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Toxic effect of extracts of Peganum harmala and Euphorbia guyoniana on Androctonus australis (Scorpiones; Buthidae)

Linda Rouari[1,2], Abdelmalek Rouari[3], Farouk Benaceur[2,4], Ilham Messahli[4], Elhafnaoui Lanez[5], Mohammed Larbi Benamor[5], Hicham Benhammouda[1], Zineb Souilem[6], Amel Rouari[1], Nacira Boukhari[1], Salah Eddine Sadine[1]

[1]Laboratory of Valorization and Conservation of Arid Ecosystems (LVCEA), Faculty of Natural and Life Sciences, University of Ghardaïa, Ghardaïa 47000, Algeria.
[2]Research Unit on Medicinal Plants (URPM), Biotechnology Research Center (CRBt), Constantine 25000, Algeria.
[3]Laboratory of Saharan Bioresources: Preservation and Valorization, Faculty of Natural and Life Sciences, University of Ouargla, Ouargla 30000, Algeria.
[4]Laboratory of Biological and Agronomic Sciences (LBAS), Amar Telidji University, Laghouat 03000, Algeria
[5]Laboratory of Valorisation and Technology of Saharian Resources (VTRS), Department of Process Engineering and Petrochemistry, Faculty of Technology, University of El Oued, El Oued 39000, Algeria.
[6]Laboratory of Mathematics and Applied Sciences (LMSA), Faculty of Science and Technology, University of Ghardaïa, 47000 Ghardaïa, Algeria.
Corresponding Author: Linda Rouari
Corresponding Author Email: rouari.linda@gmail.com

ABSTRACT: Peganum harmala and Euphorbia guyoniana are two medicinal plants known for their phytochemical constituents and biological activities. This study aimed to investigate the toxic potential of ethanolic extracts of both plants against the scorpion Androctonus australis collected from the Ghardaïa region, Algeria.

Phytochemical screening and high-performance liquid chromatography (HPLC) were carried out to characterize the extracts. The toxicological activity was evaluated at five concentrations (10, 15, 20, 25, and 30 mg/mL), using 5% DMSO as a control, by monitoring mortality and locomotor inhibition over 72 h. Molecular docking was also performed to investigate the interactions between selected phytochemicals and acetylcholinesterase.

The extraction yield was higher for E. guyoniana (5.10%) than for P. harmala (3.44%). Phytochemical screening revealed the presence of several secondary metabolites, particularly flavonoids, tannins, alkaloids, and phenolic compounds. The results showed that mortality increased with extract concentration and exposure time, reaching approximately 60% at the highest concentrations after 72 h. Locomotor inhibition was also more pronounced at higher concentrations. Molecular docking results suggested that several phytochemicals could interact with acetylcholinesterase.

Overall, the results indicated that the ethanolic extracts of P. harmala and E. guyoniana exerted toxicological and behavioral effects against A. australis under the experimental conditions tested, suggesting their potential as sources of bioactive compounds for further investigation.

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1. INTRODUCTION

Scorpions reproduce mainly in environments with high temperatures, which favors the development. This suggests that climate change and rising temperatures in certain regions could increase their activity and proliferation, as indicated by several studies on the impact of climate on arthropods [1-3].

According to [4], scorpion envenomation represents a public health issue in several regions worldwide, including North Africa, the Middle East, Asia, and Latin America. In these areas, venomous scorpion stings can cause symptoms ranging from localized pain to severe systemic reactions, including neurological disorders, respiratory failures, and sometimes death. Scorpion envenomation remains a significant public-health concern in North Africa, with epidemiological studies documenting substantial morbidity and mortality associated with scorpion stings [5]. The Algerian Sahara is considered an important area for scorpionism, owing to the diversity and abundance of medically important scorpion species [6].

Managing scorpion populations, particularly in regions where they pose a threat to human health, involves various control strategies. Chemical methods have shown some effectiveness through the use of pesticides [7].

The use of plants as natural pesticides is gaining increasing interest due to their natural origin and potential ecological benefits. Plants produce various bioactive compounds that can be used as biopesticides [8].

While scorpion control is necessary in certain regions due to their danger, an approach combining chemical, biological methods, and habitat management seems to be the most promising approach. In this context, the aim of this study is to evaluate the effect of ethanolic extracts of two plant species, *Peganum harmala* and *Euphorbia guyoniana*, from the Ghardaïa region (Algeria) against the scorpion species *Androctonus australis* from the same region.

2. MATERIALS AND METHODS

2.1 Animal model

Adult specimens of the scorpion species *Androctonus australis* (Buthidae) selected for this study were collected in the northern portion of the Northern Sahara (32° 27' N, 32° 32' N and 3° 37' E, 3° 44' E). The specimens were identified according to [9]. The collected scorpions were placed in controlled mass-breeding conditions. Each individual was isolated in a separate plastic box filled with a layer of sand to prevent cannibalism. Their diet consisted of nutrient-rich Tenebrio molitor larvae, provided regularly. In addition, a moistened cotton pads were placed in each box to provide access to water.

2.2 Plant material

The aerial parts (leaves, flowers, and stems) of the Saharan plants *Peganum harmala* and *Euphorbia guyoniana* were selected for this study. The plant species were identified by Mr Bnesamaune, an Assistant Professor in the Department of Biology, University of Ghardaïa, using the identification keys of [10]. Voucher specimens were deposited at the Department of Biology, University of Ghardaïa, Ghardaïa, Algeria, under the voucher numbers MP-2024-10 for *P. harmala* and MP-2024-11 for *E. guyoniana*.

After collection, the selected parts were dried in the shade, away from direct sunlight, at room temperature for a period of 25 days. Once fully dried, the plant parts were ground into a fine powder. The resulting powder was then stored in a paper bag and kept in a dark environment.

2.3 Extraction

A total of 200 g of plant powder from each plant was subjected to cold maceration in 1000 mL of 96% ethanol for 24 hours at room temperature under continuous stirring using WIS-10 Shaking Incubator, Korea.

After maceration, the mixture was vacuum filtered. The obtained organic extracts were then concentrated by evaporation to dryness using a rotary evaporator set at 45°C to remove the solvent. The dry extract was collected, weighed, and stored in amber glass flasks at 4°C.

2.4 Phytochemical screening

Phytochemical screening was carried out using qualitative tests based on color changes and precipitation reactions to identify the main chemical families present in the extracts [11, 12]. The analysis focused on the presence of flavonoids, tannins, coumarins, free quinones, alkaloids, reducing compounds, saponins, steroids, and anthocyanins.

