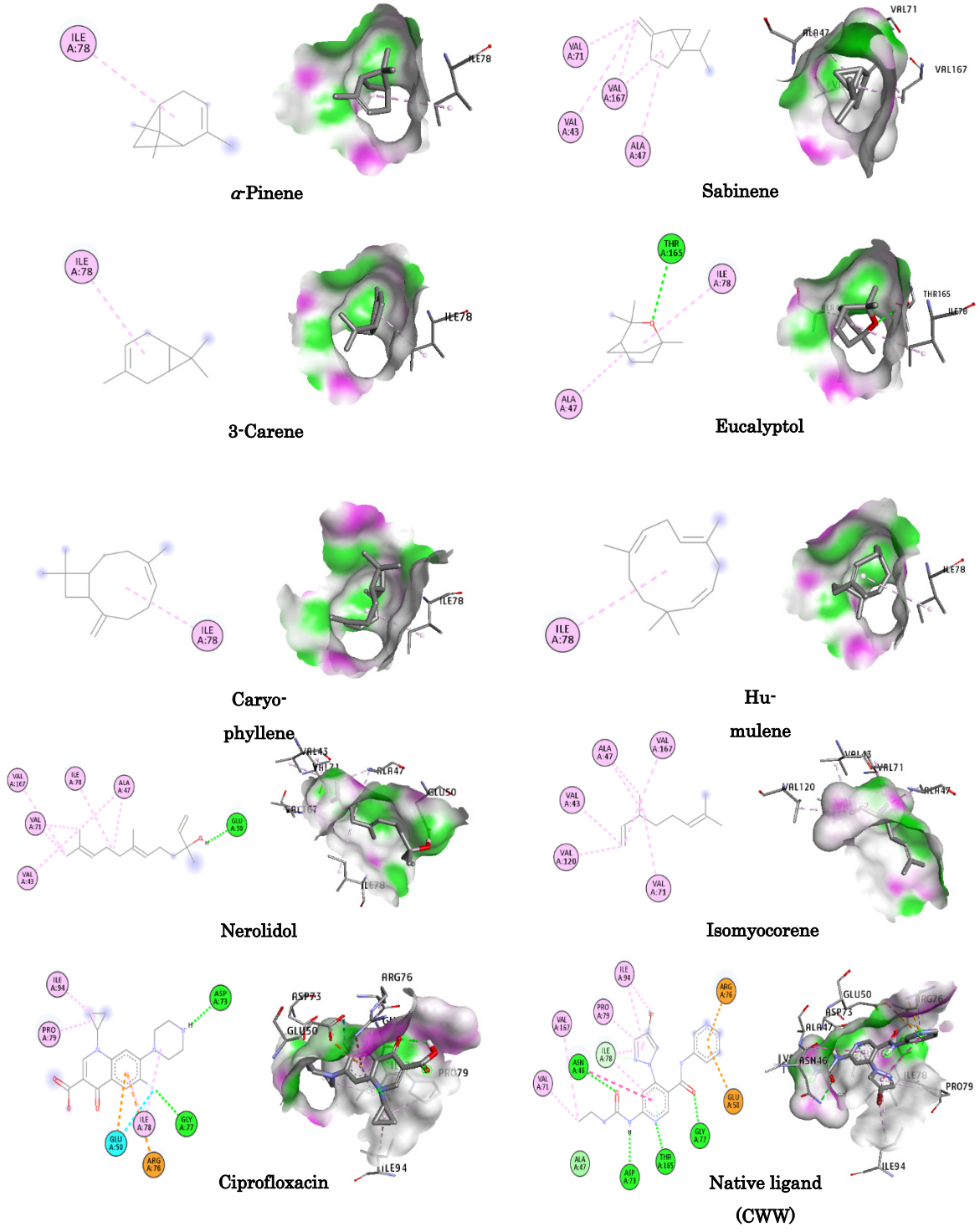

Fig. 3 2D and 3D visualization of ciprofloxacin, native ligand (CWW), and eight target compounds on the 6F86 protein.

Table 7 Docking interactions of selected compounds with α-glucosidase (PDB ID: 3AJ7).

