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Insecticidal Activity and Phytochemical Profiling of *Oudneya Africana* Extract against *Tenebrio Molitor*

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Abstract: Stored-product insects contribute substantially to post-harvest losses worldwide, highlighting the urgent demand for sustainable alternatives to synthetic insecticides. The increasing use of chemical insecticides has generated concerns regarding environmental contamination and the development of resistant insect populations, reinforcing the need for safer pest management strategies. Plant-derived bioactive compounds have attracted considerable attention because of their biodegradability, low environmental persistence, and reduced toxicity toward non-target organisms. *Oudneya africana* R.Br. (Brassicaceae) is recognized for its diverse phytochemical profile. To our knowledge, no previous study has investigated the insecticidal activity of *Oudneya africana* against *Tenebrio molitor*. This study assessed the contact toxicity of an ethanolic extract of *Oudneya africana* against larval and adult stages of *Tenebrio molitor*. Bioassays were performed at concentrations ranging from 10 to 50 mg mL^{-1}, with mortality monitored for 72 h. Qualitative phytochemical screening detected alkaloids, steroids, flavonoids, quinones, coumarins, and terpenoids. HPLC profiling tentatively indicated the presence of quercetin, gallic acid, catechin, caffeic acid, nicotinic acid, rutin, adonitol, chlorogenic acid, coumaric acid, and ascorbic acid through comparison of their retention times with those of reference standards. Mortality increased progressively with extract concentration in larvae,

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whereas adults exhibited a weaker and non-significant concentration-dependent response. Larvae showed lower LC50 (19.65 mg mL^{-1}) than adults (LC50 = 42.96 mg mL^{-1}). Lethal time analysis indicated faster toxicity in larvae (LT50 = 9.82 h) than in adults (LT50 = 77.37 h). GLM analysis revealed significant effects of extract concentration and developmental stage, with no significant interaction. Molecular docking predicted favorable interactions of quercetin and catechin with *Tenebrio molitor* α-amylase, suggesting a possible interaction with this digestive enzyme. These results indicate that *Oudneya africana* may represent a potential source for the development of more environmentally sustainable botanical insecticides.

Key words: Insecticidal activity, *Oudneya Africana*, phenolic compounds, *Tenebrio molitor*, Ghardaïa.

Post-harvest infestation by storage pests poses a serious challenge to food security worldwide, with cereal losses reported to reach approximately 10 to 40% of total global production (Hagstrum and Athanassiou, 2019). The yellow mealworm, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), is a common insect pest found in stored agricultural products, including flour, cereals, bran, and other starch-based commodities. Its high reproductive capacity, combined with its ability to withstand typical storage conditions, enables this species to establish and persist in stored-product environments. (Ahmad *et al.*, 2021).

For many years, the protection of stored commodities has depended heavily on synthetic insecticides, including organophosphates, pyrethroids, methyl bromide, and phosphine. However, the intensive and prolonged use of these chemicals has generated increasing concerns about environmental contamination, unintended effects on beneficial and non-target organisms, and the development of insecticide resistance. In particular, reduced sensitivity to phosphine has been reported in several insect pests associated with stored agricultural products, limiting the effectiveness of this widely used fumigant (Benhalima *et al.*, 2004). In this context, botanical insecticides are increasingly being considered as viable alternatives that are consistent with the principles of integrated pest management and sustainable agriculture (Alouani *et al.*, 2025). Plant-derived bioactive compounds have received growing scientific attention as sustainable tools for pest management, owing to their relatively low environmental impact and broad spectrum of insecticidal activities (Wink, 2003; Isman, 2006).

Oudneya africana R.Br. (Brassicaceae) is a drought-tolerant plant naturally found in the arid and semi-arid regions of North Africa, with its distribution extending across Algeria, Tunisia, Libya, and Morocco. (Nabti and Belhattab, 2016). It contributes to soil stabilization and dune fixation and contains bioactive compounds, particularly phenolic compounds and flavonoids, with potential biological activity (Ould El Hadj *et al.*, 2003). Phenolic compounds are known to interfere with insect growth and development by modulating hormonal levels, disrupting molting processes, and inducing oxidative stress (Acheuk and Doumandji-Mitiche, 2013).

Despite the biological activities previously reported for *Oudneya africana*, including its effects on dipteran larvae, no study has, to our knowledge, investigated its insecticidal activity against *Tenebrio molitor* to date. In view of the bioactive phenolic compounds detected in the plant extract, *Oudneya africana* may represent a promising botanical resource for further investigation as a potential source of plant-based insecticides.

Tenebrio molitor is also widely used as a model organism in toxicological and physiological studies due to its ease of rearing, short life cycle, high reproductive capacity, and well-characterized biochemical markers (Vigneron *et al.*, 2019). These characteristics make it suitable for evaluating the biological effects and potential mechanisms of plant-derived substances.

