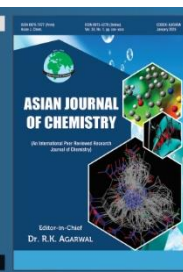




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Chemical Composition, Biological Activities and Molecular Docking Study of *Euphorbia pulcherrima* Leaf Essential Oil from Dak Lak, Vietnam

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Euphorbia pulcherrima is widely cultivated in tropical and subtropical regions, although information on the chemical composition and bioactivities of its essential oil remains limited. In this study, the chemical constituents, antioxidant, anti-inflammatory and angiotensin-converting enzyme (ACE) inhibitory activities of *E. pulcherrima* leaf essential oil from Dak Lak Province, Vietnam, were investigated together with molecular docking and *in silico* ADMET analyses. GC-MS analysis identified 24 compounds representing 93.40% of the total oil, with menthone, longifolene, bornyl acetate, α -terpinolene, aromadendrene and β -bourbonene as the major constituents. The essential oil showed moderate antioxidant and anti-inflammatory activities, with IC₅₀ values of 40.98 and 41.88 μ g/mL, respectively, and weak ACE inhibitory activity (IC₅₀ = 836.75 μ g/mL). Docking analysis identified favourable interactions between several terpenoids and ACE- and inflammation-related targets, providing a preliminary basis for further study of the essential oil and its constituents without establishing individual compound activity.

Keywords: *Euphorbia pulcherrima*, Essential oil, GC-MS, Antioxidant activity, Anti-inflammatory activity, ACE inhibition.

INTRODUCTION

Euphorbia pulcherrima Willd. ex Klotzsch, commonly known as poinsettia, belongs to the family Euphorbiaceae and is widely cultivated as an ornamental and medicinal plant in tropical and subtropical regions. The species is native to Mexico and has been widely introduced elsewhere for its colourful bracts and adaptability to varied environmental conditions [1,2]. In Vietnam, *E. pulcherrima* is commonly distributed in the Central Highlands and southern provinces, particularly in Dak Lak Province, where favourable climatic conditions support its growth throughout the year. In traditional medicine, different parts of the plant have been used for the treatment of skin disorders, constipation, inflammation and several other ailments [3]. Previous phytochemical studies have identified various secondary metabolites in *E. pulcherrima*,

such as terpenoids, flavonoids, steroids and triterpenes, which may contribute to its pharmacological effects [4-6].

Natural products rich in volatile terpenoids have received considerable attention for their antioxidant and anti-inflammatory properties. Excessive production of reactive oxygen species (ROS) causes oxidative stress and contributes to the development of chronic conditions, particularly inflammation and cardiovascular disorders [7,8]. Essential oils with radical scavenging activity may, therefore, represent valuable natural sources for antioxidant agents. Their potential to modulate inflammatory responses provides a further basis for investigating terpenoid-rich essential oils in inflammation-related models.

Inflammation involves the activation of immune cells and the release of several inflammatory mediators, among which nitric oxide (NO) has an important role. Excessive NO pro-

duction by inducible nitric oxide synthase (iNOS) in activated macrophages can contribute to tissue injury and the progression of inflammatory diseases [9,10]. For this reason, inhibition of NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages is widely used to assess the anti-inflammatory potential of natural products [11,12]. Several terpenoid-rich essential oils have been reported to suppress NO production through modulation of inflammatory signaling pathways [13]. Beyond their effects on oxidative and inflammatory processes, such natural products may have relevance to cardiovascular health, where angiotensin-converting enzyme (ACE) represents an important therapeutic target.

ACE catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor involved in blood pressure regulation [14]. Although synthetic ACE inhibitors such as captopril are effective, their prolonged use may cause adverse effects, prompting interest in natural ACE inhibitors from medicinal plants and essential oils [14-16]. Despite the reported traditional and biological significance of *E. pulcherrima*, its essential oil remains poorly studied, particularly its chemical composition and ACE-inhibitory activity in leaf oil collected from Vietnam.

Although GC-MS profiling, *in vitro* bioassays, molecular docking and absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction are established approaches in essential-oil research, the chemical and biological profile of *E. pulcherrima* leaf essential oil collected in Dak Lak Province, Vietnam, remains insufficiently documented. The present study focuses on characterizing the GC-MS profile of locally collected *E. pulcherrima* leaf essential oil and evaluating its DPPH radical-scavenging, LPS-stimulated NO inhibitory and ACE-inhibitory activities. To our knowledge, ACE inhibition by *E. pulcherrima* leaf essential oil has not been specifically reported. Molecular docking and *in silico* ADMET analyses were used for contextualizing the experimental findings, rather than as methodological innovations.

EXPERIMENTAL

A homologous series of *n*-alkanes (C7-C30) used for the calculation of retention indices was obtained from Sigma-Aldrich (St. Louis, MO, USA). Lipopolysaccharide (LPS), Dulbecco's Modified Eagle Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Griess reagent, angiotensin-converting enzyme from rabbit lung (ACE, EC 3.4.15.1), hippuryl-L-histidyl-L-leucine (HHL), hippuric acid, benzene sulphonyl chloride (BSC), captopril and DPPH were purchased from Sigma-Aldrich (St. Louis, MO, USA). The RAW264.7 macrophage cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All solvents and chemicals used throughout the study were of analytical grade, including methanol, diethyl ether, pyridine, hydrochloric acid, boric acid, dimethyl sulfoxide (DMSO) and anhydrous sodium sulphate. Helium (99.999%) used as the carrier gas for GC-MS analysis was supplied by Messer Vietnam Co., Ltd. (Ho Chi Minh City, Vietnam).

Plant material: Fresh leaves of *E. pulcherrima* were harvested in March 2026 from Ea Kao Commune, Dak Lak Province, Vietnam (12°56'52.0" N, 108°16'45.0" E). Plant

authentication was performed and a voucher specimen (EP-L.01032026) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Dak Lak Province, Vietnam, for future taxonomic reference.

Essential oil isolation: Fresh leaves of *E. pulcherrima* were carefully washed to remove surface contaminants, rinsed with distilled water and left to drain at ambient temperature for approximately 2.5 h prior to air-drying for an additional 2 h. Subsequently, 100 g of finely cut plant material was introduced into a Clevenger-type apparatus and hydrodistilled with 1000 mL of distilled water for 80 min under controlled heating conditions. After condensation, the distillate was separated into aqueous and essential oil layers. The essential oil fraction was recovered using diethyl ether, dehydrated over anhydrous sodium sulphate and concentrated under reduced pressure to obtain the crude essential oil.