Compounds	Docking score (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	Other interactions
α-Pinene	–5.55	- [a]	TRP238, PHE314, ALA418, ILE419	LYS156
Sabinene	–4.99	-	PHE314, ALA418, ILE419	-
3-Carene	–4.97	HIS423	TRP238, PHE314, ALA418, ILE419	LYS156
Eucalyptol	–5.71	-	PHE314, ALA418, ILE419	-
Caryophyllene	–6.62	-	-	LYS156
Humulene	–6.35	-	-	LYS156
Nerolidol	–5.76	ASP233	TRP238, PHE314, ALA418, ILE419	-
Isomyocorene	–4.52	HIS423	TRP238, PHE314, ALA418, ILE419	LYS156
Acarbose	–11.39	ASN235, ASN317, HIS423, GLU429	-	-

[a] Not forming hydrophobic interaction

Among the essential oil constituents, caryophyllene exhibited the strongest binding affinity (approximately –6.62 kcal/mol), which was mainly stabilized by hydrophobic contacts and a weak electrostatic interaction involving Lys156 (**Fig. 4**). Other compounds showed weaker binding energies, consistent with their reduced interaction profiles. In contrast, acarbose, the reference *α*-glucosidase inhibitor, demonstrated a markedly stronger binding affinity (approximately –11.39 kcal/mol), forming multiple hydrogen bonds with key catalytic residues such as Asn235, Asn317, His423, and Glu429 (**Fig. 4**). These extensive hydrogen bonding interactions explain the superior inhibitory potency of acarbose compared to the essential oil constituents.

Taken together, the docking analysis suggests that the moderate *α*-glucosidase inhibitory activity of the essential oil constituents is mainly associated with hydrophobic binding, whereas extensive hydrogen bonding accounts for the superior potency of the reference inhibitor acarbose.

The physicochemical properties, lipophilicity, and drug-likeness parameters of the studied compounds were predicted using the SwissADME web tool[32], and the results are summarized in **Table 8**. Most essential oil constituents exhibited relatively low molecular weights (138.25–222.37 g/mol) and moderate to high lipophilicity, with predicted LogP values ranging from 3.38 to 5.24, which is consistent with their terpenoid nature. These compounds generally showed low topological polar surface area (TPSA ≤ 38.69 Å²) and limited hydrogen-bonding capacity, features that are commonly associated with favorable membrane permeability.

With the exception of eucalyptol and nerolidol, which fully complied with Lipinski's rule of five[31]; however, minor lead-likeness violations were observed, mainly related to lipophilicity. Most hydrocarbon terpenes exhibited one Lipinski violation, mainly attributed to elevated lipophilicity. Nevertheless, all essential oil constituents displayed acceptable predicted bioavailability scores (0.55), suggesting a reasonable potential for oral absorption. In contrast, the reference *α*-glucosidase inhibitor acarbose showed multiple Lipinski violations, a very high TPSA value (329.01 Å²), and a low predicted bioavailability score (0.11), reflecting its poor membrane permeability and limited oral absorption, in agreement with its known pharmacokinetic behavior (**Table 8**).

Ciprofloxacin, used as the antibacterial reference compound, fully satisfied Lipinski's criteria and exhibited favorable physicochemical parameters, supporting its established drug-likeness. Regarding lead-likeness, most essential oil constituents showed moderate deviations primarily related to lipophilicity, whereas acarbose was classified as non-lead-like due to two lead-likeness violations associated with its high molecular complexity and polarity. Overall, the SwissADME predictions indicate that several essential oil constituents, particularly oxygenated terpenoids such as nerolidol and eucalyptol, possess physicochemical profiles compatible with drug-like behavior, supporting their further consideration as bioactive candidates.