Accordingly, this study aimed to assess the insecticidal effects of an ethanolic extract of *Oudneya africana* on the larval and adult stages of *Tenebrio molitor*, characterize its phytochemical profile through qualitative screening and HPLC analysis, and explore the possible binding interactions of selected phenolic constituents with α-amylase using molecular docking.

Materials and Methods

Plant sampling and botanical identification

Leaves, flowers, and stems of *Oudneya africana* were collected in the Ghardaïa region. Botanical identification was performed according to the identification keys provided by Quezel and Santa (1963). After collection, the aerial parts were air-dried at room temperature in a shaded and well-ventilated environment until a constant weight was reached. The dried plant material was subsequently ground into a fine powder and stored under appropriate conditions until extraction.

Insect rearing conditions

Larvae and adults of *Tenebrio molitor* were maintained in plastic rearing containers under controlled laboratory conditions. The rearing environment was regulated at 27 ± 2°C and 65 ± 5% relative humidity, with a photoperiod of 12 h light and 12 h darkness. Semolina served as both the rearing medium and primary food source, whereas fresh potato pieces were added at regular intervals to supply moisture and supplementary nutrients.

Preparation of the botanical extract

For the extraction procedure, 200 g of finely powdered *Oudneya africana* material was macerated in 1 L of ethanol 70% at room temperature for 24 h. Throughout the extraction period, the suspension was continuously agitated using a shaking incubator (Precise Shaking Incubator WIS-10, Korea). The macerated material was subsequently subjected to three successive vacuum-filtration steps. The resulting filtrate was evaporated under reduced pressure at 45°C using a rotary evaporator until complete solvent removal. The resulting dry extract was weighed and maintained at 4°C until further analysis.

Phytochemical screening

The phytochemical profile was assessed through conventional qualitative assays, in which characteristic color reactions and/or precipitate formation were used as indicators for specific classes of secondary metabolites (El-Haoud *et al.*, 2018; Rouari *et al.*, 2022). The screening covered flavonoids, tannins, coumarins, free quinones, alkaloids, reducing compounds, saponins, steroids, and anthocyanins.

Chromatographic Profiling of the Extract

For the chromatographic determination, 0.5 mg of the dried extract was dissolved in 5 mL of HPLC-grade methanol. The solution was subsequently filtered and transferred to an autosampler vial prior to injection. Analyses were performed using a Thermo Scientific Vanquish UHPLC system fitted with an autosampler and a reversed-phase C18 column. Isocratic elution was performed with a mobile phase consisting of methanol, acetonitrile, and purified water at a volumetric ratio of 40:15:45. The chromatographic run was conducted at a flow rate of 0.8 mL min^{-1}, while the column was maintained at 25°C. Detection was carried out at 254 nm, and the injection volume was 20 µL. The compounds detected in the extract were tentatively identified by comparing their retention times with those of corresponding authentic reference standards analyzed under the same chromatographic conditions.

Insecticidal bioassay

Contact toxicity was evaluated by exposing *Tenebrio molitor* larvae and adults to the ethanolic extract of *Oudneya africana* at concentrations of 10, 20, 30, 40, and 50 mg mL^{-1}. For each concentration, 1 mL of the prepared extract solution was applied to a filter paper placed in a 100 mm Petri dish. Five insects were then placed directly onto the treated filter paper. Each concentration was tested in three independent replicates, with one Petri dish per replicate, resulting in 15 insects tested per concentration and developmental stage. The negative control was prepared using 20% DMSO and was tested under the same experimental conditions, with three replicate Petri dishes containing five insects each. Mortality was assessed after 12, 24, 48, and 72 h of exposure. At each assessment, insects were classified as dead when they exhibited complete absence of spontaneous movement and did not respond to gentle mechanical stimulation of the body or appendages.

A 20% DMSO solution was selected as the solvent control, as this concentration was necessary to obtain complete solubilization of the ethanolic extract at the concentrations tested. For the control treatment, 1 mL of the

DMSO solution was applied to filter paper placed inside the Petri dishes, using the same application procedure as that employed for the extract-treated groups. Throughout the 72-h exposure period, no mortality was observed in insects exposed to 20% DMSO alone. This result indicated that, under the experimental conditions used in this study, the selected DMSO concentration did not produce detectable mortality. Accordingly, mortality recorded in the treated groups was assessed relative to the corresponding solvent control.

Mortality (%) was calculated as follows: (Number of insects that died / Total number of insects tested) × 100 (Rouari *et al.*, 2022, Kemassi *et al.*, 2018).

