



Chemical profiling, phytotoxicity, and in silico evaluation of *Eremobium aegyptiacum* subsp. *longisiliquum*: volatile compounds and fatty acids as potential natural herbicidal agents

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Abstract

Weeds are a major challenge in agriculture, and plant-derived natural compounds offer a sustainable alternative to synthetic herbicides. *Eremobium aegyptiacum* subsp. *longisiliquum*, a native species growing in the semi-arid regions of Ghardaia, Algeria, remains largely unexplored for its bioactive potential. This study aimed to investigate the volatile compounds, fatty acids composition, and phytotoxicity of *n*-hexane and dichloromethane extracts using wheat germination and leaf puncture assays, complemented by molecular docking analyses. SPME-GCMS and fatty acid profiling revealed a chemically rich composition dominated by hexahydrofarnesylacetone (45%), undecane (27.2%), dihydroactinidiolide (14%), oleic acid (49.8%), and palmitic acid (30.8%). HS-SPME-GC-MS analysis revealed a volatile fraction dominated by hexahydrofarnesylacetone (45.0%), undecane (27.2%), and dihydroactinidiolide (14.0%), while fatty acid profiling identified oleic acid (49.8%) and palmitic acid (30.8%) as the major lipid constituents. Both extracts exhibited dose-dependent inhibition of wheat seed germination and induced necrotic lesions on *Oxalis corniculata*, *Urtica dioica*, and *Brachypodium sylvaticum*, with the *n*-hexane extract showing the strongest activity. Molecular docking suggested that the major constituents may interact with key plant enzymes, including acetohydroxyacid synthase (AHAS), 4-hydroxyphenylpyruvate dioxygenase (HPPD), and protoporphyrinogen oxidase (PPO). These interactions may be associated with the disruption of amino acid biosynthesis and pigment-related pathways.

Keywords *Eremobium aegyptiacum* subsp. *longisiliquum* · Volatile compounds · Fatty acids · Phytotoxicity · Molecular docking · AHAS · HPPD · PPO · Natural herbicide

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Introduction

Weeds represent one of the major challenges in agriculture, causing substantial reductions in crop yields and often forcing farmers to rely heavily on synthetic herbicides (Duke 2012; Li et al. 2015). Although these chemicals are effective, their widespread use can have serious environmental impacts and raise concerns about human health, highlighting the urgent need for safer, sustainable alternatives in crop protection (Dayan et al. 2009; Zhou et al. 2025).

Plants are remarkable chemical factories, producing a vast diversity of compounds that help them survive, defend against pests and pathogens, and interact with neighboring plants (Bennett and Wallsgrove 1994; Bankova 2014). These include primary metabolites, such as fatty acids that are essential for growth and energy storage, and secondary metabolites, including volatile organic compounds, terpenes, and aromatic ketones, which are often involved in defense mechanisms (Harborne 1998; Chowdhary and Tank 2023). The diversity and abundance of these bioactive molecules vary among species, developmental stages, and environmental conditions, offering a rich source of natural products with phytotoxic, antifungal, and insecticidal activities (Duke and Romagni 2000; Dayan et al. 2000).

Eremobium aegyptiacum subsp. *longisiliquum*, a native species growing in the semi-arid regions of Ghardaia, Algeria, thrives under harsh climatic conditions and nutrient-poor soils, where plants often evolve chemical defenses to survive, compete, and resist pests (Bourlière 1953–1962). Despite its remarkable adaptation, *E. longisiliquum* remains largely understudied, and its potential as a source of natural compounds for crop protection has not been fully explored. Investigating its biological activity may provide new insights into plant-derived phytotoxic compounds and contribute to the development of alternative strategies for weed management and sustainable agricultural practices.

To date, no studies have reported the phytotoxic or allelopathic properties of *E. aegyptiacum* subsp. *longisiliquum*, although the species is frequently mentioned in floristic and taxonomic accounts. This study represents the first comprehensive evaluation of its biological activity against ecologically relevant weed species.

In this work, we examined the volatile compounds, fatty acids, and phytotoxic potential of *n*-hexane and dichloromethane extracts of *E. aegyptiacum* subsp. *longisiliquum* using selected crop models. Furthermore, we aimed to explore the potential herbicidal mode of action of its major phytochemical constituents by focusing on three key plant enzymatic targets: acetohydroxyacid synthase (AHAS),

4-hydroxyphenylpyruvate dioxygenase (HPPD), and protoporphyrinogen oxidase (PPO). These enzymes play essential roles in amino acid biosynthesis, carotenoid formation, and porphyrin metabolism. The predominant compounds identified in the extracts were evaluated for their ability to interact with these targets through molecular docking analyses, providing mechanistic insights that could support their future development as natural herbicidal agents.

Materials and methods

Plant material collection and extract preparation

Aerial parts of *Eremobium aegyptiacum* subsp. *longisiliquum* were collected in April 2025 from Ghardaia, Northern Algeria (32.490° N, 3.670° E), an arid region with sandy-calcareous soils, low rainfall, and significant temperature fluctuations, characterized by drought-tolerant plant families including Asteraceae, Chenopodiaceae, and Poaceae (Zatout et al. 2025a). Healthy plants were harvested at the flowering stage to maximize secondary metabolite content, and taxonomic identification was confirmed with regional floras; a voucher specimen was deposited under EL-2025. For extraction, 50 g of dried, powdered plant material was soaked in 400 mL of the selected solvent (*n*-hexane or dichloromethane, DCM) at room temperature for 72 h with intermittent gentle agitation. Extracts were filtered and concentrated at 40 °C, and the dried residues were stored in amber vials at 4 °C until biological analyses.

HS-SPME-GC-MS analysis

The volatile chemical profile of *Eremobium aegyptiacum* dry powder was determined using HS-SPME sampling technique followed by GC-MS analysis. About 0.5 g of the powder was placed inside a 4 mL glass vial (the matrix/headspace ratio was 1:3) with PTFE-coated silicone septum. After closing the vial with the screw cap, it was protected with parafilm to prevent any loss of volatiles. The extraction process of volatiles was carried out on a SPME device from Supelco (Bellefonte, PA) with 1 cm fiber coated with 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane). Before use, the fiber was conditioned at 270 °C for 30 min. The equilibration time was achieved by heating the sample 60 °C for 15 min. After this time, the fiber was exposed to the headspace of the samples for 20 min at 60 °C to capture and concentrate the volatiles. Between sampling runs, the fiber was regenerated by

exposing it in the GC injector at a temperature of 250 °C for 20 min to avoid possible contamination. Lastly, the analytes were desorbed thermally in the GC injector maintained at 250 °C for 2 min in splitless mode. The analysis was carried out on Clarus 500 model (Perkin Elmer, Waltham, MA, USA) gas chromatograph equipped with a flame ionization detector (FID) and coupled with a mass spectrometer. The capillary column used a Varian Factor Four VF-5 (60 m x 0.25 mm x 1.0 µm). The applied temperature programme was as follows: from 45 °C to 220 °C at 6 °/min and finally held for 15 min. Helium was used as carrier gas at a constant rate of 1 mL/min. MS scans were recorded within the range 35–450 m/z using EI ionization (energy 70 eV). The identification of compounds was based on the comparison of the mass spectra of pure components stored in the Wiley and Nist libraries database and on the comparison of the Linear Retention Indices (LRIs) calculated using a series of alkane standards (C₈–C₂₅ *n*-alkanes) with that available retention data reported in the literature. The relative amounts of the constituents were expressed as percentages (mean of three replicates).