GC-MS analysis: The chemical composition of *E. pulcherrima* essential oil was analyzed using a GC-MS system consisting of an Agilent 8890 GC System coupled with a 7000E triple quadrupole mass spectrometer (7000E GC/TQ, Agilent Technologies, Santa Clara, CA, USA). Separation of volatile constituents was performed on an HP-5MS fused-silica capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Data acquisition and processing were carried out using MassFinder software version 4.0. The gas chromatograph was operated under the following conditions: injector temperature, 250 °C; transfer line temperature, 260 °C; oven temperature programmed from 60 to 260 °C at a rate of 4 °C/min; helium as the carrier gas at a constant flow rate of 1.0 mL/min and an 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 at 70 eV. The interface, ion source and quadrupole temperatures were maintained at 280 °C, 230 °C and 200 °C, respectively and mass spectra were recorded over an *m/z* range of 35-650 amu [17]. Retention indices (RI) were calculated relative to a homologous series of *n*-alkanes (C7-C30) analyzed under identical chromatographic conditions. Compound identification was performed by comparison of the obtained mass spectra with those from the NIST 20 mass spectral library and by matching the calculated RI values with published literature data. Authentic reference standards were not employed in the present study. The relative abundance of each component was expressed as percentage peak area normalization without applying response correction factors.

DPPH radical scavenging assay: The antioxidant activity of *E. pulcherrima* essential oil was evaluated using the DPPH radical scavenging assay with slight modifications [18]. DPPH is a stable free radical in methanol with a maximum absorbance at 517 nm. Different concentrations (20, 35, 50, 65 and 80 µg/mL) of the essential oil dissolved in methanol, together with ascorbic acid used as the positive control, were mixed with 3 mL of 0.1 mM DPPH methanolic solution. The reaction mixtures were vigorously shaken and incubated in the dark at room temperature for 30 min to allow the reaction to proceed completely. The absorbance of the resulting solutions was measured at 517 nm using an Agilent Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) against a blank solution containing methanol without

sample or ascorbic acid. All experiments were performed in triplicate. The percentage of DPPH radical scavenging activity was calculated according to the following eqn. 1:

$$\text{Inhibition (\%)} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100 \quad (1)$$

where $\text{OD}_{\text{control}}$ is the absorbance of the control solution without sample, $\text{OD}_{\text{sample}}$ is the absorbance of the tested sample and OD_{blank} is the absorbance of the blank methanol solution.

The antioxidant activity was expressed as IC_{50} value, defined as the concentration required to scavenge 50% of DPPH radicals under the assay conditions. A lower IC_{50} value indicates stronger antioxidant activity.

Inhibition of NO production in RAW264.7 macrophages: The anti-inflammatory activity of *E. pulcherrima* essential oil was evaluated based on the inhibition of NO production in LPS-stimulated RAW264.7 macrophages according to previously reported methods with slight modifications [9,10]. RAW264.7 cells were cultured in DMEM supplemented with 10% fetal bovine serum under humidified conditions at 37 °C in a 5% CO_2 atmosphere for 48 h. The cells were then seeded into 96-well plates at a density of 2.5×10^5 cells/well. After incubation, the cells were stimulated with 2 μL of LPS solution (0.1 mg/mL) for 24 h in the presence of various concentrations (16, 32, 64, 128 and 256 $\mu\text{g/mL}$) of the tested samples. Cardamonin was used as the positive control. Following treatment, the culture supernatants were mixed with Griess reagent and the nitrite concentration was determined using sodium nitrite as a standard reference. The absorbance of the reaction mixtures was measured at 570 nm using a microplate reader. The percentage inhibition of NO production was calculated according to the following eqn. 2:

$$\text{Inhibition (\%)} = \frac{\text{NO}_{\text{sample}} - \text{NO}_{\text{LPS}}}{\text{NO}_{\text{control}} - \text{NO}_{\text{LPS}}} \times 100 \quad (2)$$

where NO represents the average NO concentration calculated from the NaNO_2 standard calibration curve.

Cell viability was evaluated using the MTT assay. After removal of the supernatants for NO determination, the remaining cells were treated with MTT solution (0.5 mg/mL in PBS) and incubated for 4 h at 37 °C in a humidified 5% CO_2 atmosphere. The resulting formazan crystals were dissolved in DMSO and absorbance was recorded at 540/720 nm using an Infinite F50 microplate reader (Tecan, Switzerland).

Cell viability was expressed as the percentage survival relative to the untreated control according to the following eqn. 3:

$$\text{Cell survival (\%)} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100 \quad (3)$$

All experiments were carried out in triplicate and the results were expressed as mean $\pm$ standard deviation (SD).

ACE inhibitory assay: The ACE inhibitory activity of *E. pulcherrima* essential oil was evaluated according to reported methods with slight modifications [14,16]. The assay was performed in flat-bottom 96-well microplates with a total reaction volume of 50 μL containing 20 μL of test sample at various concentrations (62.5, 125, 250, 500 and 1000 $\mu\text{g/mL}$),

10 μL of ACE solution (100 mU/mL) and 20 μL of HHL (12.5 mM) as the substrate. The reaction mixtures were incubated at 37 °C for 30 min. The enzymatic reaction was terminated by the addition of 40 μL of 1 M HCl. Subsequently, 80 μL of pyridine and 40 μL of BSC were added to the reaction mixtures. The mixtures were vortexed on ice for 1 min and the absorbance was measured at 410 nm using a TECAN GENios microplate reader (Tecan Group Ltd., Switzerland). The percentage inhibition of ACE activity was calculated using the following eqn. 4:

$$\text{Inhibition (\%)} = \frac{\text{A410}_{\text{control}} - \text{A410}_{\text{sample}}}{\text{A410}_{\text{control}} - \text{A410}_{\text{blank}}} \times 100 \quad (4)$$

where the control wells contained solvent instead of the test sample, while the blank wells contained reaction buffer in place of the HHL substrate. Captopril was used as the positive control.

The IC_{50} value, defined as the concentration required to inhibit 50% of ACE activity under the assay conditions, was calculated using TableCurve software (Systat Software Inc., San Jose, CA, USA).

Molecular docking and in silico ADMET prediction: Target structure selection was based on the relevance of each protein to the biological endpoints. The reported X-ray resolution and the availability of a co-crystallized ligand defining a relevant binding region, where applicable. iNOS was represented by PDB ID 3E7G, an X-ray structure of the human iNOS oxygenase domain determined at 2.20 Å resolution with the inhibitor AR-C95791 [19]. This structure was selected since iNOS is directly involved in NO production by activated macrophages [9,10]. COX-2 was represented by PDB ID 5IKQ, a 2.41 Å X-ray structure of human COX-2 in complex with meclofenamic acid [20] and was included as a complementary inflammation-related enzyme. IL-8/CXCL8 was represented by PDB ID 5D14, a 1.00 Å X-ray structure of human IL-8 [21]. As this structure lacks a co-crystallized small-molecule ligand, its docking site was selected using the DoGSiteScorer-predicted pocket [22] and the corresponding results were considered exploratory. ACE was represented by PDB ID 1O86, a 2.00 Å X-ray structure of human testicular ACE in complex with lisinopril [23]. This structure was selected because ACE was directly evaluated in the HHL-based assay and the lisinopril complex provides a structurally characterized inhibitor-binding region. Human structures were used as structurally characterized mammalian surrogates for the murine RAW264.7 cellular model and the rabbit-lung ACE preparation used in the assays; therefore, the docking results were not interpreted as species-specific mechanistic proof. The selected structures were downloaded from the RCSB Protein Data Bank. Prior to docking simulations, all protein structures were processed in UCSF Chimera version 1.19 by removing crystallographic water molecules and co-crystallized inhibitor molecules, followed by the addition of polar hydrogens and assignment of Gasteiger partial charges using the Dock Prep utility.