As no mortality was recorded in the solvent control, no correction using Abbott's formula was necessary, and the observed mortality values were used directly for subsequent analyses.

Probit regression was applied to determine the concentrations required to cause 50% and 90% mortality (LC50 and LC90, respectively), as well as the exposure periods associated with 50% and 90% mortality (LT50 and LT90). Mortality percentages were converted into Probit values, and concentrations or exposure times were transformed into logarithmic values to establish dose-response relationships.Bas du formulaire

Molecular Docking

The three-dimensional structure of larval α-amylase from *Tenebrio molitor* was obtained from the Protein Data Bank (PDB ID: 1JAE) and used as the target receptor for molecular docking simulations. This enzyme was selected as a biological target because it plays a fundamental role in carbohydrate digestion and energy supply during larval development. α-Amylase catalyses the hydrolysis of starch into simple sugars required for growth and metabolism; therefore, disruption of this enzymatic activity may impair nutrient utilization and contribute to insect mortality (Strobl *et al.*, 1998; Sagu *et al.*, 2021).

Selected compounds identified by HPLC analysis, namely quercetin, gallic acid, catechin, and caffeic acid, were selected for docking evaluation. Molecular structures for the selected compounds were retrieved from PubChem and subsequently prepared in appropriate file formats for the docking analysis. Temephos was included as a reference insecticidal ligand to provide a comparative computational benchmark; its docking score was not interpreted as a measure of insecticidal efficacy.

Prior to docking, the α-amylase receptor was prepared in AutoDock Tools by deleting water molecules associated with the crystal structure, introducing polar hydrogen atoms, and applying Kollman atomic charges. The selected ligands were subjected to energy minimization, followed by hydrogen addition and assignment of Gasteiger partial charges to obtain suitable structures for computational analysis.

Molecular docking was subsequently conducted with AutoDock Vina to predict the interaction of each ligand with the enzyme active site. The search space was positioned around the catalytic region of α-amylase and defined with dimensions adequate to encompass the relevant binding pocket. For each ligand, the predicted binding affinity was reported as binding free energy (kcal/mol); increasingly negative values were interpreted as indicative of more favorable predicted ligand-enzyme interactions (Trott and Olson, 2009).

Based on the docking results, quercetin and catechin were retained for a more detailed examination of their binding modes. The most favorable poses were subsequently inspected to characterize the interactions established with the protein, particularly hydrogen bonding and hydrophobic contacts. Molecular interaction profiles were visualized with BIOVIA Discovery Studio Visualizer v21 (Aouiffat *et al.*, 2025).

Statistical treatment of experimental data

All experimental data were processed and statistically analysed using Microsoft Excel and R software (version 4.5.1). Probit analysis was used to estimate lethal concentrations (LC50 and LC90) and lethal times (LT50 and LT90). A P-value < 0.05 was considered statistically significant.

Results and Discussion

Plant-based bioactive compounds are increasingly being investigated as potential alternatives to conventional synthetic pesticides,

particularly because of their biodegradability, relatively limited environmental persistence, and generally lower toxicity to non-target organisms (Guarrera, 1999; Lokesh *et al.*, 2010; El-Naggar *et al.*, 2017). In the present study, the ethanolic extract of *Oudneya africana* exhibited promising insecticidal activity and was characterized by the presence of several groups of secondary metabolites, notably flavonoids, alkaloids, terpenoids, and other phenolic constituents.

Extraction yield

The ethanolic extract of *Oudneya africana* yielded 5.33%, corresponding to the proportion of extract obtained relative to the initial plant mass. The extraction yield obtained in this study is consistent with previous reports on *Oudneya africana*, where similar yields were recorded for ethanolic extracts (Rouari *et al.*, 2022; Hajlaoui *et al.*, 2019). The efficiency of plant extraction can vary considerably according to the polarity of the solvent used, the nature of the plant material, and the specific conditions applied during the extraction process (Turkmen *et al.*, 2006; Koruthu *et al.*, 2011; Hapsari *et al.*, 2022). Ethanol, as a moderately polar solvent, is particularly effective in extracting phenolic compounds, which are often associated with biological activity.

Table 1. Qualitative screening of phytochemical classes in Oudneya africana extract

Phytochemical compounds	Oudneya africana
Flavonoids	++
Tannins	-
Terpenoids	++
Coumarins	+++
Free quinones	+
Alkaloids	++
Reducing compounds	-
Saponins	-
Steroids	+++

- negative; + weakly positive; ++ moderately positive; +++ strongly positive.

Phytochemical composition

The phytochemical analysis presented in Table 1 showed that the ethanolic extract of *Oudneya africana* contained a diverse range of secondary metabolites, with flavonoids, terpenes, coumarins, alkaloids, and steroids detected in the extract.