Fatty acids

Fatty acid content was determined by GC/MS analysis after lipid extraction process and synthesis of fatty acid methyl esters (FAMES). Specifically, the samples (0.5 g) were dissolved in 10 mL of chloroform/methanol (2:1 v/v). Then, 1 mL of each extract was dried with nitrogen and transmethylated with BF₃·CH₃OH at 70 °C for 30 min. The extraction of the obtained FAMES was carried out with *n*-hexane. 2 µL of the extract was injected into the column in splitless mode. The gas chromatographic conditions were as follows: the injector was set at 280 °C and the oven temperature program started from 170 °C and increased up to 260 °C with a rate of 3 °C/min and held constant for 15 min. Component identification and quantification were performed as described in the previous section. The analyses were performed in triplicate (Zatout et al. 2026).

Phytotoxicity assay

Germination and early seedling growth assay

The phytotoxic effects of *n*-hexane and dichloromethane (DCM) extracts of *E. aegyptiacum* subsp. *longisiliquum* on wheat (*Triticum aestivum* L.) were evaluated during seed germination and early seedling development. Wheat seeds were surface-sterilized and placed in Petri dishes (90 mm

diameter) lined with filter paper. Each Petri dish contained 10 seeds. The filter paper was moistened with the respective extract solutions at concentrations of 0.5, 1.0, and 2.0 mg/mL. Control seeds were treated with 4% methanol (MeOH). Petri dishes were incubated at 25 ± 2 °C under a 12 h light/12 h dark photoperiod. Germination percentage, radicle length, and plumule length were recorded after 5 days. Each treatment was performed in triplicate, and data were expressed as mean ± standard deviation (SD). Differences among treatments were analyzed using Tukey's HSD test at $p \leq 0.05$.

Leaf puncture assay

The phytotoxic potential of *n*-hexane and DCM extracts was further assessed using a leaf puncture assay on three weed species: *Oxalis corniculata*, *Urtica dioica*, and *Brachypodium sylvaticum*. Fully expanded leaves were punctured and treated with extract solutions at concentrations of 0.5, 1.0, and 2.0 mg/mL, while control leaves received 4% MeOH (Zatout et al. 2021b). Treated leaves were incubated under controlled conditions, and necrotic areas measured in mm² after 48 h. All treatments were performed in triplicate, and results were expressed as mean ± SD. Statistical significance was determined using Tukey's HSD test ($p \leq 0.05$) (Zatout et al. 2025b).

Statistical analysis

Data from both assays were analyzed using two-way analysis of variance (ANOVA) to evaluate the effects of extract type, concentration, and their interaction. Multiple comparisons were performed using Tukey's HSD test. Statistical analyses were conducted using XLSTAT software (Addinsoft SARL, New York, NY, USA), and differences were considered statistically significant at $p \leq 0.05$.

Molecular docking simulations

Three herbicide-related plant enzymes were selected as biological targets based on their well-established roles in essential metabolic pathways. The crystallographic structures of acetohydroxyacid synthase (AHAS, PDB ID: 1YHZ), 4-hydroxyphenylpyruvate dioxygenase (HPPD, PDB ID: 6J63), and protoporphyrinogen oxidase (PPO, PDB ID: 1SEZ) were retrieved from the Protein Data Bank. Each structure was prepared by removing water molecules, co-crystallized ligands, and heteroatoms not involved in catalysis, followed by addition of hydrogen atoms and assignment

of partial charges. Protein preparation and energy minimization were carried out using UCSF Chimera (version 1.6) (Benslama et al. 2023).

The major phytotoxic constituents identified were selected for docking. Their two-dimensional and three-dimensional structures were retrieved from PubChem, and energy minimization was performed prior to docking. All ligands were processed to generate the appropriate protonation states at physiological pH, and partial charges were assigned using Open Babel (version 3.1.1) (Bramki et al. 2024).

To validate the docking workflow, the co-crystallized ligands associated with each target enzyme were first extracted and re-docked into their respective active sites. The accuracy of the docking protocol was evaluated by calculating the root mean square deviation (RMSD) between the experimental and predicted poses. All RMSD values were found to be below 1.957 Å, confirming the reliability of the docking settings for subsequent ligand screening (Mansouri et al. 2021).

Docking calculations were performed using AutoDock Vina (version 1.2.0). Grid boxes were centered on the active sites of each enzyme based on the coordinates of the co-crystallized ligands and were defined with dimensions sufficiently large to fully encompass the binding pockets. The exhaustiveness parameter was set to 8 (default), and ten binding poses were generated for each ligand. The binding energies reported in this study correspond to docking scores predicted by AutoDock Vina (kcal/mol) and should not be interpreted as absolute thermodynamic free energies of binding. The best-ranked poses according to predicted binding affinity were retained for interaction analysis (Dammene Debbih et al. 2024).

The docked complexes were examined to identify hydrogen bonds, hydrophobic contacts, aromatic interactions, and

other non-covalent forces contributing to binding stability. Structural visualization and interaction mapping were conducted using BIOVIA Discovery Studio Visualizer (version 21.1) (Benslama et al. 2025).

Results and discussion

Chemical composition of *Eremobium aegyptiacum* subsp. *longisiliquum*

The chemical analysis of *E. aegyptiacum* subsp. *longisiliquum* revealed a complex profile of volatile compounds and fatty acids. This composition reflects the plant's ecological adaptations and its potential biological activities.

Volatile composition

The volatile profile of *E. aegyptiacum* subsp. *longisiliquum* was dominated by non-terpenoid compounds, while monoterpenes were present at lower levels. Seven volatile constituents were identified, accounting for 100% of the detected fraction, as summarized in Table 1.