The principal volatile constituents identified in the *E. pulcherrima* essential oil, together with the reference compounds cardamonin and captopril, were generated from their corresponding PubChem entries using the Build Structure module in UCSF Chimera. Ligand preparation involved

hydrogen addition, charge assignment and geometry optimization through energy minimization. The optimized protein and ligand structures were then converted into PDBQT format for molecular docking calculations.

Docking analyses were conducted using AutoDock Vina version 1.2.5 integrated within the UCSF Chimera platform. Putative binding pockets were identified using the DoGSite-Scorer server and, where available, cross-checked against the binding regions occupied by the co-crystallized ligands. For PDB 5D14, which lacks a co-crystallized small-molecule ligand, the selected site was treated as a predicted surface pocket and the corresponding docking results were interpreted as exploratory [22]. Docking grid boxes were defined to encompass the predicted active regions and interacting amino acid residues. The docking procedure employed the Broyden-Fletcher-Goldfarb-Shanno (BFGS) optimization algorithm under default AutoDock Vina settings, generating up to ten binding poses for each ligand. The resulting complexes were ranked according to binding affinity values and the conformations with the lowest binding energies were chosen for further interaction analysis.

All computational studies were performed on a workstation operating under Windows 10 Pro with an Intel® Core™ i5 processor (2.53 GHz). Drug-likeness characteristics of the

investigated compounds were assessed based on Lipinski's rule of five [24]. Pharmacokinetic behaviour and ADMET-related properties were predicted using the SwissADME web platform [25], whereas acute oral toxicity estimation was carried out using the ProTox-3 online server [26].

Statistical analysis: All experimental assays were performed in triplicate and the results are expressed as mean $\pm$ standard deviation (SD). The percentage inhibition values for DPPH radical scavenging, NO production, cell viability and ACE activity were calculated relative to the corresponding control groups. The IC₅₀ values, defined as the concentration required to produce 50% inhibition, were determined from the concentration–response curves using nonlinear regression analysis.

RESULTS AND DISCUSSION

The essential oil of *E. pulcherrima* was obtained by hydro-distillation, with a yield of 0.58% (w/w, based on fresh weight). The chemical composition of the essential oil was subsequently determined by GC-MS and the results are shown in Table-1. A total of 24 compounds were identified, representing 93.40% of the total essential oil composition. The essential oil was predominantly composed of oxygenated monoterpenes

TABLE-1
LIST OF CHEMICAL COMPOSITION OF *E. pulcherrima* ESSENTIAL OIL DETERMINED BY GC-MS

Peak	Retention time (min)	Compound	Molecular formula	RI exp.	RI lit.	Relative content (%)
1	7.12	6-Methyl-5-hepten-2-one	C ₈ H ₁₄ O	985	982	1.52
2	10.36	α -Terpinolene	C ₁₀ H ₁₆	1081	1088	9.19
3	11.44	Nonanal	C ₉ H ₁₈ O	1110	1100	0.85
4	13.07	Menthone	C ₁₀ H ₁₈ O	1150	1153	16.83
5	15.12	Decanal	C ₁₀ H ₂₀ O	1201	1200	0.72
6	18.06	Bornyl acetate	C ₁₂ H ₂₀ O ₂	1281	1285	14.85
7	18.70	Undecanal	C ₁₁ H ₂₂ O	1286	1300	0.78
8	22.73	β -Bourbonene	C ₁₅ H ₂₄	1382	1384	7.61
9	23.35	Dodecanal	C ₁₂ H ₂₄ O	1397	1400	1.95
10	23.66	Longifolene	C ₁₅ H ₂₄	1404	1408	16.61
11	25.07	Aromadendrene	C ₁₅ H ₂₄	1439	1441	8.92
12	26.65	Tridecanal	C ₁₃ H ₂₆ O	1499	1500	1.10
13	27.63	Pentadecane	C ₁₅ H ₃₂	1503	1500	0.66
14	28.91	Nerolidol	C ₁₅ H ₂₆ O	1536	1530	1.73
15	31.43	Hexadecane	C ₁₆ H ₃₄	1602	1600	1.52
16	31.55	Tetradecanal	C ₁₄ H ₂₈ O	1605	1600	0.65
17	35.10	Heptadecane	C ₁₇ H ₃₆	1701	1700	0.89
18	35.21	Pentadecanal	C ₁₅ H ₃₀ O	1704	1700	0.81
19	38.72	Octadecane	C ₁₈ H ₃₈	1804	1800	0.56
20	39.98	Hexadecanal	C ₁₆ H ₃₂ O	1802	1800	0.90
21	42.11	Nonadecane	C ₁₉ H ₄₀	1906	1900	1.06
22	43.47	Phytol	C ₂₀ H ₄₀ O	1948	1946	1.69
23	45.21	Icosane	C ₂₀ H ₄₂	2002	2000	0.86
24	48.41	Heneicosane	C ₂₁ H ₄₄	2102	2100	1.14
Monoterpene hydrocarbons (%)						9.19
Oxygenated monoterpene (%)						33.20
Sesquiterpene hydrocarbons (%)						33.14
Oxygenated sesquiterpenes (%)						1.73
Other compounds (%)						16.14
Total identified compounds (%)						93.40

RI exp: experimentally determined retention index on an HP-5MS capillary column relative to a homologous series of *n*-alkanes (C7–C30); RI lit: retention index values reported in the literature under similar chromatographic conditions; Relative content (%): percentage peak area obtained by GC normalization without correction factors.

(33.20%) and sesquiterpene hydrocarbons (33.14%), followed by other compounds (16.14%), monoterpene hydrocarbons (9.19%) and oxygenated sesquiterpenes (1.73%). Menthone (16.83%) was the major constituent, followed by longifolene (16.61%), bornyl acetate (14.85%), α -terpinolene (9.19%), aromadendrene (8.92%) and β -bourbonene (7.61%). Together, these major constituents represented approximately 74% of the oil. The data establish a terpene-rich chemical profile, although the crude oil composition does not allow the biological effects to be assigned to any individual constituent.

Several aliphatic aldehydes and hydrocarbons occurred at lower abundances. The detected aldehydes comprised nonanal (0.85%), decanal (0.72%), undecanal (0.78%), dodecanal (1.95%), tridecanal (1.10%), tetradecanal (0.65%), pentadecanal (0.81%) and hexadecanal (0.90%). Long-chain hydrocarbons such as pentadecane (0.66%), hexadecane (1.52%), heptadecane (0.89%), octadecane (0.56%), nonadecane (1.06%), icosane (0.86%) and heneicosane (1.14%) were present as minor constituents. Nerolidol (1.73%) and phytol (1.69%) represented additional oxygenated terpenoids detected in the oil.

The experimentally determined retention indices obtained using the HP-5MS column were generally consistent with reported literature values, which supported the identification of the detected compounds. The high proportions of oxygenated monoterpenes and sesquiterpene hydrocarbons provide a chemical basis for describing the volatile profile of the oil. The present data, nonetheless, do not establish a direct relationship between these chemical groups and any particular biological activity.