2.5 High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed on a Thermo Scientific Vanquish UHPLC system equipped with an autosampler and a reversed-phase C18 analytical column. An aliquot of 0.5 mg of the dried extract was accurately dissolved in 5 mL of HPLC-grade methanol, filtered, and transferred into an autosampler vial. The mobile phase consisted of methanol, acetonitrile, and purified water (40:15:45, v/v/v), delivered under isocratic conditions at a flow rate of 0.8 mL/min. The column temperature was maintained at 25 °C, and UV detection was performed at 254 nm with an injection volume of 20 µL. The chromatographic constituents were tentatively identified by comparing their retention times with those of authentic reference standards analysed under identical chromatographic conditions.

2.6 Toxicity and locomotor activity assays

The toxicity of *Peganum harmala* and *Euphorbia guyoniana* extracts was evaluated against adult *Androctonus australis* by contact application at five concentrations (10, 15, 20, 25, and 30 mg/mL), prepared in 5% dimethyl sulfoxide (DMSO). For each extract, six experimental groups were established: five concentration groups and one vehicle-control group treated with 5% DMSO. Each group comprised three independent biological replicates of 20 scorpions each (n = 60 scorpions per concentration or control group; 360 scorpions per extract across the six groups). Each scorpion was housed individually in a plastic box (15 × 20 × 11 cm) containing a thin layer of sand. A volume of 1 mL of the corresponding extract preparation was applied directly to each scorpion, while control individuals received 1 mL of 5% DMSO. The same exposure procedure and maintenance conditions were used for all groups.

Toxicological and behavioural responses were monitored for 72 h. Mortality and locomotor inhibition were assessed at 2, 4, 6, 12, 24, 48, and 72 h after treatment, with the same individuals monitored at each time point. Mortality was recorded as the absence of movement following gentle stimulation, whereas locomotor inhibition was assessed as the absence of a locomotor or defensive response following the standardized pencil stimulus described below. The mortality rate was calculated using the following formula:

$$\text{Mortality rate} = \frac{\text{number of scorpions that died after treatment}}{\text{number of scorpions introduced}} \times 100$$

The lethal concentrations (LC25, LC50, and LC90) were determined to evaluate the effects of the extracts on scorpion mortality. Mortality percentages were converted into Probit values, while extract concentrations were transformed into decimal logarithms. Dose–log concentration regression equations were then established to describe the relationship between Probit values and the logarithmic concentrations.

Scorpion locomotor activity and defensive responses were assessed by gently touching each individual with a pencil to minimize external disturbance and stress. The response was recorded as either an attempted sting (defensive response) or remaining immobile following stimulation (locomotor inhibition). The vehicle control was assessed using the same observation schedule and behavioural procedure as the extract-treated groups.

Locomotor inhibition was determined using the following formula:

$$\text{Locomotor inhibition} = \frac{\text{number of scorpions that immobile after treatment}}{\text{number of scorpions introduced}} \times 100$$

2.7 Molecular docking study

Molecular docking was carried out to explore the possible interaction of the major phytochemicals identified in the ethanolic extracts of *Peganum harmala* and *Euphorbia guyoniana* with acetylcholinesterase (AChE), a neurotoxicity-related enzymatic target involved in cholinergic neurotransmission [13]. The analysis was used as a complementary mechanistic approach to support the biological observations and to assess whether the detected compounds could interact with an arthropod AChE binding pocket [14].

The ligands were selected according to the HPLC profile obtained in the present study. The investigated compounds were quercetin, catechin, catechol (pyrocatechol), vanillin, rutin, α-tocopherol, adonitol, tannic acid, chlorogenic acid, and ascorbic acid. The three-dimensional structures of the ligands were retrieved from the PubChem database in SDF format [15]. Each ligand was converted to PDB format, protonated where appropriate, and energy-minimized using the MMFF94 force field in

Open Babel [16]. The minimized structures were subsequently converted to PDBQT format after assigning Gasteiger partial charges and defining rotatable bonds.

The crystallographic structure of *Drosophila melanogaster* acetylcholinesterase complexed with the tacrine derivative 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine was retrieved from the RCSB Protein Data Bank (PDB ID: 6XYY) [17, 18]. This structure was selected as an arthropod AChE model because no experimentally resolved AChE structure is currently available for *Androctonus australis*, and because the presence of a co-crystallized inhibitor allows active-site definition and docking protocol validation [19]. The receptor was prepared using Auto Dock Tools by retaining chain A, removing crystallographic water molecules, glycosylation residues, ions, and non-essential heteroatoms [20]. The co-crystallized ligand was retained only for defining the binding site and redocking validation, then removed before docking the phytochemicals. Polar hydrogen atoms and Kollman charges were added, and the prepared receptor was saved in PDBQT format [21].

Docking simulations were performed using Auto Dock Vina [22]. The docking grid was centred on the centroid of the co-crystallized tacrine derivative and was large enough to include the catalytic gorge and adjacent peripheral binding region of AChE. The generated docking parameters are presented in Table 1.

Table 1. Docking grid and calculation parameters used for acetylcholinesterase molecular docking.

Parameter	Value
Protein target	Drosophila melanogaster acetylcholinesterase
PDB ID	6XYY
Selected chain	A
Reference crystallographic ligand	760
Grid centre X (Å)	32.651
Grid centre Y (Å)	67.579
Grid centre Z (Å)	9.604
Grid size X (Å)	28.000
Grid size Y (Å)	28.000
Grid size Z (Å)	28.000
Exhaustiveness	32
Number of binding modes	20
Energy range (kcal/mol)	4
Receptor treatment	Rigid
Ligand treatment	Flexible

Before docking the phytochemicals, the docking protocol was validated by redocking the co-crystallized ligand into the AChE active site. The predicted pose was superimposed onto the crystallographic orientation, and the protocol was considered acceptable when the heavy-atom RMSD was below 2.0 Å [21]. After validation, all selected phytochemicals were docked using the same grid and docking conditions. The best-ranked pose of each ligand was selected according to the lowest predicted binding energy, followed by visual inspection of its orientation within the AChE binding pocket [23].

Protein–ligand interactions were analysed using Discovery Studio Visualizer and PyMOL [24, 25]. Hydrogen bonds, π–π stacking, π–alkyl contacts, hydrophobic interactions, and electrostatic contacts were recorded. The results were expressed as binding affinity values (kcal/mol), interacting residues, interaction types, and interaction distances.

2.8 Statistical analysis

Experimental data were compiled in Microsoft Excel and analysed using R software (version 4.5.1). Probit regression was used to estimate lethal concentrations (LC50 and LC90) from mortality responses, with mortality percentages converted to Probit values and concentrations transformed to decimal logarithms. Mortality was also analysed using a generalized linear model. Plant/extract, concentration, exposure time, and their interactions were included as explanatory variables; likelihood-ratio χ^2 tests were used to assess main effects and interaction terms. Statistical significance was set at $P < 0.05$. The control group was included to assess possible effects of the 5% DMSO vehicle under the same experimental conditions.