Hexahydrofarnesylacetone was the most abundant compound (45.0%). This compound is widely distributed in higher plants and it is often formed through oxidative degradation of carotenoids or isoprenoids. It has been previously associated with antimicrobial and phytotoxic activities (Abd-ELGawad et al. 2021; Niu et al. 2024; Pan et al. 2024). Undecane was the second most abundant constituent (27.2%). Aliphatic hydrocarbons such as undecane are frequently reported in plants adapted to arid environments and are known to participate in plant–insect and plant–microbe interactions, including insect-repellent and allelopathic effects (Harborne 1997; Dudareva et al. 2013; Mangoba and Alwindia 2023). Dihydroactinidiolide accounted for 14.0% of the volatile fraction. This norisoprenoid originates from carotenoid degradation and has been reported to exhibit antioxidant and antimicrobial properties, indicating the involvement of oxidative metabolism in shaping the volatile profile (Winterhalter and Rouseff 2002; Rodríguez-Bustamante and Sánchez 2007). Tridecane (5.2%) is another aliphatic hydrocarbon that functions as a semiochemical in ecological signaling and has been implicated in insect deterrence and allelopathic interactions (Muhammad et al. 2005; Bruce and Pickett 2011). The monoterpenes 1,8-cineole (4.4%), limonene (2.8%), and α -thujene (1.4%), although present in low proportions, are well known for their antimicrobial, insecticidal, and plant growth-inhibitory activities. These compounds may enhance the overall bioactivity of the

Table 1 Chemical volatile composition of *E. aegyptiacum* (percentage mean values $\pm$ standard deviation)

N.	Compound (CAS No.) ¹	LRI ^{calc}	LRI ^{lit}	%
1	α -thujene (2867-05-2)	900	902	1.4 $\pm$ 0.06
2	limonene (138-86-3)	1028	1031	2.8 $\pm$ 0.17
3	1,8-cineole (470-82-6)	1035	1033	4.4 $\pm$ 0.22
4	undecane (1120-21-4)	1106	1100	27.2 $\pm$ 1.92
5	tridecane (629-50-5)	1295	1291	5.2 $\pm$ 0.06
6	dihydroactinidiolide (17092-92-1)	1561	1568	14.0 $\pm$ 1.13
7	hexahydrofarnesylacetone (502-69-2)	1842	1846	45.0 $\pm$ 5.02
	Sum			100.0

The components are reported according to their elution order on apolar column; Linear Retention Indices measured on apolar column; Linear Retention indices from literature

Table 2 Fatty acids content of *Eremobium aegyptiacum* subsp. *longisiliquum* (percentage mean values±standard deviation)

N.	Fatty acids	LRI ^{calc}	LRI ^{lit}	%
1	tridecanoic acid, <i>C13:0</i>	1661	1664	0.4±0.02
2	myristic acid, <i>C14:0</i>	1748	1752	1.7±0.09
3	palmitic acid, <i>C16:0</i>	1969	1973	30.8±2.95
4	linoleic acid, <i>cis, cis</i> -9,12-octadecadienoic acid, <i>C18:2Δ9,12 (n-6)</i>	2095	2099	7.0±0.51
5	oleic acid, <i>C18:1Δ9 (n-9)</i>	2168	2171	49.8±4.10
6	stearic acid, <i>C18:0</i>	2175	2178	7.0±0.53
7	arachidonic acid*, <i>C20:4Δ5,8,11,14 (n-6)</i>	2320	2324	3.3±0.15
	Sum			100.0
	Saturated FAs			39.9
	Unsaturated FAs			60.1

The components are reported according to their elution order on apolar column (VF-5ms); Linear Retention Indices measured on apolar column; Linear Retention indices from literature; * tentative identification.

volatile mixture through synergistic effects (Bakkali et al. 2008; Isman 2020; Zatout et al. 2025c). Overall, the volatile profile is characterized by a predominance of aliphatic and oxygenated compounds rather than a terpene-rich pattern, reflecting specific ecological adaptations of this subspecies. However, highly and lowly volatile compounds may not be captured by the fiber used for adsorption of the compounds. To our knowledge, this represents one of the first reports on the volatile constituents of *E. aegyptiacum* subsp. *longisiliquum*.

Fatty acids

The fatty acid composition was dominated by unsaturated fatty acids, with seven identified components collectively accounting for 100% of the lipid fraction (Table 2).

Oleic acid was the principal fatty acid (49.8%). This monounsaturated fatty acid plays a key role in membrane structure and stress tolerance in plants and has been reported to exhibit antimicrobial, phytotoxic, and insecticidal activities (Harwood 2020; Marusich et al. 2020; Hashem et al. 2025). Palmitic acid was the second most abundant component (30.8%). As a major saturated fatty acid in plant lipids, it contributes to membrane stability and energy storage and has been linked to antimicrobial and allelopathic effects when present at high levels (Desbois and Smith 2010). Stearic acid and 9,12-octadecadienoic acid (linoleic acid, *C18:2n-6*) were detected at equal proportions (7.0% each). Stearic acid enhances membrane rigidity, whereas 9,12-octadecadienoic acid (linoleic acid, *C18:2n-6*) has been associated with growth-inhibitory and antimicrobial

Table 3 Effects of *n*-hexane and dichloromethane (DCM) extracts on germination percentage, radicle length, and plumule length of wheat (*Triticum aestivum* L.)

Extract	Concentration (mg/mL)	Germination (%)	Radicle length (cm)	Plumule length (cm)
<i>n</i> -Hexane	0.5	8.67±0.58 ^a	4.00±0.10 ^b	4.20±0.10 ^b
	1.0	6.67±0.58 ^b	3.10±0.10 ^d	3.27±0.15 ^d
	2.0	3.67±0.58 ^{cd}	1.70±0.10 ^f	1.80±0.10 ^f
DCM	0.5	6.67±0.58 ^b	3.60±0.10 ^c	3.80±0.10 ^c
	1.0	4.67±0.58 ^c	2.23±0.15 ^e	2.50±0.10 ^e
	2.0	2.33±0.58 ^d	1.03±0.06 ^g	1.20±0.10 ^g
Control	–	10.00±0.00 ^a	4.67±0.12 ^a	5.13±0.15 ^a

Values are expressed as mean±standard deviation (SD) of three replicates. Control seeds were treated with 4% MeOH. Different letters within the same column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$)

activities (Moreno 2015; Salsinha et al. 2018). Minor fatty acids included arachidonic acid (3.3%), myristic acid (1.7%), and tridecanoic acid (0.4%). Arachidonic acid, although rare in higher plants, has been reported in some taxa and is involved in signaling and defense responses. Myristic and tridecanoic acids, even at low concentrations, may contribute to antimicrobial and ecological interactions (Kabara et al. 1972; Walters 2015; Wasternack and Feussner 2018).