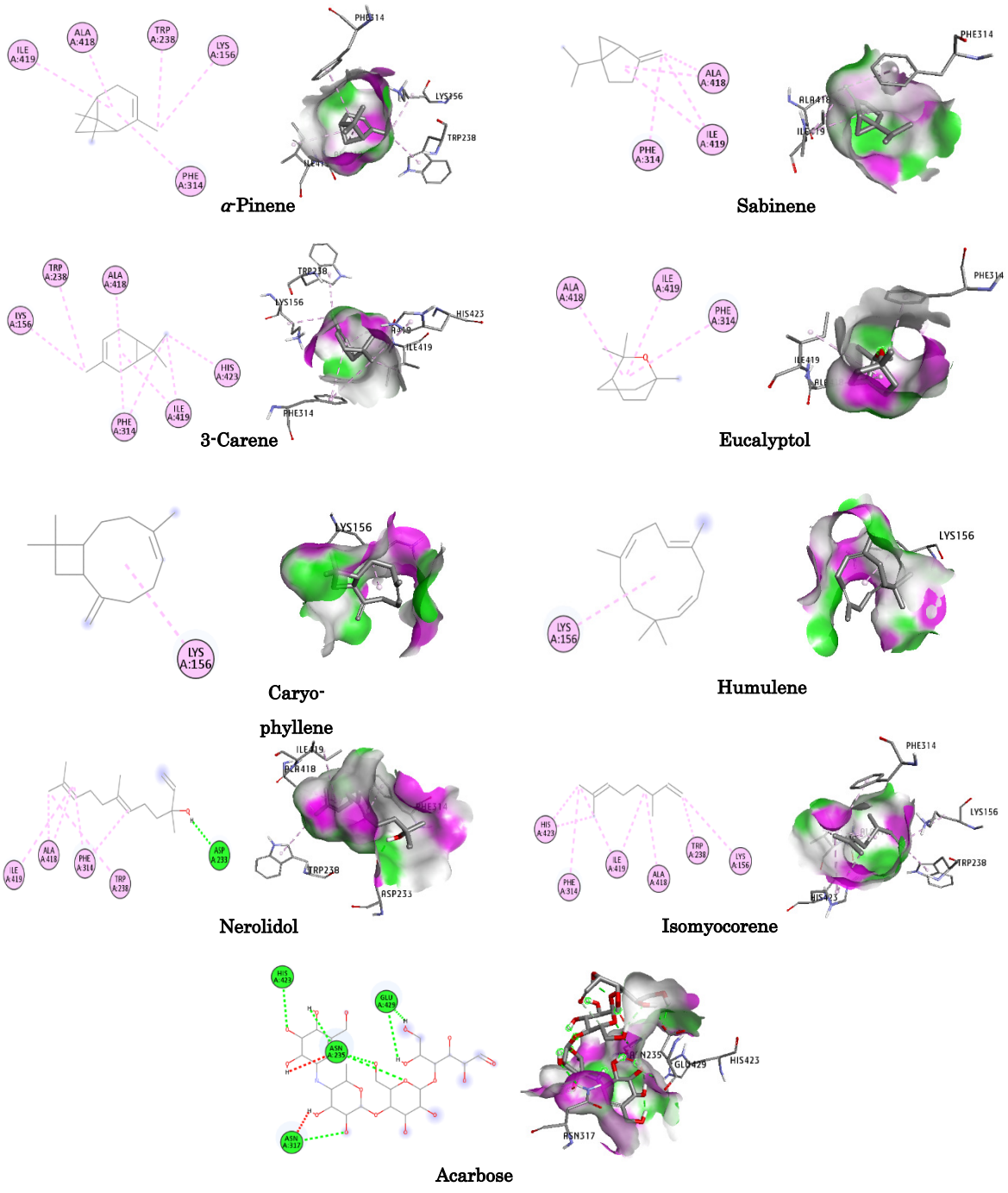

Fig. 4 2D and 3D visualization of acarbose and eight target compounds on the 3AJ7 protein.

The pharmacokinetic properties of the studied compounds were predicted using the SwissADME platform[33], and the results are summarized in **Table 9**. Most essential oil constituents exhibited moderate predicted skin permeability, with LogKp values ranging from –2.54 to –4.37 cm/s, which is consistent with their lipophilic terpenoid structures. In contrast, ciprofloxacin and acarbose showed markedly lower skin permeability (LogKp –8.53 and –16.55 cm/s, respectively), reflecting their higher polarity and molecular complexity.

Table 8 Physicochemical and solubility-related properties of selected compounds predicted by SwissADME.

Compound	MW (g/mol)	LogP	nHBD	nHBA	TPSA (Å²)	Lipinski violation	Bioavailability score	Leadlikeness violations
α-Pinene	138.25	4.03	0	0	0	1	0.55	2
Sabinene	138.25	3.42	0	0	0	1	0.55	2
3-Carene	138.25	3.39	0	0	0	1	0.55	2
Eucalyptol	154.25	2.70	0	1	9.23	0	0.55	1
Caryophyllene	208.38	4.88	0	0	0	1	0.55	2
Humulene	210.40	5.24	0	0	0	1	0.55	2
Nerolidol	222.37	4.19	1	1	20.23	0	0.55	2
Isomyocorene	142.28	4.03	0	0	0	1	0.55	2
Ciprofloxacin	333.36	0.93	2	5	72.88	0	0.55	0
Acarbose	645.60	−3.27	14	19	329.01	3	0.17	2

MW: molecular weight, LogP: octanol/water partition coefficient, nHBD: number of hydrogen bond donors, nHBA: number of hydrogen bond acceptors, TPSA: topological polar surface area.