HPLC analysis of plant extract

HPLC analysis of the ethanolic extract of *Oudneya africana* revealed 11 distinct peaks (Fig. 1), corresponding to phytochemical constituents. Among these, 10 compounds were tentatively identified by comparison with reference standards, including

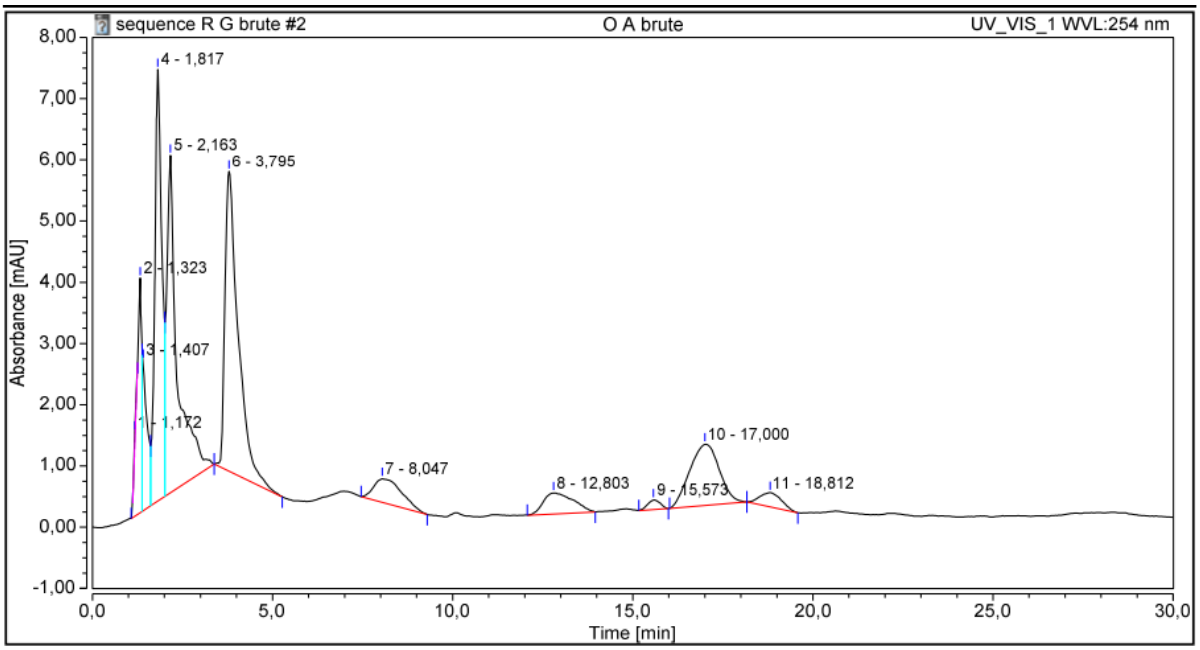


Fig 1. HPLC chromatogram of ethanolic extract of Oudneya africana. Peaks correspond to detected phytochemical constituents in the extract.

quercetin (RT = 1.172 min), gallic acid (RT = 1.323 min), catechin (RT = 1.407 min), caffeic acid (RT = 1.817 min), nicotinic acid (RT = 2.163 min), rutin (RT = 3.795 min), adonitol (RT = 8.047 min), chlorogenic acid (RT = 12.803 min), coumaric acid (RT = 17.000 min), and ascorbic acid (RT = 18.812 min). One peak at RT = 15.573 min could not be assigned to any of the available reference standards and was therefore reported as NI (not identified). These results provide a qualitative overview of several phenolic and related compounds detected in the ethanolic extract; however, their individual concentrations and relative abundances were not quantified in the present study.

Among the tentatively identified compounds, quercetin, catechin, gallic acid, and caffeic acid are widely reported to exhibit insecticidal and physiological effects in insects. Quercetin has been shown to affect insect growth and behaviour, particularly in herbivorous species (Riddick, 2021). Gallic acid contributes to plant defence responses through the activation of metabolic pathways associated with resistance mechanisms (Zhang *et al.*, 2022). Caffeic acid has also been associated with alterations in insect physiological functions and immune responses, with the magnitude of these effects depending on the concentration applied