Comparison with previous reports highlights differences in the chemical composition of *E. pulcherrima* preparations. Dominguez *et al.* [4] reported triterpenoids and sterols such as germanicol, β -amyrin, pseudotaraxasterol, pulcherrol and β -sitosterol in extracts of the species. Khan *et al.* [5] found that the fixed oil was dominated by fatty acid methyl esters, particularly palmitic acid methyl ester (66.52%) and stearic acid methyl ester (20.62%). The Dak Lak essential oil examined in the present study differed from these preparations, with volatile monoterpenes and sesquiterpenes forming the major chemical fraction. Menthone, longifolene, bornyl acetate, α -terpinolene, aromadendrene and β -bourbonene were the principal constituents. Such differences may arise from variation in plant material, geographical origin, environmental conditions, and extraction procedures. The chemical profile is also compatible with the presence of terpenoids previously reported in *E. pulcherrima* extracts by Sharif *et al.* [6]. Possible interactions

among constituents have been proposed in the literature, but they were not examined experimentally in the present work. The terpene-rich composition should therefore be regarded as a characteristic of the investigated oil rather than evidence for the activity of any particular compound.

Furthermore, phytol was detected at 1.69%, a lower proportion than reported for several related *Euphorbia* species, such as *E. pilosa* (5.75%), *E. thymifolia* (10.36%) and *E. characias* (13.5%) [27]. Differences in the phytol abundance may be associated with plant developmental stage, extraction method, plant organ, and environmental conditions. In poinsettia, bract maturation involves chlorophyll degradation and anthocyanin accumulation, and the breakdown of chlorophyll may contribute to variations in phytol levels [28]. Similarly, the detection of bicyclic sesquiterpenes such as β -bourbonene and aromadendrene further reflects the chemical diversity of the oil. Yu *et al.* [29] reported structurally diverse terpenoid metabolites from *E. pulcherrima*, including Eupulcherol A.

Antioxidant activity: The antioxidant activity of *E. pulcherrima* essential oil was evaluated using the DPPH radical scavenging assay and the results are shown in Table-2. The essential oil showed concentration-dependent radical-scavenging activity across the tested range of 20-80 $\mu\text{g/mL}$. At 20 $\mu\text{g/mL}$, the oil produced $29.35 \pm 1.16\%$ inhibition of DPPH radicals, which increased to $80.97 \pm 0.32\%$ at 80 $\mu\text{g/mL}$. The essential oil exhibited an IC_{50} value of 40.98 ± 0.39 $\mu\text{g/mL}$, consistent with moderate antioxidant activity under the assay conditions. Ascorbic acid, used as the positive control, had a lower IC_{50} value of 15.95 ± 0.16 $\mu\text{g/mL}$ and a correspondingly higher radical-scavenging capacity than the essential oil.

The antioxidant activity may be related to the terpenoid-rich composition of the essential oil, particularly its oxygenated monoterpenes and sesquiterpene hydrocarbons [30]. Terpenoid-containing essential oils have been associated with antioxidant effects through hydrogen-atom transfer and free-radical stabilization [31]. Thus, the relatively high proportion of oxygenated monoterpenes in the present oil may contribute to its DPPH radical-scavenging activity, although the contribution of individual constituents was not established experimentally.

Comparison with previous studies of the *Euphorbiaceae* family provides additional context. Le *et al.* [32] reported DPPH radical-scavenging activity for *E. tirucalli* extracts collected in Binh Thuan Province, with IC_{50} values ranging from 12.91 ± 0.70 to 528.33 ± 25.15 $\mu\text{g/mL}$ [32]. The IC_{50} value

TABLE-2
DPPH RADICAL SCAVENGING ACTIVITY DATA OF *E. pulcherrima* ESSENTIAL OIL

Essential oil concentration ($\mu\text{g/mL}$)	Replicate 1	Replicate 2	Replicate 3	Inhibition (%)	IC_{50} ($\mu\text{g/mL}$)
20	29.68	28.06	30.32	29.35 ± 1.16	40.98 ± 0.39
35	43.55	42.90	43.23	43.23 ± 0.32	
50	59.35	60.00	59.68	59.68 ± 0.32	
65	70.65	71.29	70.97	70.97 ± 0.32	
80	80.65	81.29	80.97	80.97 ± 0.32	
Ascorbic acid					15.95 ± 0.16

Values are expressed as mean $\pm$ SD of three independent experiments ($n = 3$). IC_{50} values represent the concentration required to scavenge 50% of DPPH radicals under the assay conditions. Ascorbic acid was used as the positive control.

obtained for *E. pulcherrima* essential oil was 40.98 ± 0.39 $\mu\text{g/mL}$. Sharma *et al.* [13] reported $27.5 \pm 7.8\%$ DPPH inhibition for *E. hirta* at 100 μL , whereas the present essential oil produced $80.97 \pm 0.32\%$ inhibition at 80 $\mu\text{g/mL}$. The antioxidant activity of *E. pulcherrima* essential oil was comparable to that reported for related *Euphorbiaceae* species, although differences in plant material and assay conditions may affect direct comparisons.

Anti-inflammatory and ACE inhibitory activities: The anti-inflammatory activity of *E. pulcherrima* essential oil was evaluated through inhibition of NO production in LPS-stimulated RAW264.7 macrophages and the results are shown in Table-3. The essential oil exhibited concentration-dependent inhibition of NO production over the tested concentration range of 16–256 $\mu\text{g/mL}$. At the lowest tested concentration (16 $\mu\text{g/mL}$), the essential oil inhibited NO production by $35.35 \pm 1.86\%$, whereas the inhibition increased to $77.56 \pm 2.67\%$ at 256 $\mu\text{g/mL}$. An IC_{50} value of 41.88 $\mu\text{g/mL}$ was obtained for the essential oil, compared with 2.65 $\mu\text{g/mL}$ for cardamomin, which exhibited substantially higher NO-inhibitory activity.

The observed NO inhibition may arise from the combined composition of the essential oil, but the crude-oil assay does not establish the role of any individual terpenoid. Compared with previous reports on *E. hirta*, another species belonging to the genus *Euphorbia*, the essential oil of *E. pulcherrima* collected from Dak Lak Province exhibited comparatively stronger inhibition of NO production. Sharma *et al.* [13] reported that *E. hirta* extracts showed significant NO inhibitory activity only at relatively high concentrations of approximately 200 $\mu\text{g/mL}$. In contrast, the essential oil of *E. pulcherrima* in the present study exhibited measurable inhibitory activity even at a low concentration of 16 $\mu\text{g/mL}$, with an inhibition rate of $35.35 \pm 1.86\%$, which further increased in a concentration-dependent manner to $77.56 \pm 2.67\%$ at 256 $\mu\text{g/mL}$. Moreover, the *E. pulcherrima* essential oil exhibited an IC_{50} of 41.88 $\mu\text{g/mL}$ for NO inhibition, with activity increasing with concentration.