Phytotoxicity assays

Germination and early seedling growth

The effects of *n*-hexane and dichloromethane (DCM) extracts of *E. aegyptiacum* subsp. *longisiliquum* on wheat (*Triticum aestivum* L.) germination and early seedling growth were evaluated. Both extracts caused significant reductions in germination percentage and significant inhibition of radicle and plumule elongation compared with the control treatment (4% MeOH). The results are presented in Table 3.

The results show that wheat seed germination significantly decreased with increasing concentrations of both *n*-hexane and DCM extracts, indicating a clear dose-dependent response. The strongest inhibitory effects were observed at 2.0 mg/mL for both extracts. However, at the lowest concentration (0.5 mg/mL), the *n*-hexane extract did not significantly differ from the control in terms of germination percentage. Significant inhibition was observed only at higher concentrations. At comparable concentrations, the *n*-hexane extract generally exhibited stronger inhibitory effects than the DCM extract, particularly with respect to radicle and plumule elongation. Radicle growth was more

Fig. 1 The necrotic areas induced by organic extracts: **A** methanol (MeOH 4%), **B** DCM extract at 2 mg/mL, **C** DCM extract at 2 mg/mL, **D** *n*-hexane extract at 1 mg/mL, **E** *n*-hexane extract at 2 mg/mL, **F** methanol (MeOH 4%), **G** DCM extract at 1 mg/mL, **H** DCM extract at 2 mg/mL, **I** *n*-hexane extract at 1 mg/mL, **J** *n*-hexane extract at 2 mg/mL, **K** methanol (MeOH 4%), **L** DCM extract at 1 mg/mL, **M** DCM extract at 2 mg/mL, **N** *n*-hexane extract at 1 mg/mL, **O** *n*-hexane extract at 2 mg/mL

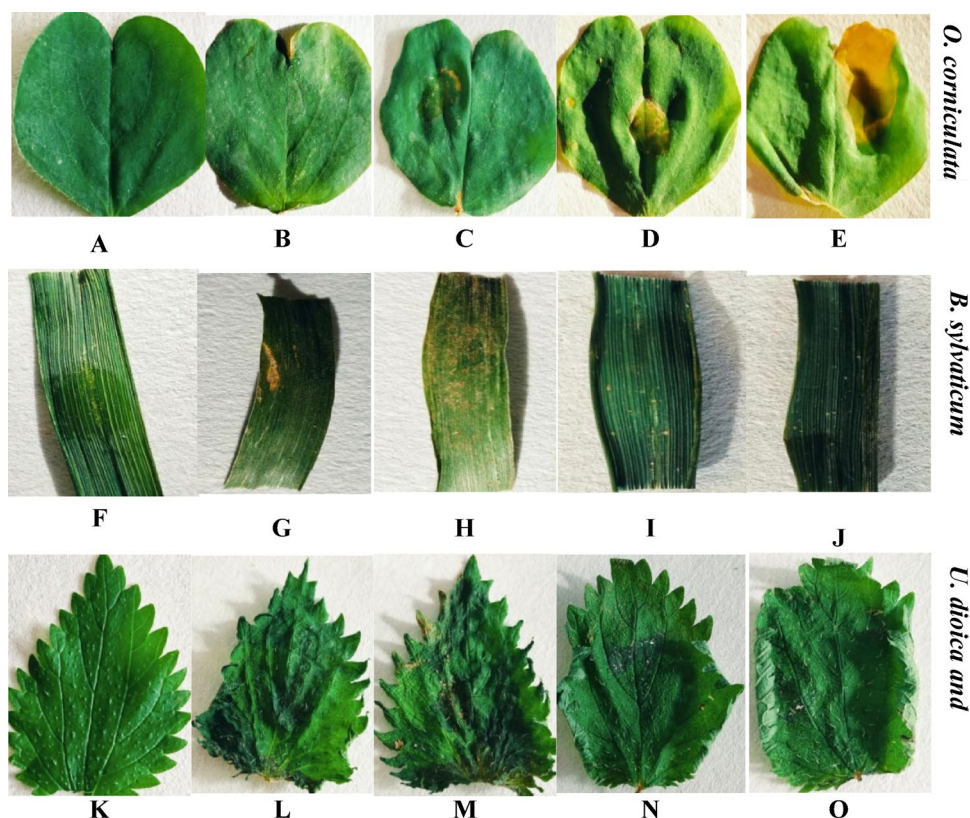


Table 4 Phytotoxic effects of *Eremobium aegyptiacum* subsp. *longisiliquum* extracts on three weed species assessed by a leaf puncture assay

Extract	Concentration (mg/mL)	Necrotic area (mm ²)		
		<i>Oxalis corniculata</i>	<i>Urtica dioica</i>	<i>Brachypodium sylvaticum</i>
<i>n</i> -hexane	0.5	18.26 ± 0.50 ^e	14.66 ± 0.61 ^e	13.06 ± 0.30 ^d
	1.0	34.00 ± 0.26 ^c	27.67 ± 0.45 ^c	23.00 ± 0.75
	2.0	48.83 ± 0.85 ^a	46.57 ± 0.40 ^a	43.23 ± 0.66 ^a
	Control	0.50 ± 0.01 ^g	0.50 ± 0.01 ^g	0.43 ± 0.06 ^f
DCM	0.5	13.90 ± 0.20 ^f	11.30 ± 0.40 ^f	10.40 ± 0.40 ^e
	1.0	26.73 ± 0.40 ^d	23.17 ± 0.32 ^d	22.33 ± 0.71 ^c
	2.0	44.27 ± 0.66 ^b	41.33 ± 0.61 ^b	36.50 ± 1.18 ^b
	Control	0.53 ± 0.06 ^g	0.46 ± 0.06 ^g	0.43 ± 0.06 ^f

Values are expressed as mean ± standard deviation (SD) of three replicates. Control leaves received were treated with 4% MeOH. Different letters within a column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$)

sensitive than plumule growth, with significant reductions observed even at the lowest concentration (0.5 mg/mL). These findings suggest that root tissues are more susceptible to allelochemicals, likely due to their direct exposure and high metabolic activity during early germination (Chung et al. 2001; Macías et al. 2020). Similar responses have been

widely reported in allelopathy studies, where radicle growth is often considered a more sensitive indicator of phytotoxicity than shoot growth (Macías et al. 2019; Scavo et al. 2021). Overall, the concentration-dependent reduction in germination percentage and seedling elongation reflects a clear phytotoxic response induced by the plant extracts.

Leaf puncture assay

The phytotoxic activity of *E. aegyptiacum* subsp. *longisiliquum* was further evaluated using leaf puncture assays against three weed species (*Oxalis corniculata*, *Urtica dioica*, and *Brachypodium sylvaticum*). The necrotic areas induced by *n*-hexane and dichloromethane (DCM) extracts at different concentrations are presented in Fig. 1; Table 4.