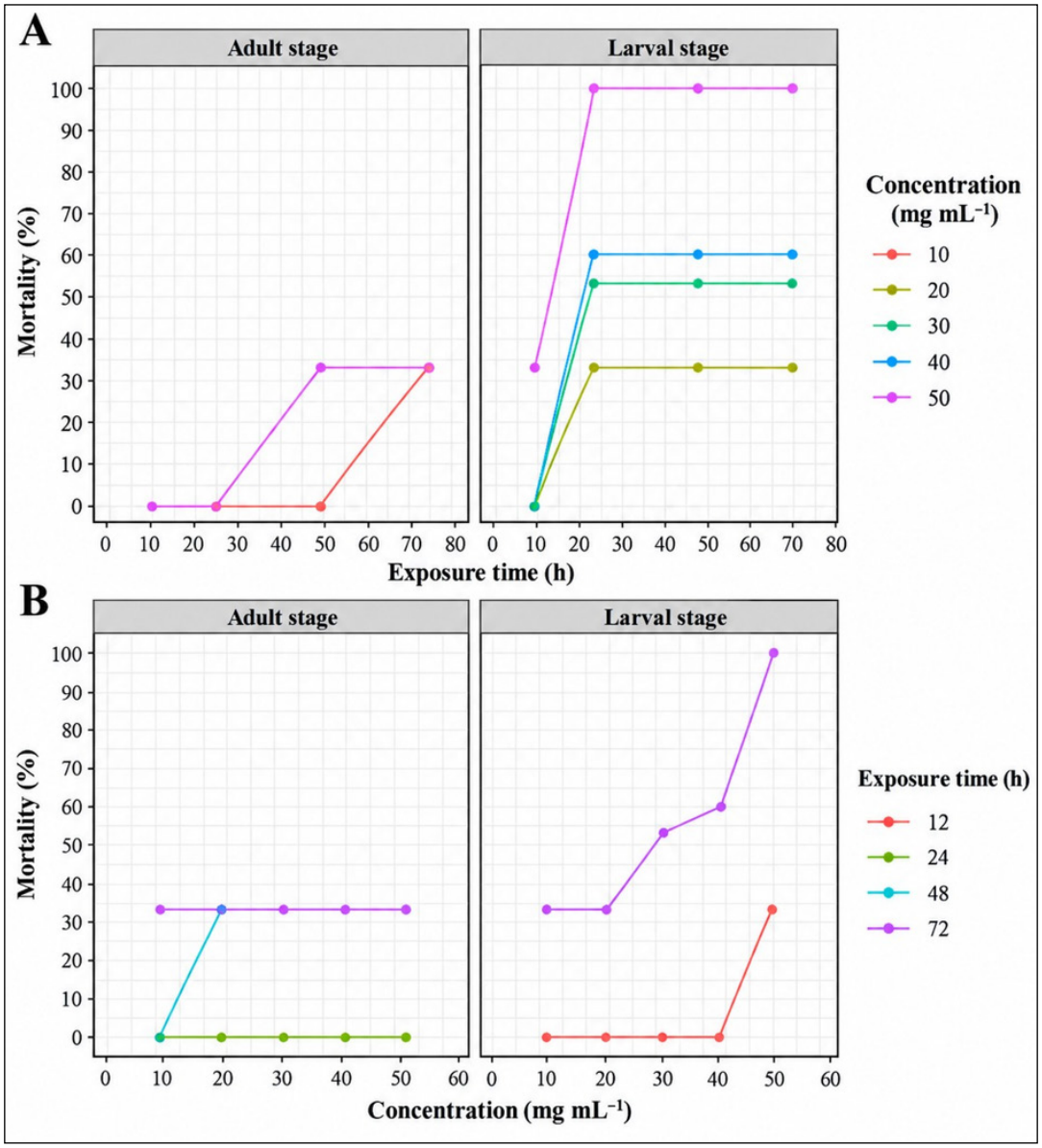


Fig. 2. Variation in mortality rates of Tenebrio molitor in response to Oudneya africana extract. A - Effect of exposure time on mortality (%) of adults and larvae; B - Effect of extract concentration on mortality (%) of larvae and adults.

Table 2. Lethal concentration values of an ethanolic extract of Oudneya africana against Tenebrio molitor

Stage	Exposure time	Equation	R^2	LC50 [mg mL^{-1}]	LC90 [mg mL^{-1}]
Adult	48 h	y = 6.22x - 5.16	0.71	42.96	69.04
Larvae	48 h	y = 3.36x + 0.65	0.53	19.65	47.23

LC50: lethal concentration (mg mL^{-1}) that kills 50% of the exposed *Tenebrio molitor*; LC90 (mg mL^{-1}): lethal concentration that kills 90% of the exposed *Tenebrio molitor*; R^2: Coefficient of determination; Y: Probits of percentage mortality X: logarithm of concentration.

