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Novel tools into phytochemical profile, bioinsecticidal properties, and molecular docking analysis of Tunisian *Diplotaxis erucoides* (L.) DC. subsp. *erucoides*

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ABSTRACT

This study evaluated the insecticidal effects of Tunisian *Diplotaxis erucoides* (DXe) against *Tribolium confusum*. Active substances present in the extracts were identified through UHPLC-Q-Orbitrap-MS/MS. Repellency and mortality were assessed using different concentrations of the methanolic extract at various time points. The results revealed that *D. erucoides* is rich in bioactive phytochemicals, including delphinidin, eriodictyol, fisetin, gluconapine, isorhamnetin, petinidin 3-O-glucoside, quercetin, and sakuranetin. The findings showed a clear dose-dependent relationship for the repellency effects of the *Diplotaxis* extract. Specifically, larvae and adult beetles displayed repellency index (RI) values of 0.85 and 0.43, respectively, when treated with a low dose of DXe. At 0.318 μL/cm^2, the extract demonstrated a similar level of attractancy across both developmental stages of the beetle, with RI values >1. Further analysis revealed that the median lethal time (LT50) was approximately 1.5 days. Notably, the extract exhibited a significant toxic effect, with LT50 values for larvae and adults of 1.58 and 1.83 days, respectively, at concentrations of 0.238 and 0.389 μL/cm^2.. Remarkably, *D. erucoides* was observed to kill 85.00 ± 0.22% of larvae and 65.0 ± 0.1% of adult beetles within a 48-hour exposure period. Determination of the lethal dose (LD50) established a value of 0.13 μL/cm^2 for larvae and 0.29 μL/cm^2 for adults, indicating statistically ($P < 0.05$) higher lethality in larvae compared to adults. In addition, *in silico* docking studies were conducted against the '*Tribolium castaneum* Arylalkylamine N-acyltransferase' (PDB: 6V3T), yielding significant findings. This report revealed the bioinsecticidal potential of the *D. erucoides* species.

1. Introduction

Post harvest storage of wheat grains is affected by various insect pests, leading to significant increased wastage, compromised food security, and financial losses. *Tribolium confusum* DuVal is a widespread pest that infests stored grains and flour (Ogreten et al., 2023). It attacks a wide range of crushed grains and related products, including spices, biscuits, flours, dried fruits chocolate, nuts, and peas (Pasha and Abd-Eltawab, 2022). Attention has shifted towards safer and more eco-friendly alternatives, particularly plant- derived bioinsecticides, which are considered promising tools for sustainable pest management (Tabouri et al., 2026). In recent years, efforts have focused on identifying safer alternatives to chemical insecticides for sustainable pest management. Habitat manipulation, a component of conservation biological control, aims to provide floral resources, alternative prey and shelter for predators and parasitoids to enhance and sustain natural pest

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suppression. The use of plant extracts as botanical insecticides is also an important provisioning ecosystem service. Selection of plant species for habitat manipulation has mainly focused on species with floral traits that support natural enemies (Amoabeng et al., 2019). The genus *Diplotaxis* is characterized by remarkable diversity, which manifests in various ways, including geographic distribution, morphological traits, molecular markers, and chromosome numbers. This genus is primarily centered in Europe, where the Mediterranean region stands out as a key area of richness and variety in species. One notable species among them is the wall rocket (*D. erucoides* (L.) DC. subsp. *erucoides*) (2n=14), an edible plant worth considering for its potential culinary uses and nutritional benefits. *Diplotaxis*, commonly known as white rocket or false rocket, is a flowering plant that belongs to the *Brassicaceae* (*Cruciferae*) family. This annual herb is widely found in Mediterranean regions of Europe (Spain, Italy, France, Romania, Malta, Serbia, Belgium, etc…), Africa (Tunisia, Algeria, Morocco, Egypt, etc…), and the Middle East (Syria, Jordan, Lebanon, etc…), and is valued as a wild edible vegetable (Guijarro-Real et al., 2019a; Bella and Wagstaff, 2019). Recognized for its distinctively pungent flavor, wall rocket has traditionally been consumed in these areas. Despite its culinary uses, there is limited information on its viability as a new crop (Guijarro-Real et al., 2020). There is significant potential for wall rocket to be marketed as a baby-leaf vegetable for salads (Di Gioia et al., 2018), aligning with the principles of the Mediterranean diet. Various species of rocket, including *Eruca sativa* L., *Diplotaxis* and *Bunias orientalis* L., are enjoyed worldwide at different growth stages, often in salads and soups (Bennett et al., 2006). The wall rocket plant can reach its flowering stage relatively quickly, though this timing can vary with the season and geographic location. It is advisable to harvest the species before floral buds begin to appear, a practice that is typical in rocket cultivation (Guijarro-Real et al., 2019b). Fresh leaves from a variety of edible plants serve as flavorful and nutritious components in meals such as salads, pizzas, sandwiches and soups. These leaves can also be processed to extend their shelf life, allowing greater versatility in culinary applications. Furthermore, both the whole plant and its extracts can be used to fortify foods or develop nutraceutical products, which are designed to deliver health benefits that go beyond standard dietary nutrition. It is often consumed raw in salads but can also be incorporated into dishes such as couscous in Tunisia, and pasta, soups and omelettes in Europe. The edible part of the plant consists of its tender leaves, which are typically harvested during the vegetative stage. These leaves are valued for their pungent, slightly bitter flavor, reminiscent of the spicy, even burning taste found in certain members of the Brassicaceae family. Recent research has highlighted an innovative approach, demonstrating that the incorporation of dried plant materials into bread not only boosts its nutritional profile but also provides additional health benefits (Ressurreição et al., 2024).