Table 9 SwissADME-predicted pharmacokinetic properties of selected compounds.

Compound	$LogK_p$ (cm/s)	HIA	BBB per	Inhibitor interaction					
				P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
α-Pinene	−4.37	Low	Yes	No	No	No	Yes	No	No
Sabinene	−4.37	Low	Yes	No	No	No	Yes	No	No
3-Carene	−4.37	Low	Yes	No	No	No	Yes	No	No
Eucalyptol	−5.30	High	Yes	No	No	No	No	No	No
Caryophyllene	−3.09	Low	No	No	No	Yes	Yes	No	No
Humulene	−2.54	Low	No	No	No	No	Yes	No	No
Nerolidol	−4.23	High	Yes	No	Yes	No	Yes	No	No
Isomyocorene	−3.58	Low	Yes	No	No	No	No	No	No
Ciprofloxacin	−8.53	High	No	Yes	No	No	No	No	No
Acarbose	−16.55	Low	No	Yes	No	No	No	No	No

HIA: human intestinal absorption, BBB per: blood–brain barrier permeability, P-gp: P-glycoprotein, CYP: cytochrome P enzymes.

Table 10 ProTox 3.0-based acute oral toxicity profiles and toxicity classification of major essential oil constituents.

Compound	LD_{50} (mg/kg)[a]	Classification
α-Pinene	15380	VI
Sabinene	15380	VI
3-Carene	15380	VI
Eucalyptol	2480	V
Caryophyllene	2450	V
Humulene	750	III
Nerolidol	5000	V
Isomyocorene	750	III

[a] LD_{50}: lethal dose, 50 %

Predictions of human intestinal absorption (HIA) indicated that oxygenated compounds such as eucalyptol and nerolidol possess high intestinal absorption, whereas most hydrocarbon terpenes were predicted to exhibit lower absorption potential. Several essential oil constituents, including *α*-pinene, 3-carene, nerolidol, and isomyocorene, were predicted to cross the blood-brain barrier (BBB), which can be attributed to their low molecular weight and high lipophilicity. In contrast, ciprofloxacin and acarbose were not predicted to penetrate the BBB (**Table 9**).

None of the essential oil constituents were predicted to be substrates of P-glycoprotein. Although some compounds

were predicted to inhibit CYP2C9, these *in silico* results should be interpreted with caution and require further experimental validation[30]. Overall, the SwissADME predictions suggest that selected essential oil constituents exhibit pharmacokinetic profiles compatible with their observed *in vitro* bioactivities, while highlighting the limitations associated with high lipophilicity and potential metabolic interactions.

The toxicity profiles of the major constituents of *A. conyzoides* essential oil were evaluated using the ProTox 3.0 (DL-AOT-based) prediction platform, and the results are summarized in **Table 10**. The predicted LD_{50} values of the essential oil constituents showed noticeable variation, ranging from 750 to 15380 mg/kg body weight, depending on the chemical structure of the compounds. According to the ProTox classification system, *α*-pinene, sabinene, and 3-carene were assigned to toxicity class VI (LD_{50} = 15380 mg/kg), indicating very low acute oral toxicity. Eucalyptol, caryophyllene, and nerolidol were classified as class V (LD_{50} = 2450–5000 mg/kg), suggesting low acute toxicity. By comparison, humulene and isomyocorene showed reduced predicted LD_{50} values of 750 mg/kg and were classified under toxicity class III, corresponding to a moderate level of acute toxicity.

Overall, the ProTox 3.0 (DL-AOT-based) predictions suggest that most *A. conyzoides* essential oil constituents exhibit low to very low acute oral toxicity, although structural differences among the compounds result in varying toxicity classes. When considered together with the favorable physicochemical and pharmacokinetic properties predicted by SwissADME, these findings provide supportive *in silico* evidence regarding the safety profile of the essential oil constituents. Nevertheless, further experimental studies are required to validate these toxicity predictions and to better define safe exposure levels.