3. RESULTS AND DISCUSSION

3.1 Extraction yield

The extraction yield differed between the two plant species. *Euphorbia guyoniana* yielded 5.10% of dry extract, whereas the yield obtained from *Peganum harmala* was lower, at 3.44%.

3.2 Phytochemical screening

Phytochemical screening (Table 2) revealed a significant presence of alkaloids and steroids across all tested extracts. The extracts of *E. guyoniana* demonstrated a greater diversity of secondary metabolites in comparison to *P. harmala*. *E. guyoniana* showed the highest tannin content compared to other species.

Table 2. Qualitative screening of phytochemicals in different extracts.

Phytochemical compounds	*Euphorbia guyoniana*	*Peganum harmala*
Flavonoids	+++	-
Tannins	++++	+
Coumarins	++++	-
Free quinones	+++	-
Alkaloids	+++	++
Reducing compounds	++++	-
Saponins	---	-
Steroids	++++	++++
Anthocyanins	-	-

-: negative; ++++ strongly positive; ++: Moderately positive; +: weakly positive

3.3 High-performance liquid chromatography (HPLC)

HPLC analysis of the ethanolic extracts from the aerial parts of *Peganum harmala* and *Euphorbia guyoniana* revealed distinct chromatographic profiles (Fig. 1A and B, respectively). In the *P. harmala* extract, seven compounds were tentatively assigned by comparing retention times with those of authentic reference standards: quercetin, catechin, pyrocatechol, vanillin, rutin, α-tocopherol, and adonitol. The *E. guyoniana* extract showed six compounds tentatively assigned by the same approach: quercetin, catechin, rutin, tannic acid, chlorogenic acid, and ascorbic acid. Several assignments were shared by both extracts, whereas others differed between extracts. Because identification was based on retention-time comparison, these assignments should be regarded as tentative; this analysis does not independently confirm compound identity or quantify their concentrations. The

profiles therefore provide a preliminary basis for selecting compounds for further investigation, rather than proof that any particular constituent caused the observed biological effects.

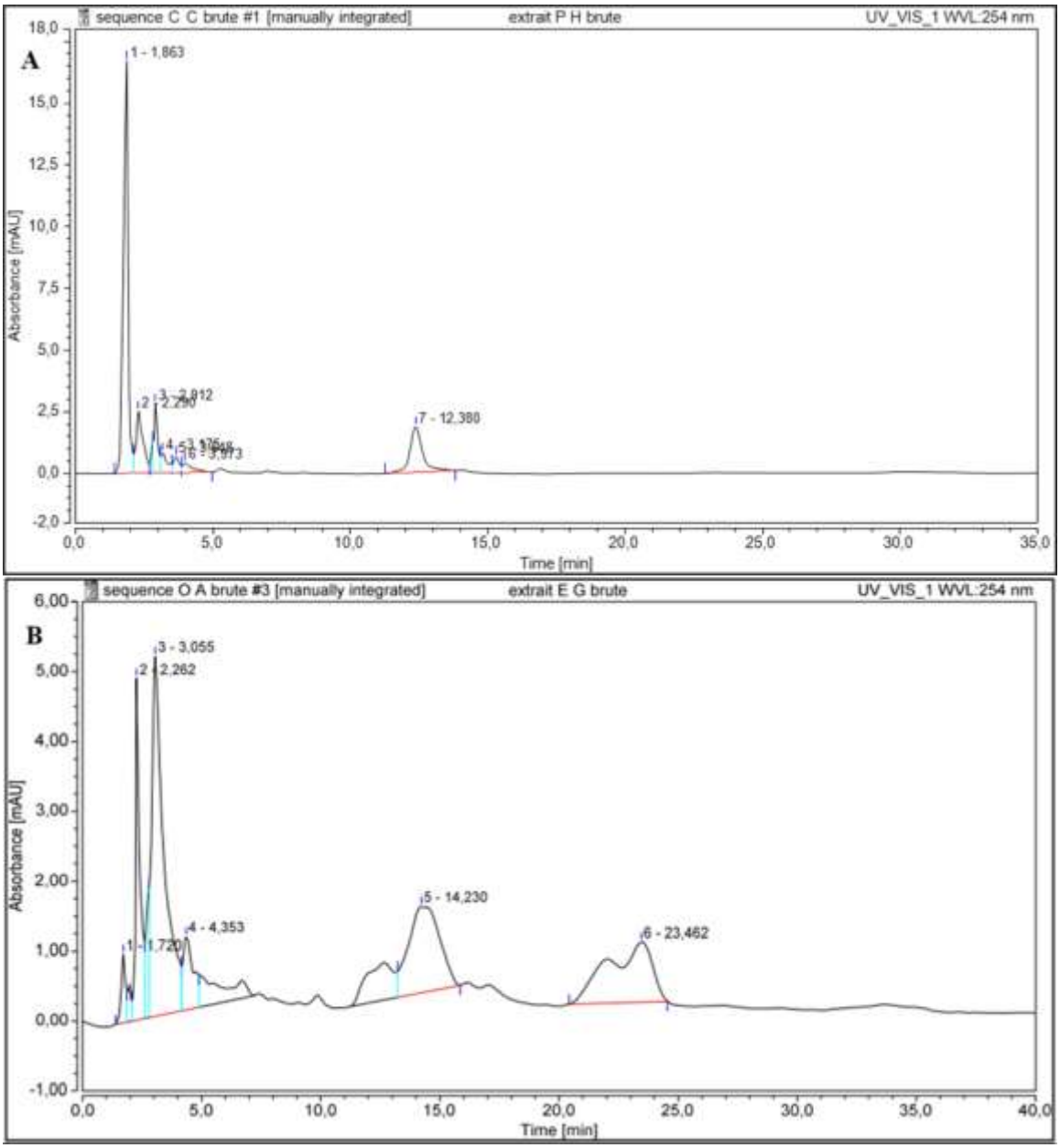


Figure 1. HPLC chromatographic profiles of the organic extracts of *Peganum harmala* (A) and *Euphorbia guyoniana* (B).

3.4 Toxicity and locomotor activity

The toxicity of the organic extracts against *Androctonus australis* varied with concentration and exposure time (Fig. 2A and B). Mortality was generally low or absent at the lowest concentrations (10 and 15 mg/mL) throughout most of the experimental period. In contrast, higher concentrations, particularly 25 and 30 mg/mL, produced greater mortality, reaching approximately 49% and 59% after 72 h for *Euphorbia guyoniana* and *Peganum harmala*, respectively. The increase in mortality observed with increasing concentration and prolonged exposure indicates that both factors contributed to the toxic response under the experimental conditions.