Table 3. Lethal time values of an ethanolic extract of Oudneya africana against Tenebrio molitor

Stage	Concentration	Equation	R^2	LT50 [h]	LT90 [h]
Adult	50 mg mL^{-1}	y = 6.99x - 8.22	0.83	77.37	118.02
Larvae	50 mg mL^{-1}	y = 3.647x + 1.3819	0.67	9.82	22.06

LT50: lethal time (h) that kills 50% of the exposed *Tenebrio molitor*; LT90 (h): lethal time that kills 90% of the exposed *Tenebrio molitor*; R^2: Coefficient of determination; Y: Probits of percentage mortality X: logarithm of exposure time.

(Punia *et al.*, 2023). Nevertheless, the available evidence does not allow the insecticidal effects observed in the present work to be attributed specifically to these individual compounds. The bioassays were conducted using the crude ethanolic extract, which contains a mixture of constituents, rather than purified or isolated molecules.

Insecticidal activity of the extract

The ethanolic extract of *Oudneya africana* induced mortality in *Tenebrio molitor* after 72 h of exposure, with higher mortality generally observed at higher concentrations (Fig. 2).

Statistical analysis revealed significant effects of both developmental stage and extract concentration on mortality. Figure 3 shows higher mortality in larvae than in adults.

Lethal concentrations and times

The toxic effects of the extract were evaluated by determining the lethal concentrations (LC50 and LC90) and lethal times (LT50 and LT90) using Probit analysis (Tables 2 and 3).

The ethanolic extract of *Oudneya africana* showed greater toxicity toward larvae than adults, as reflected by the estimated LC50 values of 19.65 and 42.96 mg mL^{-1}, respectively. The corresponding LC90 values were 47.23 mg mL^{-1} for larvae and 69.04 mg mL^{-1} for adults. At the highest tested concentration (50 mg mL^{-1}), the estimated LT50 and LT90

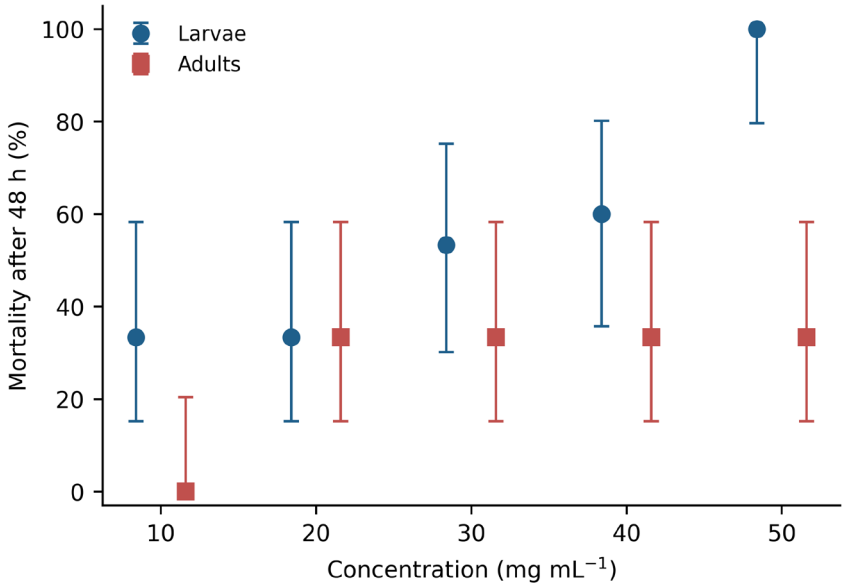


Fig. 3. Mortality of adult and larval stages of Tenebrio molitor after 48 h of exposure to different concentrations of the ethanolic extract. Points represent observed mortality, and error bars indicate 95% Wilson confidence intervals.

Table 4. Likelihood-ratio tests of the effects of extract concentration, developmental stage, and their interaction on Tenebrio molitor mortality

Effect tested	χ^2	Df	P-value
Extract concentration	16.38	1	< 0.001
Developmental stage	15.59	1	< 0.001
Concentration × developmental stage	0.32	1	0.57

for larvae were 9.82 and 22.06 h, respectively. In adults, the corresponding estimates were 77.37 and 118.02 h, both exceeding the 72 h experimental observation period. These latter values should therefore be interpreted as extrapolations derived from the fitted Probit model rather than as mortality times directly recorded during the experiment.

Statistical analysis revealed significant effects of extract concentration and developmental stage on mortality, whereas the concentration × developmental stage interaction was not significant (Table 4).

The likelihood-ratio tests revealed significant effects of both extract concentration and developmental stage on *Tenebrio molitor* mortality (χ^2 = 16.38, df = 1, P < 0.001 and χ^2 = 15.59, df = 1, P < 0.001, respectively; Table 4). In contrast, the concentration × developmental stage interaction was not significant (χ^2 = 0.32, df = 1, P = 0.57), indicating that the effect of concentration did not differ statistically between larvae and adults. Nevertheless, the observed mortality was higher in larvae than in adults, as illustrated in Figure 3.