4 Discussion

The chemical composition of *Ageratum conyzoides* essential oil from Dak Lak Province differed markedly from those reported for other geographic regions, reflecting pronounced chemotypic variability within the species. In this study, a total of 33 peaks were detected, of which 26 were identified accounting for 78.8 % of the oil were identified, with a predominance of mono- and sesquiterpenes, including α-pinene, sabinene, 3-carene, eucalyptol, caryophyllene, nerolidol, and isomyocorene (**Table 1**). In contrast, essential oils from West Africa are typically characterized by chromene-rich chemotypes, where demethoxyageratochromene (precocene I) and *β*-caryophyllene predominate, as reported for Côte d'Ivoire, Ghana, and Nigeria[19–21]. Similarly, Asian populations, including samples from South China, have shown profiles dominated by ageratochromene (precocene II), *β*-caryophyllene, and related chromene derivatives[22]. Within Vietnam, earlier studies reported chromene-dominated oils, whereas more recent investigations revealed mixed profiles containing both terpenoids and phenolic compounds[10, 11]. Compared with these reports, the Dak Lak sample exhibited a terpene-rich profile with relatively low levels of chromene derivatives, indicating a distinct chemotype. This observation suggests that the essential oil from Dak Lak may represent a terpene-rich chemotype, which may be influenced by local environmental and ecological factors. Such variation is commonly attributed to environmental and ecological factors, including climate, soil characteristics, altitude, and seasonal variation, which influence secondary metabolite biosynthesis. However, as the present study is based on a single sample, these observations should be considered preliminary, and further investigations involving multiple samples across different regions and seasons are required to confirm the influence of geographic and environmental factors. Consistent with this, significant seasonal and diurnal variations in major constituents have been reported for *A. conyzoides*[23]. A quantitative comparison further supports this distinction. In previously reported chromene-dominated chemotypes, precocene I and precocene II typically account for more than 60–80 % of the total essential oil composition[19–21], whereas Asian populations showed chromene-dominated profiles with more variable proportions[22]. In the present study, these chromene derivatives were detected only in minor amounts, while terpene constituents constituted the dominant fraction of the oil, indicating a clear shift in the relative abundance of major chemical classes. These differences further highlight the pronounced chemotypic variability of *A. conyzoides*, although the extent to which geographic origin alone accounts for this variation remains to be clarified. This pronounced difference provides strong evidence for the classification of the Dak Lak sample as a terpene-rich chemotype rather than a chromene-dominated type. Overall, the terpene-rich composition of the Dak Lak essential oil expands current knowledge of chemotypic diversity in *A. conyzoides* and provides a plausible chemical basis for the biological activities observed in this study.

Regarding antioxidant activity, the DPPH assay demonstrated that *A. conyzoides* essential oil from Dak Lak exhibited strong radical scavenging capacity, with an IC_{50} value of 6.03 ± 0.4 mg/mL, comparable to that of BHT (IC_{50} = 6.10 ± 0.10 mg/mL) (**Table 2**). This result indicates a notable antioxidant potential of the essential oil. Previous studies have reported antioxidant activity of *A. conyzoides*, although considerable variation has been observed depending on geographic origin and chemical composition. For instance, Usman et al. reported moderate DPPH scavenging activity for Nigerian

essential oil[19], which was associated with chromene- and sesquiterpene-dominated constituents, particularly precocene I and *β*-caryophyllene. In a comprehensive review, Okunade[18] emphasized the contribution of terpenoids and phenolic compounds to the antioxidant properties of this species. In many of these reports, however, the antioxidant activity was lower than that observed in the present study, likely reflecting differences in chemotype and the relative abundance of oxygenated constituents. Within Vietnam, antioxidant activity has been more frequently reported for solvent extracts rather than essential oils. Can and Trinh demonstrated that a 70 % ethanol extract of *A. conyzoides* leaves exhibited notable DPPH scavenging activity (IC_{50} = 131.74 μg/mL)[15], attributed mainly to its high polyphenol and flavonoid contents. The relatively strong activity observed for the Dak Lak essential oil may be associated with its terpene-rich composition, including caryophyllene, nerolidol, α-pinene, and oxygenated compounds such as eucalyptol, which are known to scavenge free radicals through hydrogen donation and stabilization mechanisms. Importantly, the antioxidant activity observed in the present study should not be attributed exclusively to one or two major constituents, such as precocene I or *β*-caryophyllene. Essential oils are complex multicomponent systems, and their biological activities are often influenced by additive or synergistic interactions among both major and minor constituents. Therefore, the observed DPPH scavenging activity likely reflects the combined contribution of multiple terpenoid and oxygenated constituents present in the essential oil. This observation is consistent with the chemotype analysis discussed above, where the predominance of terpenoid constituents distinguishes the Dak Lak sample from chromene-dominated chemotypes, which have generally been associated with more moderate antioxidant activity[19]. Overall, these results highlight the influence of chemotype and geographic origin on the antioxidant capacity of *A. conyzoides*.