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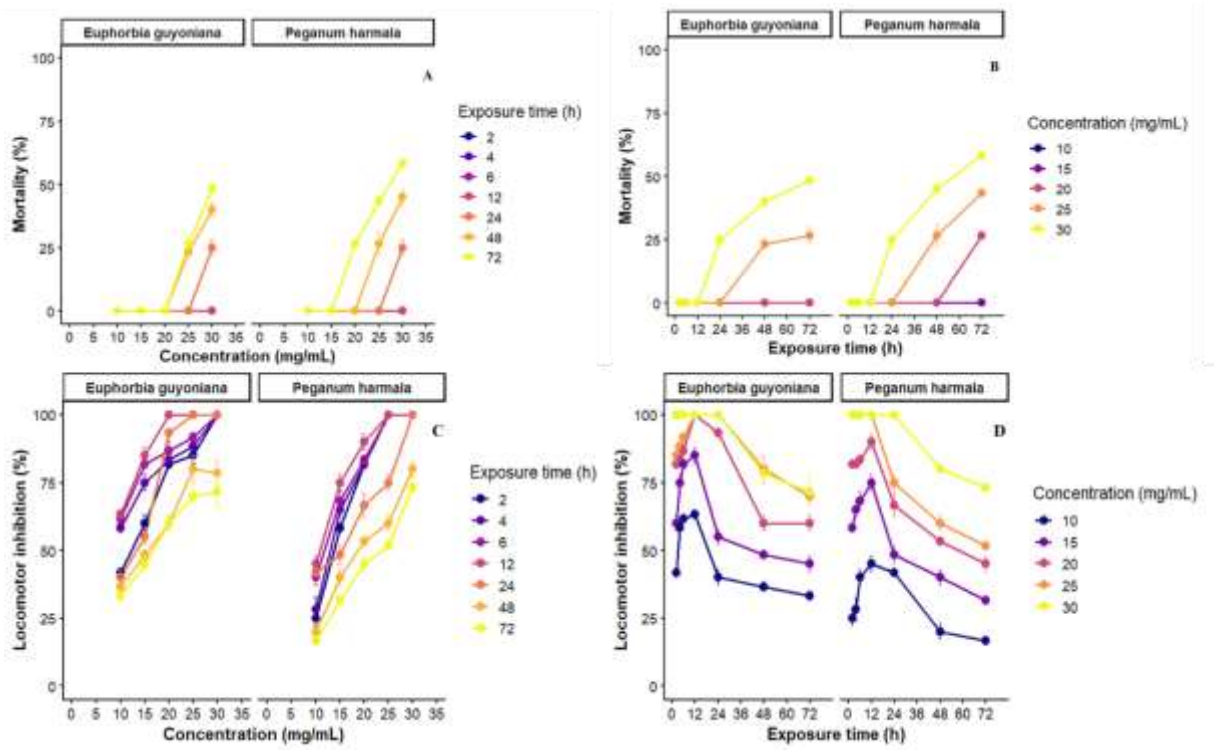


Figure 2. Toxicological effects of the organic extracts on *Androctonus australis*. A - Mortality as a function of extract concentration; B - Mortality as a function of exposure time; C -Locomotor inhibition as a function of extract concentration; D -Locomotor inhibition as a function of exposure time

No mortality or locomotor inhibition was observed in the 5% DMSO vehicle-control group during the 72 h observation period; both outcomes remained at 0% at all assessment times. Under the conditions used here, this indicates that the vehicle alone did not produce detectable mortality or locomotor inhibition in the tested scorpions.

Likelihood-ratio tests showed significant effects of plant ($\chi^2 = 9.46$, df = 1, P = 0.002), concentration ($\chi^2 = 433.91$, df = 4, P < 0.001), and exposure time ($\chi^2 = 533.52$, df = 6, P < 0.001). Significant plant × concentration ($\chi^2 = 14.73$, df = 4, P = 0.005) and concentration × exposure time interactions ($\chi^2 = 39.43$, df = 24, P = 0.025) were also detected. In contrast, neither the plant × exposure time interaction ($\chi^2 = 1.26$, df = 6, P = 0.974) nor the three-way interaction between plant, concentration, and exposure time ($\chi^2 = 0.22$, df = 24, P = 1.000) was significant.

The locomotor activity of *Androctonus australis* was progressively affected by the organic extracts (Fig. 2C and D). For both *Euphorbia guyoniana* and *Peganum harmala*, inhibition generally increased with increasing extract concentration, with the strongest responses observed at 25 and 30 mg/mL. In contrast, the temporal pattern was characterized by a pronounced inhibition during the early exposure period, followed by a gradual decrease at longer exposure times. Overall, both extracts produced comparable concentration-dependent effects on scorpion locomotor activity, while the decrease in inhibition over time suggests that this behavioral response was more pronounced during the initial phase of exposure.

Heatmap analysis revealed distinct patterns of mortality and locomotor inhibition in *Androctonus australis* following exposure to the organic extracts (Fig. 3A and B). Mortality was generally low during the early exposure periods and at lower concentrations, whereas higher mortality rates were observed at increasing concentrations and longer exposure times. For *Euphorbia guyoniana*, mortality remained absent or low up to 24 h, particularly at concentrations below 25 mg/mL, but increased from 48 h onwards and reached its highest level at 30 mg/mL after 72 h. A comparable temporal and concentration-related pattern was observed for *Peganum harmala*, with slightly higher mortality under some treatment conditions. The maximum mortality approached 60% at 30 mg/mL after 72 h for *P. harmala*. Overall, mortality increased as both concentration and exposure duration increased, indicating that these two experimental factors contributed to the observed toxic response. Although *P. harmala* tended to

produce higher mortality than *E. guyoniana*, particularly at 30 mg/mL and longer exposure periods, this difference was not statistically significant.