ACE-inhibitory activity: The ACE inhibitory activity of *E. pulcherrima* essential oil was determined using the HHL-based colorimetric assay and the results are shown in Table-3. The essential oil demonstrated dose-dependent inhibition of ACE activity within the concentration range of 62.5–1000 $\mu\text{g/mL}$. The percentage inhibition increased from $11.08 \pm 1.45\%$

at 62.5 $\mu\text{g/mL}$ to $52.67 \pm 2.12\%$ at 1000 $\mu\text{g/mL}$. The essential oil showed weak ACE-inhibitory activity, with an IC_{50} value of 836.75 $\mu\text{g/mL}$, whereas captopril exhibited much stronger activity with an IC_{50} of 3.25 $\mu\text{g/mL}$. The measurable ACE-inhibitory activity observed under the present assay conditions may be related to the total terpenoid composition of the essential oil, although the contribution of individual constituents was not determined. To our knowledge, the ACE-inhibitory activity of *E. pulcherrima* essential oil has not been previously documented. The present study provides experimental evidence of concentration-dependent ACE inhibition by leaf essential oil from Dak Lak Province, although its activity was weak compared with captopril.

Molecular docking studies: Molecular docking was performed for the major volatile constituents of *E. pulcherrima* essential oil against iNOS, COX-2, IL-8 and ACE to provide computational context for the *in vitro* anti-inflammatory and ACE-inhibitory results. Cardamomin and captopril served as reference compounds. For iNOS, β -bourbonene had the most favourable score among the tested constituents (–8.185 kcal/mol), followed by aromadendrene (–7.589 kcal/mol) and β -terpinolene (–7.045 kcal/mol). The interactions were mainly hydrophobic, while bornyl acetate formed an additional hydrogen bond with Gly371, whereas cardamomin had the strongest score (–9.370 kcal/mol).

Against COX-2, β -bourbonene (–7.009 kcal/mol), longifolene (–6.761 kcal/mol) and aromadendrene (–6.511 kcal/mol) had the most favourable scores among the oil constituents. Their binding was dominated by hydrophobic contacts, whereas menthone and bornyl acetate formed hydrogen bonds with Arg377 and Gln375. In this case, cardamomin had a score of –7.809 kcal/mol. For IL-8, the tested constituents gave lower docking scores (–4.071 to –5.402 kcal/mol). Aromadendrene had the strongest score (–5.402 kcal/mol), while cardamomin scored –5.574 kcal/mol and formed additional electrostatic interactions with Asp50 and Glu36.

Against ACE, aromadendrene (–6.959 kcal/mol) and longifolene (–6.857 kcal/mol) had the most favourable scores, followed by β -bourbonene (–6.585 kcal/mol). Hydrophobic contacts with residues such as Tyr523 and Phe457 were prominent. Menthone and bornyl acetate formed hydrogen bonds with Gln530. Several sesquiterpene hydrocarbons had more negative docking scores than captopril (–5.073 kcal/mol),

TABLE-3
ANTI-INFLAMMATORY AND ACE INHIBITORY ACTIVITIES OF *E. pulcherrima* ESSENTIAL OIL

Essential oil ($\mu\text{g/mL}$)	NO inhibition		Essential oil ($\mu\text{g/mL}$)	ACE inhibition	
	Inhibition (%)	IC_{50} ($\mu\text{g/mL}$)		Inhibition (%)	IC_{50} ($\mu\text{g/mL}$)
16	35.35 ± 1.86		62.5	11.08 ± 1.45	
32	45.90 ± 2.45		125	21.48 ± 1.87	
64	56.45 ± 1.98	41.88	250	31.88 ± 2.34	836.75
128	67.01 ± 3.50		500	42.27 ± 1.65	
256	77.56 ± 2.67		1000	52.67 ± 2.12	
Cardamomin		2.65			–
Captopril		–			3.25

Values are expressed as mean $\pm$ SD of three independent experiments ($n = 3$). IC_{50} values represent the concentration required to inhibit 50% of NO production or ACE activity under the assay conditions. Cardamomin and captopril were used as positive controls for the NO inhibitory and ACE inhibitory assays, respectively. “–” indicates not determined.

although captopril formed additional hydrogen-bond and electrostatic interactions within the ACE binding site. The representative poses are presented in Figs. 1-4, with docking scores given in Tables 4 and 5. Taken together, β -bourbonene, longifolene and aromadendrene showed favourable predicted interactions across several targets. These computational results cannot establish their contribution to the experi-

mental activity of the crude oil and individual or synergistic effects require testing with isolated compounds and defined combinations.

In silico ADMET analysis: The major volatile constituents were further assessed using SwissADME and ProTox-3 (Tables 6-8). Molecular weights ranged from 136.23 to 204.35 g/mol, while LogP values ranged from 2.66 to 4.83. Most com-