Regarding antibacterial activity, *A. conyzoides* essential oil exhibited a clear concentration-dependent inhibitory effect against *E. coli*, with inhibition zone diameters increasing from 4.0 mm at 1.00 mg/mL to 27.0 mm at 3.00 mg/mL (**Table 3**). Although ciprofloxacin showed stronger activity (IZD = 34.0 mm at 1.00 mg/mL), the essential oil still demonstrated moderate antibacterial potential against this Gram-negative bacterium. Previous studies have reported antibacterial activity of *A. conyzoides*, although the magnitude varies depending on geographic origin and chemical composition. For example, Nigerian essential oil with chromene- and sesquiterpene-rich profiles showed moderate activity against *E. coli*[19], while studies in Vietnam have mainly focused on solvent extracts rather than essential oils[12, 38]. In addition, Gram-negative bacteria are generally less susceptible due to the presence of an outer membrane barrier[16]. The observed antibacterial activity may be partially associated with the terpene-rich composition of the Dak Lak essential oil. Terpenoid constituents such as caryophyllene, nerolidol, and α-pinene have previously been reported to disrupt bacterial membranes, leading to increased permeability and impairment of essential cellular functions[18, 19]. This observation is consistent with the chemotype analysis discussed above, where the terpene-rich profile of the Dak Lak sample differs markedly from chromene-dominated chemotypes, which have generally been associated with weaker or moderate antibacterial activity[19]. Although only a single bacterial strain was evaluated, the results indicate moderate and selective antibacterial activity of the essential oil. While previous studies have reported the antioxidant and antimicrobial activities of *A. conyzoides*, the specific compounds responsible for these effects remain insufficiently explored. In this study, this gap is addressed by associating the observed bioactivities with the major terpenoid constituents based on compositional analysis and molecular docking. Further studies involving additional strains and MIC determination are needed to more comprehensively assess its antimicrobial spectrum and potency.

Regarding α-glucosidase inhibitory activity, *A. conyzoides* essential oil exhibited a concentration-dependent effect, with inhibition increasing from 14.58 % at 46.875 μg/mL to 85.84 % at 1500 μg/mL (**Table 5**). The estimated IC_{50} value of 415.4 μg/mL indicates moderate inhibitory potency compared with acarbose (IC_{50} = 18.1 μg/mL), which is expected given that acarbose is a purified drug, whereas the essential oil is a complex mixture of volatile constituents. Previous studies on *A. conyzoides* have primarily focused on antidiabetic effects of solvent extracts rather than essential oils. Okunade highlighted the role of secondary metabolites in glycemic regulation, although α-glucosidase inhibition has mainly been attributed to non-volatile constituents[18]. More generally, α-glucosidase inhibition is a key mechanism underlying the antidiabetic effects of plant-derived compounds, particularly phenolics and terpenoids[38]. Although essential oils typically exhibit weaker inhibitory activity than phenolic-rich extracts. For example, Loizzo et al. demonstrated that selected terpenes showed measurable inhibition of α-glucosidase *in vitro*[39]. In addition to the inherent activity of individual constituents, the moderate α-glucosidase inhibitory effect observed for the essential oil may also arise from synergistic interactions among its major terpenoid components. Compounds such as caryophyllene and nerolidol, which exhibited relatively stronger binding affinities in docking simulations, may contribute to the observed inhibitory effect through potential complementary hydrophobic interactions within the active site. Although each compound alone may exhibit limited inhibitory potency, their combined presence in a complex mixture could enhance the overall biological effect, as commonly observed in essential oil systems[6]. Previous SAR studies have suggested that terpenoid compounds can interact with α-glucosidase primarily through hydrophobic contacts and partial occupation of the catalytic pocket,