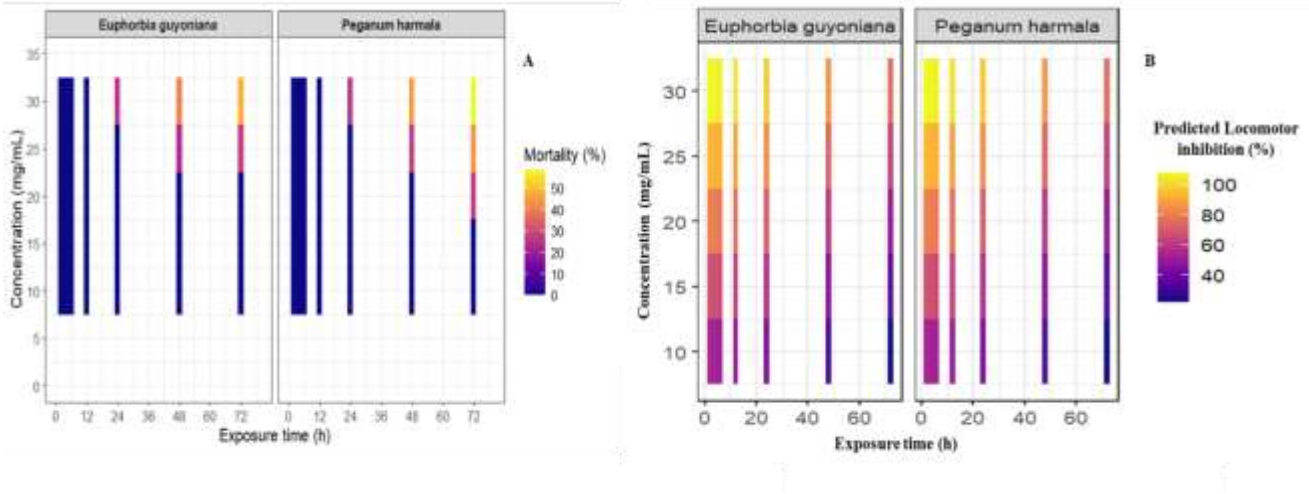


Figure 3. Heatmap representation of the effects of the organic extracts on *Androctonus australis*: mortality rates (A) and locomotor inhibition rates (B).

The locomotor inhibition heatmap showed a different temporal pattern. Locomotor impairment was most pronounced at the higher extract concentrations, particularly at 20 and 30 mg/mL, and was detectable during the early exposure periods for several treatments. In contrast to mortality, locomotor inhibition did not consistently increase with exposure time and tended to decrease at later observation times in some treatments. Similar response patterns were observed for both plant extracts, although the magnitude of inhibition varied among treatments. These results indicate that concentration was an important determinant of the behavioral response, whereas the temporal progression of locomotor inhibition was less consistent than that observed for mortality. Thus, the heatmap highlights a distinction between the relatively progressive mortality response and the more transient pattern of locomotor impairment induced by the extracts.

The boxplots showed a concentration-related increase in mortality for both *Euphorbia guyoniana* and *Peganum harmala* extracts (Fig. 4A). Mortality remained low at 10, 15, and 20 mg/mL, whereas higher concentrations, particularly 25 and 30 mg/mL, were associated with increased mortality. The highest mortality values were observed at 30 mg/mL. Although the two extracts showed broadly similar distributions, *P. harmala* produced slightly higher mortality at the highest concentration. Greater dispersion at higher concentrations indicated increased variability in individual responses under these treatment conditions.

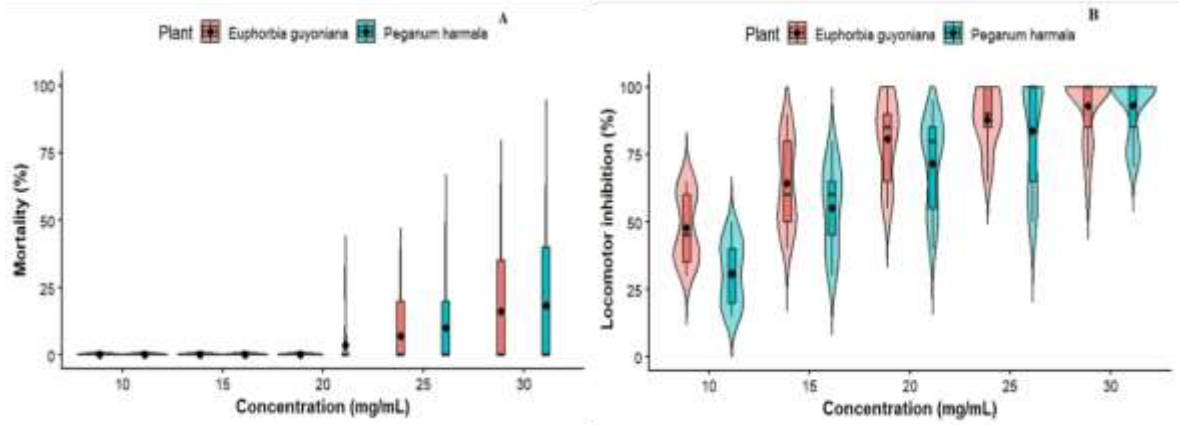


Figure 4. Boxplot representation of the effects of extract concentration on *Androctonus australis*: mortality rates (A) and locomotor inhibition rates (B).

The distribution of locomotor inhibition across extract concentrations is presented in Fig. 4B. For both extracts, inhibition generally increased with concentration, with stronger responses observed at 20, 25, and 30 mg/mL than at the lower concentrations. The distributions also showed greater variability among individuals at higher concentrations. Overall, the boxplots support the concentration-related pattern observed for locomotor inhibition, while indicating some variation in response among individual scorpions.

Correlation analysis revealed significant positive associations between mortality and extract concentration for both *Euphorbia guyoniana* and *Peganum harmala*. For *E. guyoniana*, mortality was moderately correlated with concentration ($r = 0.47$, $p = 0.0049$), while a similar association was observed for *P. harmala* ($r = 0.44$, $p = 0.0083$). Mortality was also positively correlated with exposure time, with significant associations observed for *E. guyoniana* ($r = 0.50$, $p = 0.0025$) and *P. harmala* ($r = 0.61$, $p < 0.001$). The stronger correlation observed for *P. harmala* suggests a closer association between mortality and exposure duration in this treatment. Overall, these results indicate that mortality was associated with both extract concentration and exposure time under the experimental conditions.

A stronger positive association was observed between locomotor inhibition and extract concentration for both *E. guyoniana* ($r = 0.77$, $p < 0.001$) and *P. harmala* ($r = 0.82$, $p < 0.001$). Thus, higher extract concentrations were associated with greater locomotor inhibition. In contrast, locomotor inhibition showed significant negative correlations with exposure time for *E. guyoniana* ($r = -0.45$, $p = 0.0042$) and *P. harmala* ($r = -0.48$, $p = 0.0035$), indicating that inhibition tended to decrease at longer exposure times.

3.5 Efficacy of plant extracts

The toxicological efficacy of each extract was evaluated by determining the lethal concentrations (LC50 and LC90) using the Probit method. The estimated values are presented in Table 3.

Table 3. Lethal concentration estimates corresponding to 50% and 90% mortality of *Androctonus australis* exposed to the plant extracts.

Extract	Exposure time (h)	Regression line	R^2	P_ value	LC_{50} (mg/mL)	LC_{90} (mg/mL)
Euphorbia guyoniana	48	y = 4.45x - 2.25	0.82	0.09	43 ± 1	83 ± 4
	72	y = 4.86x - 2.70	0.82	0.09	39 ± 3	71 ± 7
Peganum harmala	48	y = 4.73x -2.56	0.82	0.09	40 ± 3	75 ± 9
	72	y = 6 x -3.67	0.94	0.02	28 ± 0	46 ± 1

LC_{50}: lethal concentration (mg/mL) that kills 50% of the exposed scorpions; LC_{90} (mg/mL): lethal concentration that kills 90% of the exposed scorpions; R^2: Coefficient of determination; Y: Probits of percentage mortality X: logarithm of concentration; Values are presented as mean ± standard deviation (SD).