Both extracts induced visible necrotic lesions on the tested leaves, and the extent of tissue damage increased significantly with rising concentration, indicating a clear dose–response relationship. Two-way ANOVA revealed a highly significant effect of extract type, concentration, and their interaction on necrotic area for all species ($p < 0.001$). Increasing the extract concentration from 0.5 to 2.0 mg/mL resulted in a marked enlargement of necrotic areas for all three species. At the highest concentration (2.0 mg/mL), both extracts produced the most significant phytotoxic

effects, whereas control treatments (4% MeOH) induced negligible necrosis ($<1 \text{ mm}^2$). At equivalent concentrations, the *n*-hexane extract generally exhibited stronger phytotoxic activity than the DCM extract for most species and concentrations. For instance, at 2.0 mg/mL , the *n*-hexane extract induced necrotic areas of $48.83 \pm 0.85 \text{ mm}^2$ in *O. corniculata*, compared to $44.27 \pm 0.66 \text{ mm}^2$ for the DCM extract, with statistically significant differences between treatments ($p < 0.05$). Similar trends were observed for *U. dioica* and *B. Sylvaticum* at higher concentrations. However, no significant difference between the two extracts was observed at 1.0 mg/mL for *B. sylvaticum*, as indicated by the shared statistical grouping. Overall, the results suggest a tendency toward higher phytotoxic activity of the *n*-hexane extract, although this effect is not uniform across all conditions.

The leaf puncture assay therefore confirms the strong phytotoxic potential of *E. aegyptiacum* subsp. *longisiliquum*, as both extracts caused extensive necrotic damage to weed leaves. Although the germination and leaf puncture assays target different plant tissues and developmental stages, the consistent dose-dependent responses and the greater activity of the *n*-hexane extract in both assays suggest the involvement of common phytotoxic mechanisms.

The phytotoxic effects observed in this study are consistent with previous reports describing strong inhibitory and necrotic activities of organic plant extracts rich in volatile and lipophilic secondary metabolites. Several studies have shown that *n*-hexane extracts often exhibit higher phytotoxicity than extracts obtained with more polar solvents, such as DCM, due to their enrichment in lipophilic bioactive compounds (Gebreyohannes et al. 2023; Pinto et al. 2023; Lee et al. 2024). The pronounced necrotic responses and clear dose-dependent effects recorded here are in agreement with those reported for other allelopathic plant species evaluated using similar bioassays (Chai et al. 2015; Abdelmalik et al. 2023). To the best of our knowledge, *E. aegyptiacum* subsp. *longisiliquum* has not previously been evaluated for phytotoxic activity in peer-reviewed bioassays. This study therefore represents the first report of its phytotoxic effects using a leaf puncture assay.

The similarity in inhibitory patterns observed in both germination and phytotoxicity assays suggests a strong relationship between the chemical composition of the extracts and their biological activity. In both cases, the higher activity of the *n*-hexane extract highlights the key role of lipophilic compounds in mediating allelopathic effects, as supported by the GC–MS and fatty acid profiles.

GC–MS analysis revealed that the volatile fraction of *E. aegyptiacum* subsp. *longisiliquum* was dominated by hexahydrofarnesylacetone, undecane, and dihydroactinidiolide,

along with smaller amounts of monoterpenes such as 1,8-cineole and limonene. Hexahydrofarnesylacetone, the most abundant compound, has been reported in various plant species, and has been associated with biological activities, including phytotoxic effects (Abd-ElGawad et al. 2020; İnci et al. 2022). Due to its lipophilic nature, it may interact with biological membranes; however, its specific mechanism of action remains unclear and requires further experimental investigation. Undecane, as an aliphatic hydrocarbons, has also been reported to exert growth-inhibitory effects (Scognamiglio et al. 2013; Respatie et al. 2025) and may act synergistically with hexahydrofarnesylacetone to enhance allelopathic activity (Pardo-Muras et al. 2022). Dihydroactinidiolide, although present in lower amounts, has been associated with phytotoxic effects and may further contribute to the observed responses (Kato-Noguchi and Peters 2013; Charoenying et al. 2022). Monoterpenes such as 1,8-cineole and limonene are known to disrupt membrane integrity, inhibit seedling growth, and impair photosynthetic processes, which is consistent with the observed reductions in radicle and plumule elongation, as well as the development of necrotic lesions (Romagni et al. 2000; Abd-ElGawad et al. 2020).

It is important to note that the HS-SPME technique provides a profile of volatile compounds present in the headspace above the dried plant material. This profile reflects only the emitted volatile fraction and does not necessarily correspond to the composition of the solvent extracts (*n*-hexane and DCM) used in the phytotoxicity assays. Consequently, any direct attribution of the observed biological activity to compounds identified by HS-SPME remains speculative.

In addition to volatile compounds, the fatty acid profile of *E. aegyptiacum* subsp. *longisiliquum* revealed a high proportion of oleic and palmitic acids. Fatty acids have been reported to interfere with membrane fluidity and enzymatic activity and may contribute to the observed phytotoxicity by enhancing membrane permeability and facilitating the action of other bioactive constituents (Shiralipour et al. 1997; Fukuda et al. 2004). The combined presence of fatty acids and volatile lipophilic compounds may enhance the overall phytotoxic and allelopathic effects of the extracts.

Taken together, the germination and phytotoxicity assays provide complementary evidence that *E. aegyptiacum* subsp. *longisiliquum* produces bioactive secondary metabolites capable of inhibiting plant growth at multiple developmental stages. The *n*-hexane extract generally exhibited higher phytotoxic activity than the DCM extract, which may be related to the preferential extraction of lipophilic compounds. However, the chemical composition of the extracts

was not directly characterized in this study, and therefore any attribution of biological activity to specific compounds remains speculative and requires further analytical confirmation. Nevertheless, the observed phytotoxic effects suggest that *E. aegyptiacum* subsp. *longisiliquum* contains bioactive compounds with potential relevance for weed management.

Molecular docking

The molecular docking analysis was conducted to explore the potential interactions between the major constituents of the n-hexane extract and key herbicide-related targets. The n-hexane extract was selected due to its higher phytotoxic activity observed in the biological assays. Given the chemical complexity of plant extracts, the computational study focused on the most abundant compounds identified in the extract. Among these, oleic acid, palmitic acid, and hexahydrofarnesylacetone exhibited the most favorable predicted binding affinities across the selected targets and were therefore retained for detailed docking analysis.

It is important to note that molecular docking provides a theoretical prediction of ligand–target interactions and does not constitute direct evidence of biological activity. Therefore, the results presented here should be interpreted as indicative of potential interactions.