with oxygenated functional groups further contributing to binding stabilization[38–40]. In this context, the presence of both hydrocarbon terpenes (e.g., caryophyllene) and oxygenated terpenoids (e.g., nerolidol) in the essential oil may support a multi-component interaction mechanism, which partially explains the observed inhibitory activity despite its lower potency compared to acarbose. It should be noted that the docking analysis was performed using a yeast α-glucosidase model due to the lack of suitable human structures, and therefore the results should be interpreted as preliminary mechanistic insights rather than direct evidence of activity against human α-glucosidase. Despite structural differences, yeast α-glucosidase models have been widely used in the literature for initial screening and SAR analysis. However, the exact contribution of individual compounds requires further validation through isolation and bioassay-guided studies. This activity may be associated with the terpene-rich chemotype of the Dak Lak essential oil, as discussed above, where the predominance of mono- and sesquiterpenes distinguishes it from chromene-dominated chemotypes and may contribute to its enzyme-modulating effects. Although weaker than acarbose, such activity may still be relevant in multifunctional natural systems, where combined antioxidant, antibacterial, and enzyme inhibitory activities contribute synergistically to overall biological effects.

Molecular docking was performed to provide mechanistic support for the antibacterial and α-glucosidase inhibitory activities observed *in vitro*. However, the docking results should be interpreted only as supportive mechanistic hypotheses and do not constitute direct experimental evidence of ligand-target interactions. The major terpenoid constituents of *A. conyzoides* essential oil exhibited moderate predicted binding energies toward DNA GyrB (6F86) and α-glucosidase (3AJ7), with interactions mainly governed by hydrophobic contacts within the active-site pockets[24, 36]. For DNA GyrB, larger and oxygenated terpenoids, such as nerolidol and caryophyllene, showed more favorable interaction profiles than smaller hydrocarbon monoterpenes, which may partially contribute to the antibacterial activity observed against *E. coli*. Although the predicted binding affinities were lower than those of the reference drug ciprofloxacin, the collective interactions of multiple constituents may account for the observed antibacterial effect, which is typical of terpene-rich essential oils[18, 19]. Similarly, in the α-glucosidase model, the essential oil constituents displayed moderate binding energies, whereas acarbose formed extensive hydrogen-bonding interactions with catalytic residues, explaining its higher inhibitory potency[37, 38]. These results suggest that the enzyme inhibition observed for the essential oil arises from cumulative and relatively nonspecific interactions rather than a single high-affinity ligand, consistent with previous reports on terpene-enzyme interactions[39]. Because the biological assays in the present study were performed using the whole essential oil rather than isolated compounds, the specific contribution of each docked constituent to DNA GyrB or α-glucosidase inhibition cannot be confirmed. Therefore, the docking results should be regarded as supportive computational evidence only, and future studies using commercially available or isolated compounds are required to validate their direct enzyme inhibitory effects. Notably, these findings are in line with the chemotype analysis discussed above, where the terpene-rich profile of the Dak Lak essential oil supports a multi-component interaction mechanism underlying its biological activities. An *in silico* ADMET evaluation further indicated that most terpenoids possess low molecular weights, high lipophilicity, and limited hydrogen-bonding capacity, resulting in acceptable predicted bioavailability despite minor Lipinski violations[31, 32]. ProTox 3.0 predictions suggested low to very low acute toxicity for the major constituents[33]. However, as these results are based on computational models, further *in vivo* studies are required to confirm their safety and pharmacological relevance. The ADMET predictions further support the experimental findings by indicating that the major terpenoid constituents possess physicochemical properties favorable for membrane permeability and bioavailability. Their relatively high lipophilicity and low molecular weight may facilitate interactions with biological membranes, which is consistent with the observed antibacterial activity against *E. coli*. In addition, the predicted pharmacokinetic profiles indicate that these compounds possess physicochemical characteristics compatible with potential biological interactions, supporting the moderate α-glucosidase inhibitory activity observed *in vitro*. Nevertheless, as these predictions are based on *in silico* models, they should be interpreted with caution and require further experimental validation.