3.6 Molecular docking analysis against acetylcholinesterase

Molecular docking was performed to clarify whether the phytochemicals detected in the ethanolic extracts of *Peganum harmala* and *Euphorbia guyoniana* could interact with acetylcholinesterase (AChE), a neurotoxicity-related target frequently associated with pesticidal effects in arthropods. The docking protocol was validated by redocking the co-crystallized inhibitor into the active site of *Drosophila melanogaster* AChE. The redocked pose reproduced the crystallographic orientation with an RMSD of 1.32 Å, confirming the reliability of the docking conditions [26]. The predicted binding affinity of the co-crystallized ligand was −10.2 kcal/mol, while tacrine, used as a reference AChE inhibitor, showed a binding energy of −7.9 kcal/mol.

The predicted binding affinities of the compounds tentatively assigned by HPLC comparison with authentic standards are presented in Table 4. Rutin showed the most favourable docking score (−9.6 kcal/mol), followed by tannic acid (−9.3 kcal/mol) and quercetin (−8.4 kcal/mol). Catechin also showed a predicted affinity of −7.8 kcal/mol, while chlorogenic acid and α-tocopherol showed moderate predicted affinities. Vanillin, ascorbic acid, pyrocatechol, and adonitol showed weaker predicted

binding. These scores describe computationally predicted poses and affinities only; they do not demonstrate enzyme inhibition or establish that these compounds are responsible for the toxicity of the crude extracts [27].

Table 4. Predicted binding affinities of the identified phytochemicals against acetylcholinesterase.

Rank	Ligand	Source extract	Binding energy (kcal/mol)	Binding profile
Reference	Co-crystallised ligand 760	—	−10.2	Validation ligand
1	Rutin	*P. harmala* and *E. guyoniana*	−9.6	Strong
2	Tannic acid	*E. guyoniana*	−9.3	Strong
3	Quercetin	*P. harmala* and *E. guyoniana*	−8.4	Strong
Reference	Tacrine	Reference inhibitor	−7.9	Reference AChE inhibitor
4	Catechin	*P. harmala* and *E. guyoniana*	−7.8	Moderate-to-strong
5	Chlorogenic acid	*E. guyoniana*	−7.4	Moderate
6	α-Tocopherol	*P. harmala*	−7.2	Moderate
7	Vanillin	*P. harmala*	−5.9	Weak-to-moderate
8	Ascorbic acid	*E. guyoniana*	−5.7	Weak-to-moderate
9	Pyrocatechol	*P. harmala*	−5.5	Weak-to-moderate
10	Adonitol	*P. harmala*	−4.8	Weak

The interaction patterns of the three best-ranked phytochemicals are summarized in Table 5 and illustrated in Figure 5. Rutin, tannic acid and quercetin showed distinct but complementary binding modes. Their interaction networks were dominated by hydrogen bonding, supported by hydrophobic, π-type and electrostatic contacts. This pattern is typical of polyphenolic compounds, whose multiple hydroxyl groups and aromatic systems allow flexible recognition of enzyme-binding pockets [28].

Quercetin adopted a compact binding orientation within the AChE gorge, as shown in Figure 5A and 5B. Its binding was stabilized by an extensive hydrogen-bonding network involving TYR71, GLU80, ASN84, GLY150, GLY151, TYR162, GLU237, SER238, TYR370 and HIS480. The shortest hydrogen bonds were observed with ASN84, GLU237, SER238 and TYR370, suggesting strong polar stabilization within the binding cavity [29]. In addition, quercetin formed hydrophobic and aromatic contacts with TYR71 and TRP83, while the interaction map indicated a π-cation contribution involving HIS480. These interactions explain the favorable docking energy of quercetin and suggest that its planar flavanol scaffold is well suited for recognition by aromatic and polar residues within AChE [30].

Rutin exhibited the strongest predicted binding affinity among all tested phytochemicals. As illustrated in Figure 5C and 5D, rutin occupied a broad region of the AChE binding site, allowing simultaneous contact with several polar, charged and hydrophobic residues. The interaction profile included eleven hydrogen bonds involving HIS273, ILE327, LEU328, SER329, ILE400, LYS403, THR405, GLU408 and ARG571. Particularly favorable interactions were observed with HIS273, SER329, ILE400, LYS403, GLU408 and ARG571. In addition, rutin formed hydrophobic contacts with ALA333, LYS403 and PHE439, together with π-cation interactions involving LYS403 and HIS438. The presence of a salt bridge with LYS278 further reinforced the binding mode. The high docking score of rutin can therefore be attributed to its extended flavonoid glycoside structure, which enables multi-point anchoring across the binding gorge [31].

Tannic acid also displayed a strong predicted affinity toward AChE, with a binding energy of −9.3 kcal/mol. Its 2D and 3D binding modes are shown in Figure 5E and 5F. The ligand formed ten hydrogen bonds with HIS273, SER329, ASP344, ILE400, LYS403 and HIS438, with several short donor–acceptor distances below 3.0 Å. The interaction with HIS438 showed the shortest H–A distance (1.62 Å), while additional strong contacts were observed with ASP344, SER329 and ILE400. Tannic acid also formed salt bridges with HIS273, LYS278 and LYS403, indicating a strong electrostatic contribution to complex stabilization. However, because tannic acid is a large and highly flexible polyphenol, its favorable docking score should be interpreted cautiously. The extensive number of contacts may reflect broad surface complementarity rather than selective inhibition of AChE [32].

Table 5. Main molecular interactions of the three best-ranked phytochemicals with acetylcholinesterase.