The interactions of these compounds with the selected enzymes (AHAS, HPPD, and PPO) are summarized in Table 5, and the corresponding binding poses are illustrated in Figs. 2, 3 and 4.

Acetoxyacid synthase (AHAS), also known as acetolactate synthase, is an essential enzyme in the biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine) (Dezfulian et al. 2017). Because inhibition of AHAS disrupts protein biosynthesis and leads to rapid growth arrest, this enzyme represents a well-established target for commercial herbicides (Zhou et al. 2007). Its relevance in herbicide design fully justifies its inclusion as a priority target in this study.

The co-crystallized ligand exhibited a docking score of -8.5 kcal/mol and formed numerous stabilizing hydrogen bonds with key catalytic residues such as ASP396, PHE397, GLY448, ASP474, ASN478, and MET479, complemented by hydrophobic interactions with TYR477 and VAL480, and an attractive electrostatic interaction with ASP447 (Fig. 2).

Among the docked compounds, oleic acid showed the best affinity (-7.7 kcal/mol), forming five hydrogen bonds, mainly with SER396, ASP474, ASN478, TYR543, and GLY395, which are critical for anchoring the fatty acid moiety deep in the active site. Hydrophobic contacts with

ALA58, MET422, and MET479 further contributed to its stabilization.

Palmitic acid interacted similarly (-7.2 kcal/mol), sharing the same hydrogen-bonding network with SER396, ASP474, and ASN478 and also engaging hydrophobic residues surrounding the binding pocket.

Hexahydrofarnesylacetone, although bulkier and more hydrophobic, displayed a slightly weaker docking score (-6.7 kcal/mol). Its interaction profile was dominated by hydrophobic contacts with MET422, VAL480, and π -alkyl stacking with TYR477, and only a single hydrogen bond with SER396, explaining its slightly lower affinity.

The results indicate that AHAS possesses a binding cavity capable of accommodating long-chain fatty acids, which may be associated with the inhibition of seed germination. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme in tyrosine catabolism, catalyzing the conversion of 4-hydroxyphenylpyruvate into homogentisate, a precursor of plastoquinone and tocopherols, which are essential for protecting carotenoids against photo-oxidative damage (Zatout et al. 2025b; Xu et al. 2019). Inhibition of HPPD leads to chlorosis, bleaching, and necrosis due to the depletion of protective pigments (Ahrens et al. 2013), making it a central target for several commercial herbicides. Therefore, assessing the binding behavior of the major extract constituents toward HPPD may help to explain the observed leaf necrosis in the phytotoxicity assay.

The co-crystallized ligand bound with an affinity of -6.9 kcal/mol, forming key hydrogen bonds with HIS226, HIS308, and LYS421, while engaging in π - π stacking and π -alkyl interactions with PHE424, and a halogen interaction with ASN423 (Fig. 3).

Among the screened compounds, hexahydrofarnesylacetone showed an affinity nearly identical to the native ligand (-6.8 kcal/mol), relying almost exclusively on hydrophobic and π -alkyl interactions with residues such as PHE381, VAL228, PRO280, LYS421, MET335, HIS226, HIS308, PHE392, and PHE419. The absence of hydrogen bonding indicates that its binding is primarily driven by hydrophobic complementarity.

Palmitic acid also docked favorably (-6.8 kcal/mol). The presence of one hydrogen bond with GLN293 and a salt bridge involving ARG290 contributed to its stabilization, in addition to hydrophobic interactions with VAL269, PRO280, LYS421, HIS308, PHE381, PHE392, and PHE419.

Oleic acid showed a slightly weaker affinity (-6.6 kcal/mol), interacting mainly through hydrophobic contacts and π -alkyl interactions, with HIS226 contributing an attractive charge interaction.

Table 5 Binding affinities and interaction profiles of the major phytotoxic compounds identified in *Eremobium aegyptiacum* subsp. *longisiliquum*, docked against the three herbicide-related targets (AHAS, HPPD, and PPO)

			docking score (Kcal/mol)	Hydrogen interactions (Distance Å)	Hydrophobic interactions	Other interactions
AHAS (1YHZ)	Co-crystal- lized ligand	ICS	− 8.5	ASP396 (1.89), PHE397 (2.06), GLY448 (2.13), ASP474 (1.65), ASN478 (1.75), MET479 (2.07), THR521 (2.48), GLN419 (2.40), LYS393 (2.70), THR421 (2.65), TYR477 (2.57), PHE397 (2.19), MET422 (3.07, 3.01)	TYR477 (π -alkyl), VAL480 (alkyl),	ASP447 (attractive charge), MET422 (Sulfur-X)
	Best docked compounds	Oleic acid	− 7.7	SER396 (1.98), ASP474 (1.79), ASN478 (1.86), TYR543 (2.39), GLY395 (2.84)	ALA58 (alkyl), MET422 (alkyl), MET422 (alkyl), MET422 (alkyl), MET422 (alkyl), MET479 (alkyl)	−
		Palmitic acid	− 7.2	SER396 (2.00), ASP474 (1.76), ASN478 (1.85), TYR543 (2.34), GLY395 (2.91)	MET422 (alkyl), MET422 (alkyl), MET479 (alkyl)	−
		Hexahydrofarnesylacetone	− 6.7	SER396 (2.23)	MET422 (alkyl), MET422 (alkyl), MET422 (alkyl), VAL480 (alkyl), TYR477 (π -alkyl)	−
HPPD (6J63)	Co-crystal- lized ligand	NDT	− 6.9	HIS226 (2.98), HIS308 (2.71, 2.98), LYS421 (2.81)	PHE424 (π - π stacked), PHE424 (π -alkyl)	ASN423 (halogen bond − fluorine)
	Best docked compounds	Hexahydrofarnesylacetone	− 6.8	−	PHE381 (π -sigma), VAL228 (alkyl), PRO280 (alkyl), PRO280 (alkyl), LYS421 (alkyl), LEU265 (alkyl), LEU265 (alkyl), MET335 (alkyl), HIS226 (π -alkyl), HIS308 (π -alkyl), HIS308 (π -alkyl), PHE392 (π -alkyl), PHE419 (π -alkyl), PHE419 (π -alkyl)	−
		Palmitic acid	− 6.8	GLN293 (2.31)	VAL269 (alkyl), PRO280 (alkyl), LYS421 (alkyl), HIS308 (π -alkyl), HIS308 (π -alkyl), PHE381 (π -alkyl), PHE392 (π -alkyl), PHE419 (π -alkyl)	ARG290 (salt bridge; attractive charge)
		Oleic acid	− 6.6	−	LEU427 (alkyl), HIS308 (π -alkyl), PHE381 (π -alkyl), PHE381 (π -alkyl), PHE392 (π -alkyl), PHE424 (π -alkyl), PHE424 (π -alkyl)	HIS226 (attractive charge)