Despite the comprehensive experimental and computational approach employed, several limitations should be acknowledged. The biological activities were evaluated exclusively using *in vitro* models; therefore, the observed antioxidant, antibacterial, and α-glucosidase inhibitory effects may not fully translate to *in vivo* conditions. Although these assays provide reliable tools for preliminary screening, further pharmacological studies using appropriate animal models are required to confirm their physiological relevance. The antibacterial activity was initially assessed using a disc diffusion assay and further evaluated using MIC determination. While inhibition zone diameters provide a preliminary indication of antimicrobial effects, particularly for hydrophobic essential oils, MIC values offer a more reliable quantitative assessment. However, the absence of MBC determination represents a limitation, and future studies incorporating MBC measurements would provide deeper insight into the bactericidal potential of the essential oil. In addition, molecular docking and ADMET analyses were performed using *in silico* approaches, which provide

valuable insights into ligand-target interactions, pharmacokinetic behavior, and safety-related properties. However, these predictions do not fully capture dynamic biological processes such as metabolism, bioavailability, or potential synergistic interactions among multiple constituents. Therefore, the results should be considered indicative and require experimental validation. Finally, the essential oil analyzed in this study was obtained from a single harvest at a specific geographic location and season. Although this allows accurate characterization of a defined chemotype, it does not account for environmental variability. Given the known chemotypic diversity of *A. conyzoides*, future studies involving multiple collection sites, seasonal variation, and comparative chemotype analysis would further strengthen the general applicability of these findings.

5 Conclusion

An integrated combination of chemical characterization and biological evaluation was applied to elucidate the properties of *A. conyzoides* essential oil. GC-MS profiling revealed a terpene-dominated composition mainly consisting of mono- and sesquiterpenoids, which corresponded well with the biological responses observed. The essential oil exhibited notable free radical scavenging activity, moderate and selective antibacterial activity against *E. coli*, and moderate α-glucosidase inhibitory activity, collectively highlighting its multifunctional bioactive potential. Molecular docking analysis provided preliminary supportive mechanistic insights into the antibacterial and α-glucosidase inhibitory effects, suggesting that these activities may arise from additive hydrophobic interactions of multiple terpenoid constituents rather than from a single high-affinity compound. Importantly, the present study does not establish direct target-based inhibition of DNA GyrB or α-glucosidase by individual essential oil constituents. Further validation using isolated compounds and enzyme-based assays will be necessary to identify the principal active components. *In silico* ADMET predictions further indicated generally favorable pharmacokinetic properties and low to moderate predicted toxicity at the preliminary screening level, supporting the potential applicability of the essential oil at an exploratory stage. Notably, humulene and isomyocorene were predicted to belong to toxicity class III, indicating moderate acute toxicity. This limitation should be considered in future studies focused on safety evaluation and therapeutic development. Overall, these findings suggest that *A. conyzoides* essential oil represents a potential natural source of bioactive compounds, although its antimicrobial activity appears to be selective and moderate. Future studies focusing on MBC determination, broader antimicrobial evaluation, and detailed mechanistic investigations will further clarify its pharmacological potential. Nevertheless, further *in vivo* pharmacological and toxicological studies are required to confirm its efficacy, safety, and therapeutic potential.

Ethical Approval

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and materials

All the data generated in this research work has been included in the manuscript.

Author Contributions

Conceptualization, Truong Nhan Ngu and Dao Cuong To; methodology, Truong Nhan Ngu, Phuong Dai Nguyen Nguyen, Minh Quan Pham, Phi Hung Nguyen, Viet Tuan Trinh, Tien Khi Nguyen, and Dao Cuong To; software, Minh Quan Pham and Dao Cuong To; validation, Minh Quan Pham, Phi Hung Nguyen, and Dao Cuong To; formal analysis, Thi Kim An Nguyen, Manh Ha Nguyen, Truong Nhan Ngu, Thi Bich Hanh Dam, Minh Quan Pham, and Thi Thuy Do; investigation, Thi Kim An Nguyen, Manh Ha Nguyen, Truong Nhan Ngu, Thi Bich Hanh Dam, Minh Quan Pham, and Thi Thuy Do; resources, Truong Nhan Ngu and Thi Bich Hanh Dam; data curation, Thi Kim An Nguyen, Truong Nhan Ngu, Thi Bich Hanh Dam, Minh Quan Pham, and Dao Cuong To; writing—original draft preparation, Truong Nhan Ngu, Minh Quan Pham, and Dao Cuong To; writing—review and editing, Phi Hung Nguyen, Viet Tuan Trinh, Tien Khi Nguyen, and Dao Cuong To; visualization, Minh Quan Pham; supervision, Phuong Dai Nguyen Nguyen and Dao Cuong To; project administration, Dao Cuong To; funding acquisition, Dao Cuong To.

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