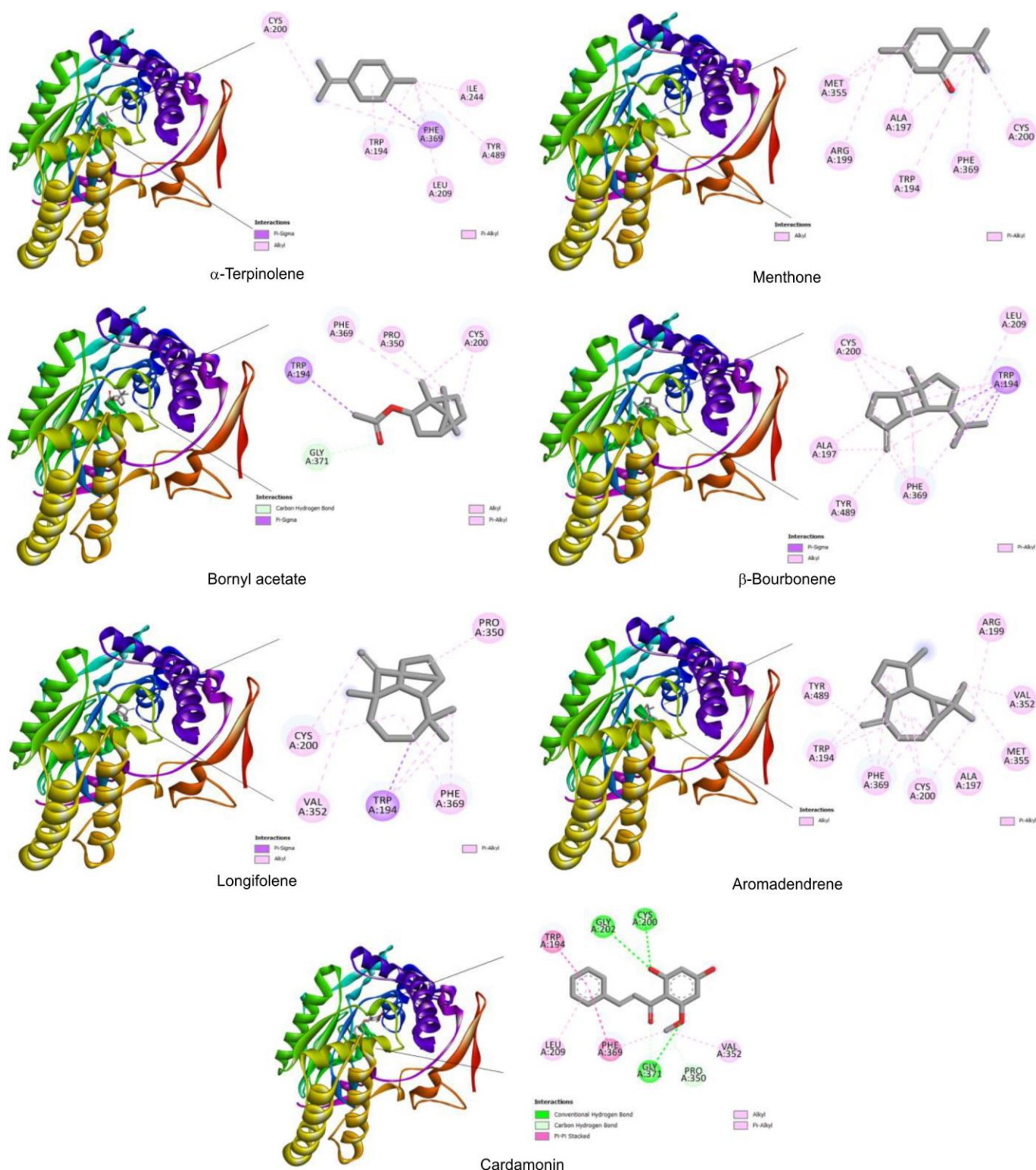


Fig. 1. 2D and 3D visualization of target compounds and cardamonin on the iNOS protein

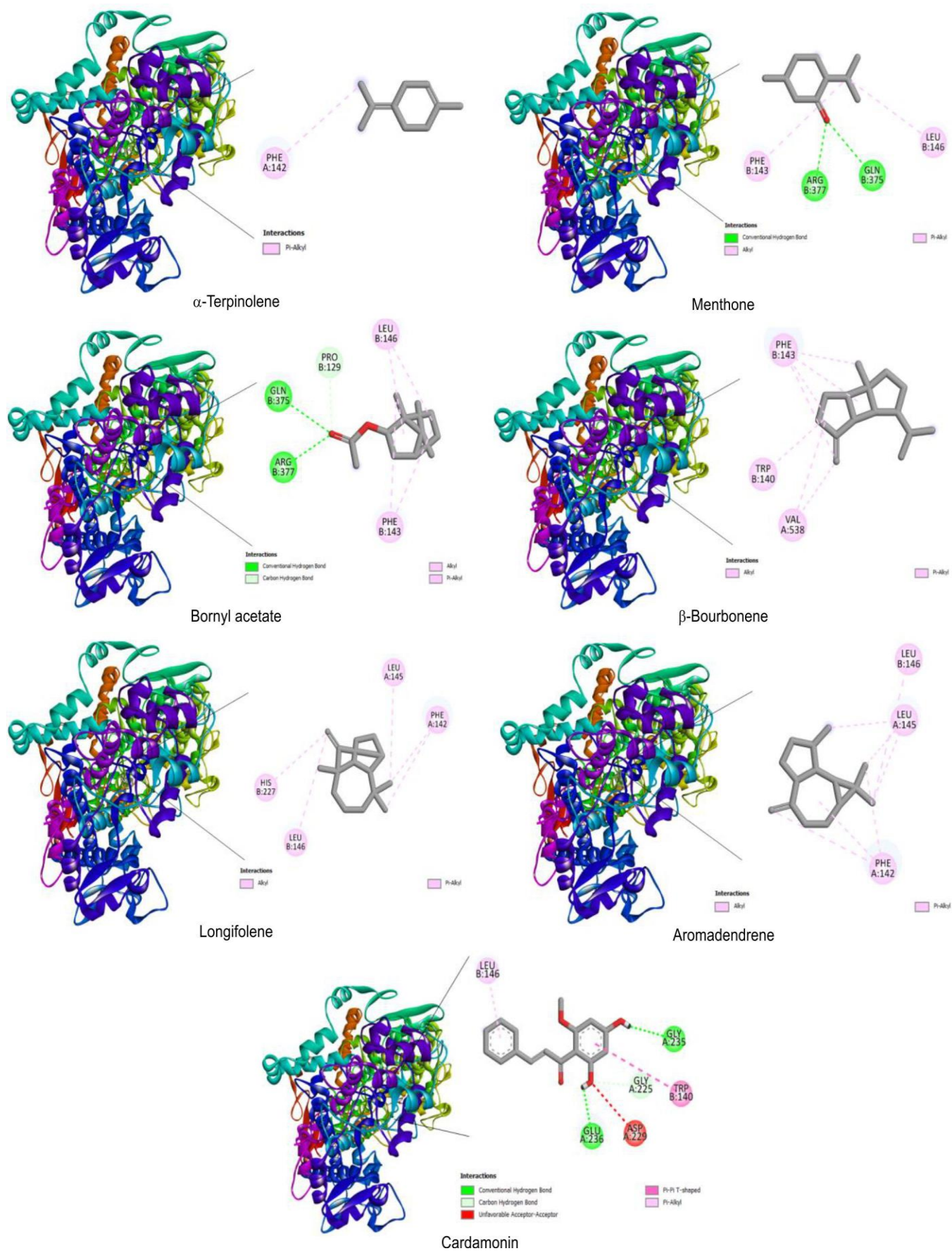


Fig. 2. 2D and 3D visualization of target compounds and cardamonin on the COX-2 protein