Ligand	Binding energy (kcal/mol)	Hydrogen bonds	Hydrophobic / π interactions	Electrostatic interactions
Quercetin	−8.4	TYR71: 2.71/3.61 Å, 147.81°; GLU80: 3.30/3.93 Å, 126.91°; ASN84: 1.80/2.35 Å, 112.36°; ASN84: 3.14/3.53 Å, 104.50°; ASN84: 2.03/2.89 Å, 146.24°; GLY150: 2.78/3.44 Å, 122.69°; GLY151: 3.44/4.00 Å, 116.22°; TYR162: 2.30/3.03 Å, 133.21°; GLU237: 1.83/2.67 Å, 142.08°; SER238: 1.88/2.74 Å, 149.09°; TYR370: 2.03/2.98 Å, 166.18°; HIS480: 3.50/3.95 Å, 109.34°; HIS480: 3.30/3.98 Å, 126.14°	TYR71: 3.56 Å; TRP83: 3.35 Å; TRP83: 3.31 Å; π-stacked and π-T-shaped interactions involving the aromatic region	π-cation interaction involving HIS480
Rutin	−9.6	HIS273: 1.87/2.74 Å, 141.59°; HIS273: 2.84/3.49 Å, 124.59°; ILE327: 3.76/4.07 Å, 100.13°; LEU328: 2.59/3.36 Å, 136.75°; SER329: 2.11/2.62 Å, 110.71°; ILE400: 2.15/3.08 Å, 159.21°; LYS403: 2.31/3.32 Å, 170.04°; THR405: 2.96/3.81 Å, 141.61°; GLU408: 2.86/3.79 Å, 161.58°; GLU408: 2.88/3.84 Å, 175.25°; ARG571: 2.18/2.67 Å, 107.24°	ALA333: 3.99 Å; LYS403: 3.70 Å; PHE439: 3.67 Å; π-cation with LYS403: 3.54 Å; π-cation with HIS438: 5.65 Å; π-alkyl and π-T-shaped contacts	Salt bridge with LYS278: 5.29 Å
Tannic acid	−9.3	HIS273: 2.16/3.08 Å, 148.57°; HIS273: 2.51/3.00 Å, 111.22°; SER329: 2.21/2.57 Å, 101.50°; ASP344: 1.99/2.88 Å, 151.91°; ASP344: 2.47/3.17 Å, 127.88°; ILE400: 1.83/2.60 Å, 133.59°; ILE400: 2.22/3.09 Å, 149.30°; LYS403: 3.26/3.94 Å, 129.22°; LYS403: 2.06/3.05 Å, 162.03°; HIS438: 1.62/2.55 Å, 149.77°	THR275: 3.03 Å; π-T-shaped interaction with PHE439; carbon–hydrogen contacts	Salt bridges with HIS273: 5.20 Å, LYS278: 5.45 Å and LYS403: 4.95 Å

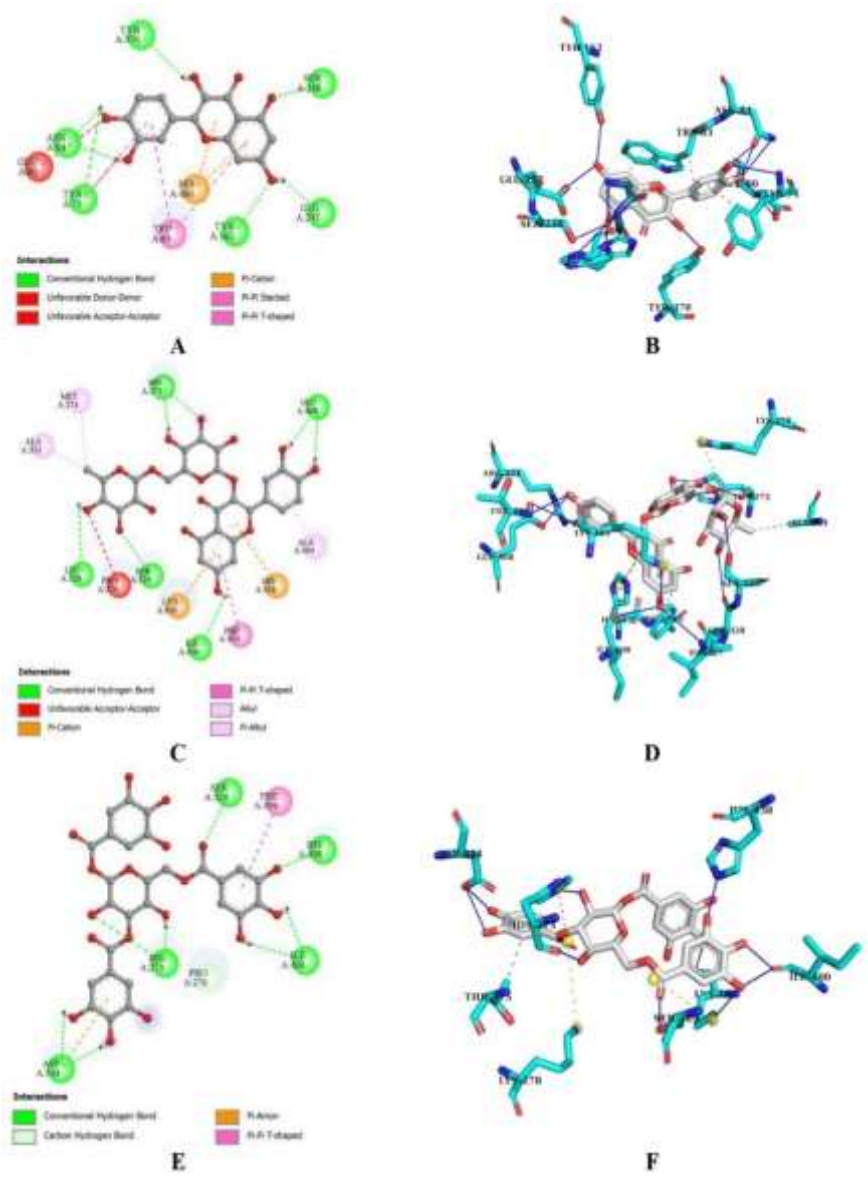


Figure 5. Two-dimensional and three-dimensional interaction diagrams of the best-ranked phytochemicals docked into AChE. A, B - Quercetin– Acetylcholinesterase complex; C, D - Rutin–AChE complex; E, F- Tannic acid– Acetylcholinesterase complex.

3.7 Discussion

Scorpion envenomation represents the primary cause of toxicity and accounts for considerable mortality in various regions of the world. This public health burden is attributed to the high frequency and/or severity of envenomation cases, which often exceed the capacity of local healthcare systems to manage effectively [4, 33].

The primary strategy for controlling scorpion populations has been the application of chemical insecticides, especially in contexts where alternative methods, such as the removal of breeding sites, woodpiles, rocks, and debris from areas surrounding human dwellings, are either impractical or ineffective [7]. In the search for effective and economically viable alternatives to chemical pesticides, researchers are actively exploring the use of novel naturally derived molecules, primarily extracted from plants [34]. Plant-based pesticides offer several advantages over synthetic pesticides. They are generally biodegradable, less costly to produce, and have a low impact on non-target organisms, including humans [35]. These attributes make plant extracts a promising avenue for the development of more sustainable and environmentally friendly pest control strategies. However,

plant origin alone does not establish that a preparation is safer, more biodegradable, less costly, or less harmful to non-target organisms than a synthetic pesticide. Such properties require direct testing of each preparation under relevant conditions.