Table 5 (continued)

			docking score (Kcal/mol)	Hydrogen interactions (Distance Å)	Hydrophobic interactions	Other interactions
PPO (1SEZ)	Co-crystal- lized ligand	OMN	− 8.1	ARG98 (1.71), GLY178 (2.92), ARG98 (3.05), GLY175 (3.07), ALA174 (2.89)	PHE392 (π – π stacked), LEU334 (alkyl), LEU356 (alkyl), ARG98 (alkyl), PHE392 (π –alkyl), LEU356 (π –alkyl), LEU372 (π –alkyl)	ARG98 (salt bridge; attractive charge), ALA174 (halogen bond), ILE175 (hal- ogen bond), GLY370 (halogen bond), THR371 (halogen bond)
	Best docked compounds	Palmitic acid	− 7.2	ARG98 (2.19, 2.93)	LYS551 (alkyl), LEU356 (alkyl), LEU372 (alkyl), PHE392 (π –alkyl), PHE392 (π –alkyl), TRP435 (π –alkyl), PHE439 (π –alkyl), PHE439 (π –alkyl)	ARG98 (salt bridge; attractive charge)
		Oleic acid	− 6.9	TYR99 (2.93), ARG98 (2.85)	PHE392 (π –sigma), ALA466 (alkyl), ALA438 (alkyl), LYS551 (alkyl), LEU356 (alkyl), LEU372 (alkyl), PHE392 (π –alkyl), PHE392 (π –alkyl), TRP435 (π –alkyl), TRP435 (π –alkyl), PHE439 (π –alkyl), PHE439 (π –alkyl)	ARG98 (attractive charge)
		Hexahydrofarnesylacetone	− 6.9	–	LYS551 (alkyl), ALA466 (alkyl), LYS551 (alkyl), LEU334 (alkyl), VAL336 (alkyl), PHE392 (π –alkyl), PHE392 (π –alkyl), TRP435 (π –alkyl), PHE439 (π –alkyl), PHE439 (π –alkyl)	–

These results suggest that HPPD can effectively accommodate both long-chain fatty acids and hydrophobic terpenoid derivatives, potentially contributing to chlorotic and necrotic symptoms observed in treated plants.

Protoporphyrinogen oxidase (PPO) is a crucial enzyme in the biosynthesis of chlorophyll and heme (Koch et al. 2004). Inhibition of PPO leads to the accumulation of photoreactive intermediates, resulting in immediate oxidative damage and rapid tissue necrosis (Ambrosini et al. 2019). Given that the n-hexane extract induced visible necrotic lesions, PPO was selected as a mechanistically relevant target.

The co-crystallized ligand displayed a strong affinity (− 8.1 kcal/mol), characterized by a dense network of hydrogen bonds involving ARG98, GLY178, GLY175, and ALA174, supported by π – π stacking with PHE392, multiple π –alkyl interactions, and several halogen bonds (Fig. 4).

Among the best docked compounds, palmitic acid exhibited the highest affinity (− 7.2 kcal/mol), stabilized by hydrogen bonds with ARG98, and hydrophobic interactions with LYS551, LEU356, LEU372, TRP435, and PHE439, in addition to an attractive charge interaction with ARG98.

Oleic acid bound slightly less strongly (− 6.9 kcal/mol) but established two hydrogen bonds with TYR99 and ARG98,

along with extensive hydrophobic and aromatic interactions involving ALA466, ALA438, LYS551, LEU356, LEU372, PHE392, TRP435, and PHE439.

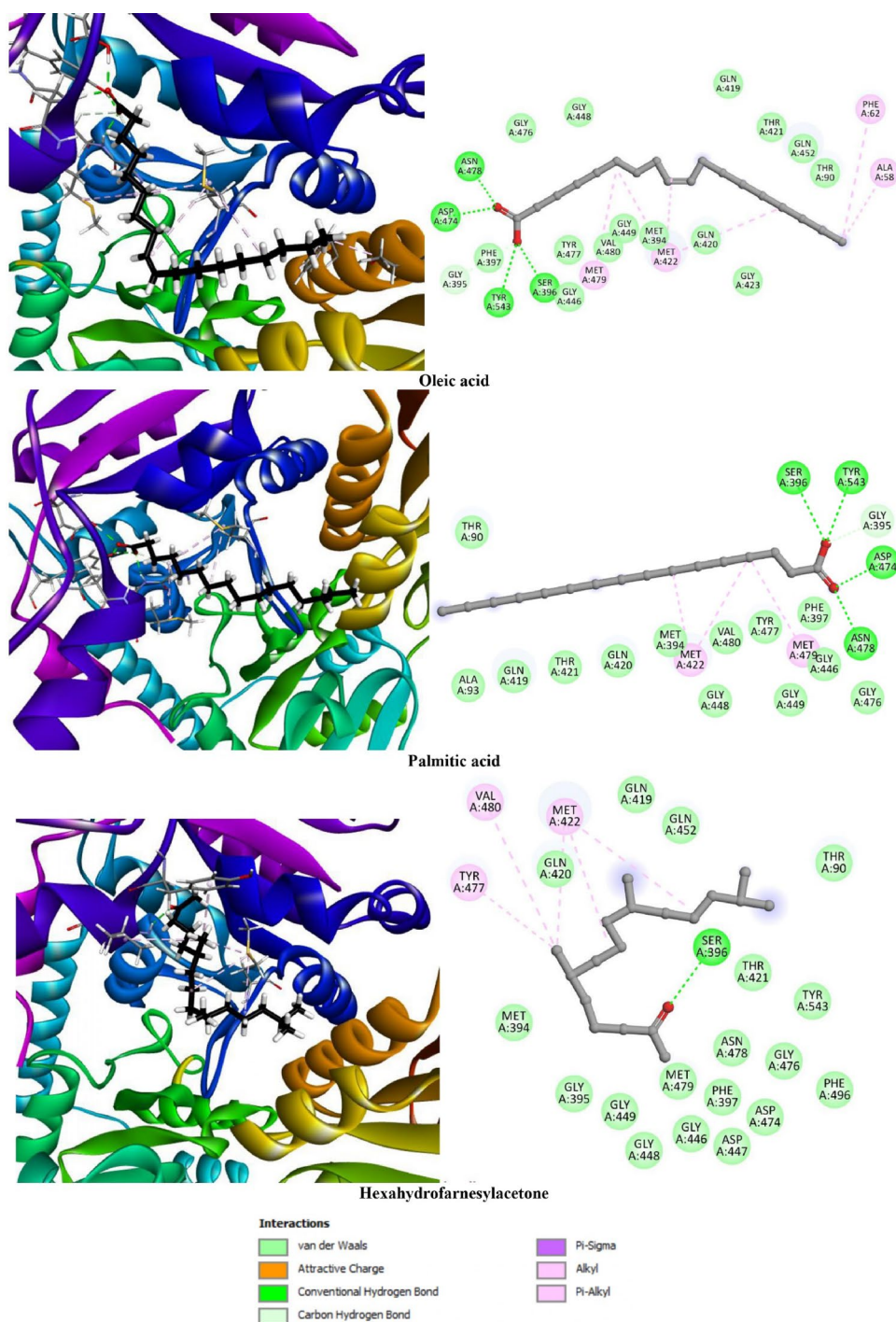
Hexahydrofarnesylacetone demonstrated similar affinity (− 6.9 kcal/mol), driven almost exclusively by alkyl and π –alkyl interactions with residues such as LYS551, ALA466, LEU334, VAL336, PHE392, TRP435, and PHE439, reflecting its non-polar nature.