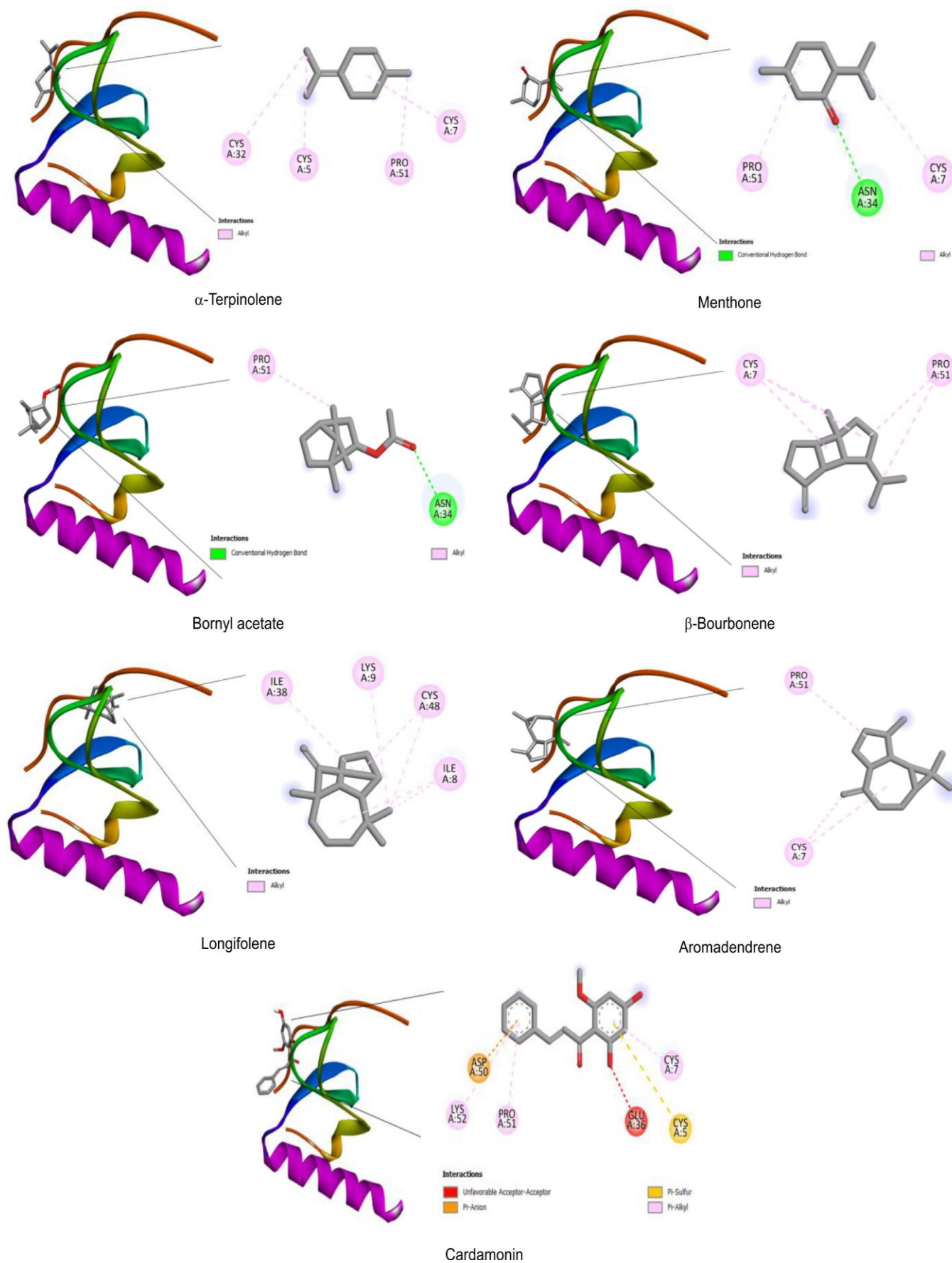


Fig. 3. 2D and 3D visualization of target compounds and cardamonin on the IL-8 protein

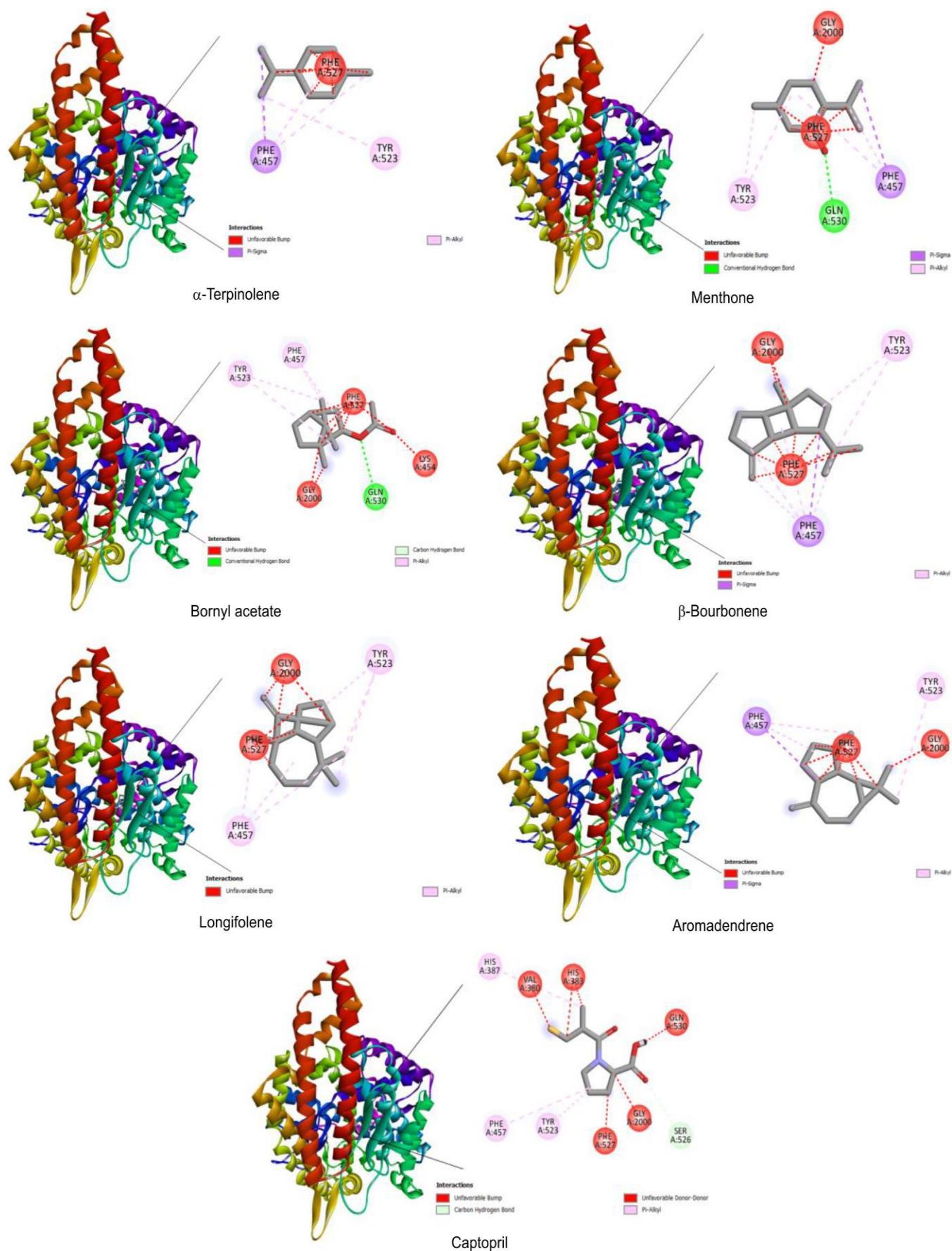


Fig. 4. 2D and 3D visualization of target compounds and captopril on the ACE protein