The main objective of this study was to propose an ecological and environmentally friendly solution to reduce the risks associated with scorpion envenomation, while also decreasing the number of stings in humans. To achieve this goal, we focused our research on the use of plant extracts, known for their bio-pesticidal effect and traditional use in arid and semi-arid regions. By evaluating the effects of these extracts on scorpions, our approach aims to identify sustainable alternatives to synthetic insecticides, while also promoting the value of local plant resources. Two species were selected for their richness in bioactive compounds: *Peganum harmala* and *Euphorbia guyoniana*.

Pesticides come into contact with invertebrates through their cuticles or tracheal mucosa [7]. In this study, toxicity may occur through the absorption of extracts, which can exert various physiological effects, notably by disrupting the nervous system, impairing respiration, or acting as hormonal inhibitors.

According to the studies conducted by [7, 33, 36], the exposure of scorpions to chemical pesticides induces an escape response, reflecting strong sensory irritation and the activation of avoidance behavior. This phenomenon is likely linked to the stimulation of cuticular or tracheal receptors by volatile chemical compounds. In contrast, the plant extracts evaluated in the present study induced a noticeable inhibition of movement, primarily during the first 12 hours of exposure. This inhibition may result from a disruptive effect on the nervous system or an alteration of energy metabolism. These extracts exhibit a mode of action distinct from that of conventional pesticides and offer promising potential as biopesticide alternatives for environmentally compatible scorpion control, by limiting their dispersion and reducing their biological activity.

The extracts produced locomotor inhibition, particularly during the early observation period. This behavioural response could be compatible with several physiological effects, including nervous-system disturbance or changes in energy metabolism, but neither pathway was measured directly in this study. Consequently, the observed immobility cannot be attributed specifically to acetylcholinesterase inhibition or to a single mode of action.

Scorpions are predominantly nocturnal and their activity patterns may be relevant to future studies of exposure and contact [9, 37, 38]. However, the present laboratory experiment did not evaluate application timing, outdoor persistence, or efficacy in natural habitats. Any recommendations about when or where to apply these extracts would require dedicated field studies.

It is advisable to avoid periods of intense sunlight when applying these extracts, as prolonged exposure to high temperatures and UV radiation can alter the chemical composition of the active ingredients in natural extracts [39]. The environmental persistence and stability of these extracts under sunlight, high temperatures, and other outdoor conditions were not evaluated in this study. Their suitability for environmental application therefore remains undetermined.

This study provides a preliminary laboratory assessment of the toxicological and behavioural effects of ethanolic extracts of *Peganum harmala* and *Euphorbia guyoniana* on *Androctonus australis*. The findings may help prioritize these locally available plant resources for further investigation. They do not, by themselves, demonstrate that either extract reduces scorpion stings, is effective under field conditions, or is suitable for practical scorpion control.

The docking results suggest that the strongest AChE interactions are mainly associated with polyphenolic constituents. Rutin and quercetin, which were detected in both plant extracts, may contribute to the neurotoxicity-related effects of both *P. harmala* and *E. guyoniana*. Tannic acid, detected in *E. guyoniana*, may also contribute to its activity through extensive polar and electrostatic interactions with AChE [40]. However, the overall mortality observed in the contact bioassay is unlikely to arise from a single compound or a single mechanism. The extracts contain several secondary metabolite families, including flavonoids, tannins, coumarins, alkaloids, quinones and steroids, which may act collectively through nervous-system disruption, oxidative stress, respiratory impairment, cuticular penetration or metabolic disturbance [32].

These findings provide a molecular explanation that is consistent with the dose- and time-dependent mortality observed experimentally. At low concentrations, the number of bioactive constituents reaching physiological targets may be insufficient to induce marked mortality. At higher concentrations and longer exposure times, cumulative penetration and interaction with neurotoxicity-related targets such as AChE may contribute to the increased mortality recorded after 48–72 h. The docking results therefore support the hypothesis that AChE interaction may be one of the mechanisms involved in the toxic effect activity of *P. harmala* and *E. guyoniana* ethanolic extracts [41, 42].

Nevertheless, docking remains predictive and does not prove enzymatic inhibition. Therefore, the present results should be considered as mechanistic support rather than direct confirmation of AChE inhibition. Further biochemical assays using purified AChE or arthropod-derived cholinesterase extracts are required to confirm the inhibitory potential of rutin, tannic acid, quercetin and the corresponding plant extracts. Despite this limitation, the docking analysis strengthens the biological interpretation of the toxic effect activity and highlights polyphenolic constituents as plausible contributors to the pesticide potential of these Saharan medicinal plants [43].

4. CONCLUSION

Under the laboratory conditions tested, ethanolic extracts of *Peganum harmala* and *Euphorbia guyoniana* produced toxicological and behavioural effects against *Androctonus australis*. Mortality was associated with extract concentration and exposure time, while locomotor inhibition was generally more pronounced at higher concentrations. These findings represent a preliminary assessment and do not establish field effectiveness or suitability for practical scorpion control.

Qualitative phytochemical screening and HPLC retention-time comparisons provided preliminary information about the chemical profiles of the extracts. The HPLC assignments remain tentative, and the observed effects cannot be attributed to specific compounds or mechanisms on the basis of the present data. Molecular docking predicted interactions between selected phytochemicals and AChE, but these predictions do not demonstrate enzyme inhibition.

Further studies should confirm compound identities and concentrations, test AChE inhibition experimentally, and assess effects on non-target organisms, environmental persistence, and efficacy under field conditions. Until such evidence is available, the extracts should be considered candidates for further investigation rather than established safe or environmentally suitable scorpion-control agents.

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AUTHOR CONTRIBUTIONS

Conceptualization, Linda Rouari and Salah Eddine Sadine; Data curation, Elhafnaoui Lanez and Linda Rouari; Formal analysis, Linda Rouari; Investigation, Linda Rouari and Elhafnaoui Lanez; Methodology, Abdelmalek Rouari, Zineb Souilem, Amel Rouari, and Nacira Boukhari; Project administration, Linda Rouari; Software, Elhafnaoui Lanez, Mohammed Larbi Ben Amor, and Farouk Benaceur; Supervision, Salah Eddine Sadine; Validation, Linda Rouari, Farouk Benaceur, Elhafnaoui Lanez, and Salah Eddine Sadine; Visualization, Linda Rouari and Salah Eddine Sadine; Writing – original draft, Linda Rouari; Writing – review and editing, Linda Rouari, Ilham Messahli, Hicham Benhammouda and Salah Eddine Sadine. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The experimental procedures involved adult scorpions; an invertebrate group not covered by the institutional regulations governing animal experimentation. Therefore, institutional ethical approval was not required. The animals were collected, maintained and handled according to applicable institutional and national guidelines.

DATA AVAILABILITY STATEMENT

The raw data supporting the findings of this study, including mortality and locomotor-response data from *Androctonus australis* exposed to ethanolic extracts of *Peganum harmala* and *Euphorbia guyoniana*, are available from the corresponding author upon reasonable request.

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