These results suggest that PPO inhibition may contribute to the observed phytotoxic activity. It is important to note that molecular docking provides only a theoretical prediction of ligand–target interactions. The obtained docking scores reflect relative binding tendencies and do not constitute experimental evidence of enzymatic inhibition. Therefore, further in vitro or in vivo validation is required to confirm the biological activity of the identified compounds.

Conclusions

This study demonstrates the phytotoxic potential of *Eremobium aegyptiacum* subsp. *longisiliquum* as a promising source of plant-derived herbicidal agents. The observed

Fig. 2 Binding mode and interaction profile of the best docked compounds, oleic acid, palmitic acid, and hexahydrofarnesylacetone, within the active site of AHAS (PDB ID: 1YHZ)



biological activity underscores the key role of lipophilic secondary metabolites in suppressing weed growth and supports their role in sustainable weed management strategies.

The findings also emphasize the value of underexplored wild species as reservoirs of bioactive compounds with

agricultural relevance. Although further investigations are needed to confirm their large-scale applicability, the present results provide a strong foundation for the future development of eco- friendly, plant-based solutions for weed control.

Oleic acid

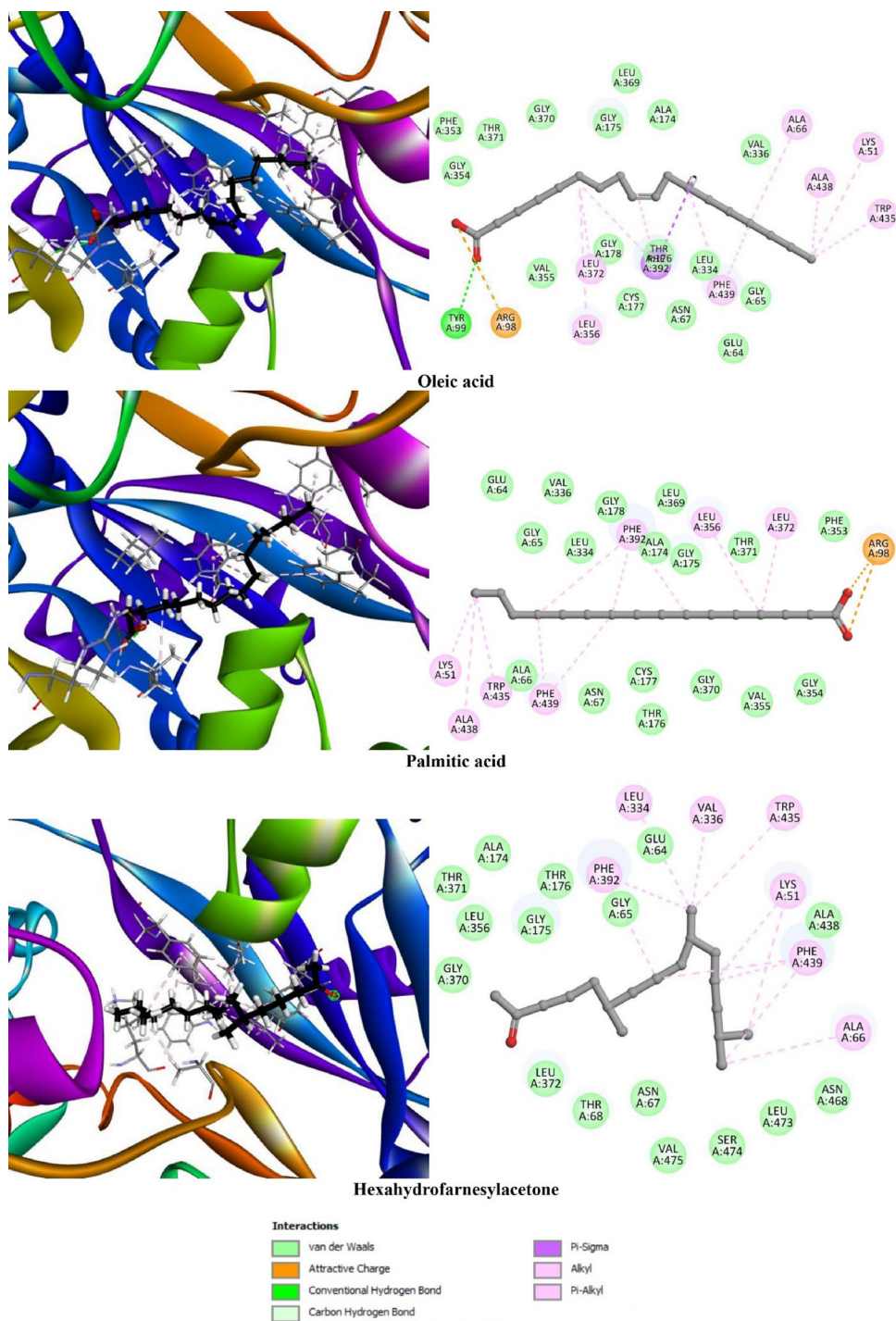
Palmitic acid

Hexahydrofarnesylacetone

Interactions

- van der Waals
- Attractive Charge
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Pi-Sigma
- Alkyl
- Pi-Alkyl

Fig. 4 Binding pose and interaction diagrams of the three major compounds, oleic acid, palmitic acid, and hexahydrofarnesylacetone, inside the PPO binding pocket (PDB ID: 1SEZ)



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Author contributions RZ: conceptualization, methodology, resources, investigation, supervision, writing original draft, writing review and editing. OB: formal analysis, data curation, writing original draft. FZM: formal analysis, writing review and editing. CZ: supervision, resources, formal analyses. SG: formal analyses, investigation, data curation, methodology, supervision, writing and revision.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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