TABLE-4
BINDING AND INTERACTIONS OF STUDIED COMPOUNDS AND
REFERENCE COMPOUNDS ON THE iNOS, COX-2 AND IL-8 PROTEINS

Compound	Docking score (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	Other interactions
iNOS protein (PDB ID: 3E7G)				
α -Terpinolene	-7.045	–	Tyr489, Leu209, Ile244, Phe369, Cys200, Trp194	–
Menthone	-5.369	–	Trp194, Cys200, Phe369, Ile244, Leu209, Tyr489	–
Bornyl acetate	-5.856	Gly371	Phe369, Cys200, Pro350, Trp194	–
β -Bourbonene	-8.185	–	Cys200, Ala197, Phe369, Trp194, Leu209	–
Longifolene	-6.228	–	Cys200, Val352, Trp194, Phe369, Pro350	–
Aromadendrene	-7.589	–	Tyr489, Trp194, Phe369, Cys200, Ala197, Met355, Val352, Arg199	–
Cardamonin	-9.370	Gly202, Cys200, Gly371, Pro350, Gly371	Leu209, Phe369, Val352, Trp194	–
COX-2 protein (PDB ID: 5IKQ)				
α -Terpinolene	-5.630	–	Phe142	–
Menthone	-4.951	Arg377, Gln375	Leu146, Phe143	–
Bornyl acetate	-5.839	Arg377, Gln375, Pro129	Phe143, Leu146	–
β -Bourbonene	-7.009	–	Phe143, Trp140, Val538	–
Longifolene	-6.761	–	His227, Leu146, Phe142, Leu145	–
Aromadendrene	-6.511	–	Leu146, Leu145, Phe142	–
Cardamonin	-7.809	Glu236A, Gly235A, Gly225A	Leu146B, Trp140B	Asp229A
Interleukin-8 (PDB ID: 5D14)				
α -Terpinolene	-4.071	–	Val32, Cys5, Pro51, Cys7	–
Menthone	-4.351	Asn34	Cys7, Pro51	–
Bornyl acetate	-4.264	Asn34	Pro51	–
β -Bourbonene	-4.880	–	Cys7, Pro51	–
Longifolene	-4.306	–	Ile38, Lys9, Cys48, Ile8	–
Aromadendrene	-5.402	–	Pro51, Cys7	–
Cardamonin	-5.574	–	Lys52, Pro51, Cys7	Asp50, Cys5, Glu36

“–” Not forming hydrogen bonds and other interactions.

TABLE-5
BINDING AND INTERACTIONS OF THE STUDIED COMPOUNDS AND REFERENCE COMPOUND ON ACE PROTEIN (PDB ID: 1O86)

Compounds	Docking score (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	Other interactions
α -Terpinolene	-5.519	–	Tyr523, Phe457	Phe527
Menthone	-5.435	Gln530	Tyr523, Phe457	Phe527, Gly2000
Bornyl acetate	-5.548	Gln530	Phe457, Tyr523	Phe527, Gly2000, Lys454
β -Bourbonene	-6.585	–	Tyr523, Phe457	Phe527, Gly2000
Longifolene	-6.857	–	Tyr523, Phe457	Phe527, Gly2000
Aromadendrene	-6.959	–	Tyr523, Phe457	Phe527, Gly2000
Captopril	-5.073	Ser526	His387, Phe457, Tyr523	Val380, His383, Gln530, Gly2000, Phe527

“–” Not forming hydrogen bonds.

pounds met Lipinski's rule of five; β -bourbonene, longifolene, and aromadendrene each had one violation associated with higher lipophilicity [24]. TPSA values were generally low and predicted water solubility was limited (Log S -2.65 to -4.31).

Menthone and bornyl acetate had high predicted gastrointestinal absorption, whereas most sesquiterpene hydrocarbons had lower absorption. Most constituents were predicted to cross the BBB, except longifolene and captopril. None were predicted as P-glycoprotein substrates. α -Terpinolene, β -bourbonene, longifolene and aromadendrene were predicted to inhibit CYP-

2C9, while cardamonin showed predicted interactions with CYP1A2, CYP2C9 and CYP3A4.

ProTox-3 predicted LD₅₀ values of 500-5000 mg/kg. β -Bourbonene, longifolene and aromadendrene had the highest predicted LD₅₀ values (5000 mg/kg), whereas menthone had the lowest (500 mg/kg). The compounds were classified as “may be harmful if swallowed” by the prediction model.

Conclusion

This study documents the chemical composition and biological activities of *E. pulcherrima* leaf essential oil collected

TABLE-6
PHYSICO-CHEMICAL PROPERTIES, LIPOPHILICITY AND WATER
SOLUBILITY OF STUDIED COMPOUNDS ANALYZED USING SWISSADME

Compound	MW (g/mol)	LogP	nHBD	nHBA	TPSA (Å ²)	MR	Lipinski violation	LogS (ESOL)	nRotB
α-Terpinolene	136.23	3.57	0	0	0.00	47.12	0	-3.50	0
Menthone	154.25	2.66	0	1	17.07	48.27	0	-2.65	1
Bornyl acetate	196.29	3.12	0	2	26.30	56.33	0	-3.63	2
β-Bourbonene	204.35	4.66	0	0	0.00	67.14	1	-4.01	1
Longifolene	204.35	4.83	0	0	0.00	66.88	1	-4.31	0
Aromadendrene	204.35	4.61	0	0	0.00	67.14	1	-4.07	0
Cardamonin	270.28	2.60	2	4	66.76	76.79	0	-3.90	4
Captopril	217.29	0.41	1	3	96.41	59.97	0	-1.14	4

MW: molecular weight; LogP: log of octanol/water partition coefficient; nHBD: number of hydrogen bond donors; nHBA: number of hydrogen bond acceptors; TPSA: topological polar surface area; MR: molar refractivity; nRotB: number of rotatable bonds.

TABLE-7
PHARMACOKINETIC PREDICTIONS OF STUDIED COMPOUNDS COMPUTED BY SWISSADME

Compound	LogK _p (cm/s)	GI Abs	BBB per	Inhibitor interaction					
				P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
α-Terpinolene	-3.96	Low	Yes	No	No	No	Yes	No	No
Menthone	-5.08	High	Yes	No	No	No	No	No	No
Bornyl acetate	-4.44	High	Yes	No	No	No	No	No	No
β-Bourbonene	-4.20	Low	Yes	No	No	No	Yes	No	No
Longifolene	-3.94	Low	No	No	No	No	Yes	No	No
Aromadendrene	-4.20	Low	Yes	No	No	No	Yes	No	No
Cardamonin	-5.46	High	Yes	No	Yes	No	Yes	No	Yes
Captopril	-7.38	High	No	No	No	No	No	No	No

GI Abs: gastrointestinal absorption; BBB per: blood-brain barrier permeability; P-gp: P-glycoprotein; CYP: cytochrome P.

TABLE-8
TOXICITY PREDICTION RESULTS BY PROTOX-3

Compound	LD ₅₀ (mg/kg)	Classification
α-Terpinolene	4390	May be harmful if swallowed
Menthone	500	Harmful if swallowed
Bornyl acetate	3100	May be harmful if swallowed
β-Bourbonene	5000	May be harmful if swallowed
Longifolene	5000	May be harmful if swallowed
Aromadendrene	5000	May be harmful if swallowed
Cardamonin	3000	May be harmful if swallowed
Captopril	2078	May be harmful if swallowed

LD₅₀: lethal dose, 50%

in Dak Lak Province, Vietnam. GC-MS analysis identified 24 compounds accounting for 93.40% of the essential oil, with menthone, longifolene, bornyl acetate, α-terpinolene, aromadendrene and β-bourbonene as the major constituents. The oil exhibited moderate DPPH radical-scavenging and NO-inhibitory activities, while ACE inhibition was weak under the present assay conditions (IC₅₀ = 836.75 µg/mL) compared with captopril (IC₅₀ = 3.25 µg/mL). The study documents the chemical profile of *E. pulcherrima* leaf oil from Dak Lak and provides experimental data on its ACE-inhibitory activity. Molecular docking and ADMET analyses offer complementary computational data on possible constituent–target interactions and predicted pharmacokinetic and toxicity profiles;

these results do not establish mechanism, pharmacological efficacy or safety.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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