The higher observed mortality in larvae may be associated with physiological and biochemical differences between developmental stages, including differences in cuticle properties and detoxification capacity. However, these mechanisms were not directly investigated in the present study. Such stage-dependent responses have been reported in other insect models exposed to botanical extracts.

The significant effect of extract concentration indicates that mortality varied significantly with increasing extract concentration. The significant effect of developmental stage indicates that mortality differed between larvae and adults. In contrast, the non-significant concentration × developmental stage interaction indicates that the concentration effect did not differ significantly between the two developmental stages. These findings suggest that both extract concentration and developmental stage contributed significantly to the observed mortality response.

The insecticidal effects reported for botanical extracts can differ substantially among plant species, reflecting differences in their phytochemical profiles as well as in the experimental conditions used for evaluation. Previous studies have documented insecticidal activity of extracts obtained from plants such as *Azadirachta indica*, *Nicotiana tabacum*, and *Ocimum sanctum* against *Tenebrio molitor* and

Table 5. Molecular docking results of catechin and quercetin with Tenebrio molitor larval a-amylase (PDB ID: 1JAE)

Complexe	Residues	Interaction type	Distances (Å)
1JAE -Catechin	GLN366	Carbon hydrogen bond	3.46
	ARG365	Pi-alkyl	5.14
		Alkyl	4.97
1JAE - Quercetin	Gly369	Carbon hydrogen bond	3.77
	GLN35	Conventional hydrogen bond	2.89
	HIS466	Pi-cation	4.88
	ALA 469	Pi-alkyl	4.66
1JAE - Temephos	VAL 359	Carbon hydrogen bond	3.46
		Carbon hydrogen bond	3.54
		Alkyl	5.26
	TRP 364	Pi-alkyl	4.83

Distances are given in angstroms (Å). Pi-alkyl, Pi-cation, carbon hydrogen bond, and conventional hydrogen bond indicate the type of interaction between ligand and protein residue

other insect pests, but the magnitude of the effects has varied across studies (Sentosa *et al.*, 2019; Darwiati *et al.*, 2020; Rodríguez-Cervantes *et al.*, 2025; Shaw and Hassan, 2025). Such differences may be related to the composition and relative abundance of bioactive constituents present in each extract, together with factors such as the extraction procedure and bioassay conditions.

In silico molecular docking results

The docking simulations showed that the selected compounds exhibited varying binding affinities toward the α-amylase of *Tenebrio molitor* (PDB ID: 1JAE), as summarized in Table 5 and illustrated in Fig. 4.

Quercetin showed the strongest predicted binding energy (-8.9 kcal mol^{-1}), followed by catechin (-8.7 kcal mol^{-1}), whereas gallic acid (-6.5 kcal mol^{-1}), caffeic acid (-6.3 kcal mol^{-1}), and temephos (-6.5 kcal mol^{-1}) showed less negative predicted binding energies. These results indicate stronger predicted ligand-target interactions for quercetin and catechin under the docking conditions used. However, docking scores cannot be directly interpreted as a measure of insecticidal efficacy. Therefore,

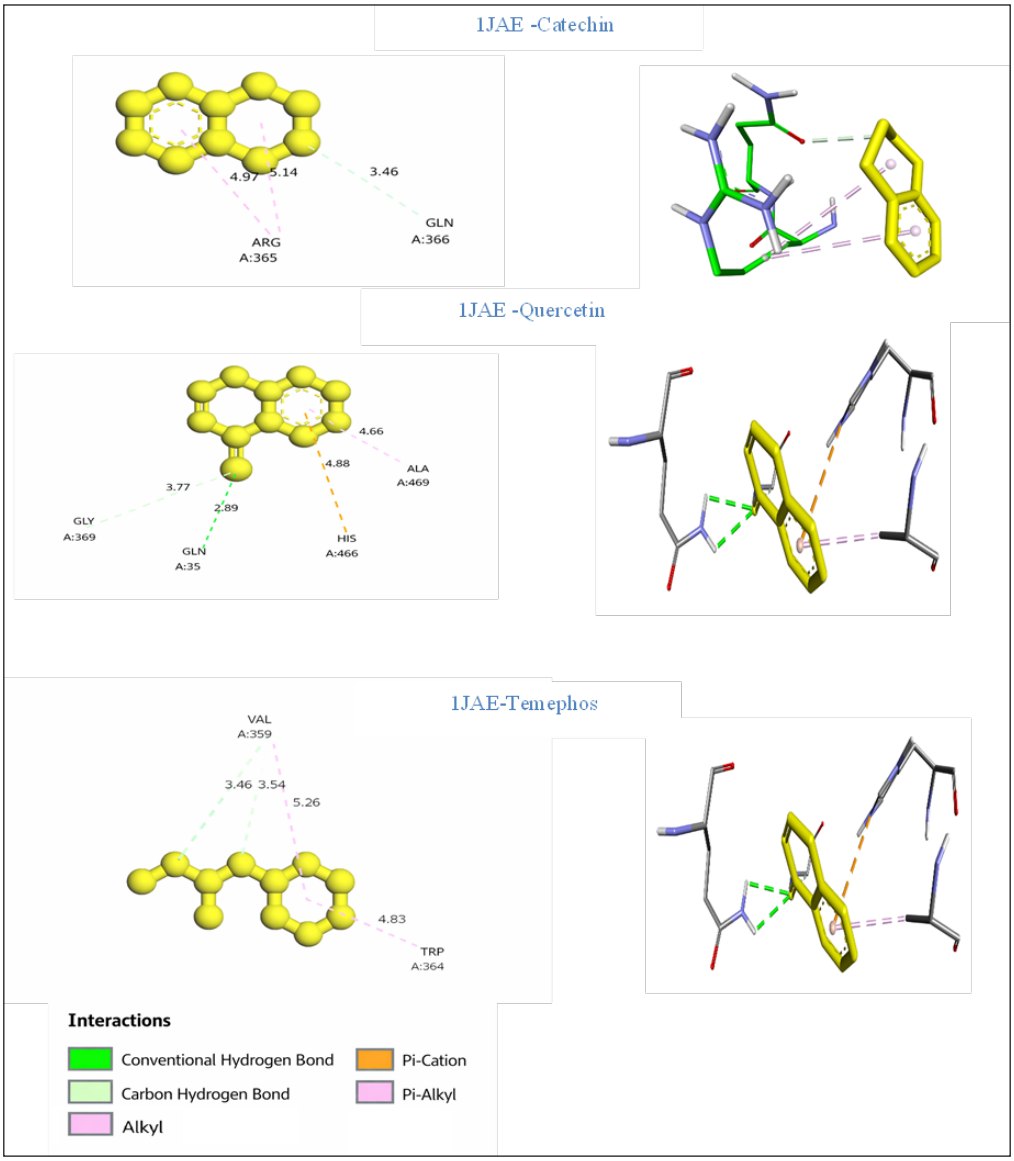


Fig. 4. Molecular interactions of catechin, quercetin, and temephos with the active site of Tenebrio molitor larval a-amylase (PDB ID: 1JAE). Docking interactions showing hydrogen bonds, Pi-alkyl, Pi-cation, and other ligand-residue interactions for catechin, quercetin, and temephos. Distances are given in angstroms (Å).

the docking results should not be considered evidence that quercetin or catechin is more insecticidal than temephos.

Quercetin formed hydrogen-bond and hydrophobic interactions with residues located near the active pocket, including GLN35, GLY369, HIS466, and ALA469. Catechin interacted with GLN366 and ARG365 through carbon-hydrogen and hydrophobic contacts. These interactions indicate favorable predicted ligand-protein interactions within the binding region.

The less negative docking score obtained for temephos indicates a weaker predicted affinity under the present docking conditions. Overall, quercetin and catechin showed more negative predicted binding energies than the other tested compounds, including temephos. These predicted interactions may indicate a possible interaction with α-amylase; however, their contribution to the insecticidal activity of *Oudneya africana* extract remains uncertain. These findings should consequently be considered as mechanistic hypotheses rather than direct evidence that α-amylase inhibition caused the observed insect mortality.

Conclusions

The results of this study demonstrate that the ethanolic extract of *Oudneya africana* induced mortality in *Tenebrio molitor*, with larvae being more susceptible than adults under the experimental conditions. Phytochemical analysis identified several compounds, including quercetin, gallic acid, catechin, and caffeic acid, which were tentatively identified by HPLC based on their retention times compared with those of reference standards. Molecular docking showed that quercetin and catechin had favorable predicted interactions with *Tenebrio molitor* α-amylase. However, these docking results alone cannot confirm enzyme inhibition or demonstrate that α-amylase is involved in the mortality observed in the bioassay. Further biochemical studies and bioassay-guided fractionation are needed to determine the contribution of individual compounds and to assess whether interactions among the constituents contribute to the insecticidal activity of the extract.

Conflict of interest:

The authors declare no conflict of interest.

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The third is semiparametric efficient estimation: efficient influence functions, double robustness, and targeted minimum loss-based estimation with cross-validated ensemble learning for nuisance components. The fourth concerns the tuning quantities such estimators carry, and the conditions under which a choice made from the data still preserves the estimand that was declared. He applies these methods to survey, health and experimental data, and contributes to interdisciplinary studies through statistical design and analysis.

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