Recent analyses using liquid chromatography coupled with electrospray ionization and high-resolution mass spectrometry (LC-ESI/HRMS) have identified 42 compounds in the ethanol extract of various plant parts, including glucosinolates, hydroxycinnamic acids, flavonoids, and oxylipins (Loizzo et al., 2021). Among these, glucosinolates and flavonols represent the primary phenolic components found in extracts derived from both flowering and non-flowering aerial parts, as well as from the flowers, leaves, roots, and seeds of *D. erucoides*. The compounds identified within the *Diplotaxis* genus predominantly include flavanols, particularly mono-, di-, and triglycosides of kaempferol, quercetin, and isorhamnetin (with variations such as 3-O, 7-O, and/or 3-O,7-O-glycosides), accompanied by sugar moieties including glucose, galactose, and rhamnose. In addition to flavonoids, other bioactive compounds such as triterpenoids, indolic alkaloids, and oxylipins have also been documented (Ressurreição et al., 2024). Glucosinolates, a class of secondary metabolites, play a protective role in plants and contribute to the characteristic peppery flavor associated with Brassica species, such as rocket. When plant tissues are damaged through cutting or chewing, glucosinolates are enzymatically transformed into isothiocyanates (ITC) by the action of myrosinase and thiocyanate S-methyltransferase (TMT). However, it is important to note that stress during harvesting can alter the glucosinolate profile of the leaves. Additionally, if storage conditions are not optimal, these beneficial compounds may degrade into anti-nutrients. For instance, glucosinolates have been shown to decrease significantly in rocket leaves after just one or two weeks of storage at 4°C (Spadafora et al., 2020). A comprehensive phytochemical investigation of *D. erucoides* (L.) D.C. led to the discovery of a new flavonoid, rhamnetin-3,3'-di-O-β-D-glucopyranoside (Ben Salah et al., 2015). Further analysis of the 70% ethanol extract derived from the aerial parts of this plant resulted in the identification of a novel flavonoid glycoside, termed diploerucoside A, with the structure kaempferol-3-O-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside]-7-O-α-L-rhamnopyranoside. In addition to this new compound, the study identified seven known compounds: one flavonoid, one phenolic glycoside, one monoterpene, one triterpene, one sitosterol, and two monoglycerolipids. Structural characterization of these compounds was achieved through extensive spectroscopic techniques, including 1D and 2D NMR (1H, 13C, 1H-1H COSY, HSQC, and HMBC), as well as high-resolution mass spectrometry (HR-ESI-MS). Comparisons with existing literature also helped confirm the structures of these compounds (Mokhtari et al., 2022).

The fatty acid profile of the *n*-hexane extract of non-flowering aerial parts of *D. erucoides* was obtained using gas chromatography and revealed three acids identified as palmitic acid (14.35%), linolelaidic acid (6.91%), and linoleic acid (29.10%) (Ben Salah et al., 2015). Although often considered a weed, it has potential as a bioinsecticide, particularly due to its chemical compounds and winter flowering cycle. The plant contains compounds that are potentially repellent or toxic to certain insects. Its abundant winter flowering can provide a food source for certain pollinating insects, but may also attract or repel certain pests.

A comprehensive analysis has been conducted to identify and characterize the phytocompounds present in extracts derived from the *D. erucoides* species. This investigation employs advanced analytical techniques, specifically UHPLC-Q-Orbitrap-MS/MS, to gain deeper insight into the plant's medicinal properties. The report examines the effect of *D. erucoides* extract on repelling and causing mortality in the larvae and adults of *T. confusum*. Molecular docking studies were carried out to investigate the interaction of major bioactive molecules with '*Tribolium castaneum* Arylalkylamine N-acyltransferase' (PDB: 6V3T), aiming to uncover a potential mechanism of action that aligns with the observed bioinsecticidal activity against stored- product pests.

2. Materials and methods

2.1. Collection of plant materials and sample preparation

In February 2024, the aerial parts of *D. erucoides* were harvested from the Zannouch region (Gafsa, Tunisia). This area, characterized by a temperate climate with temperatures ranging from 18 to 20°C, lies east of Gafsa town, between the cities of El Ksar and Sened. Aerial parts of DXe, commonly known as wall rocket, were methodically harvested and air-dried under natural ambient conditions. The dried plant material was then carefully cut and pulverized into a fine powder to facilitate subsequent analyses. This size reduction increases the surface area, enhancing the availability of volatile compounds during extraction. The powdered material was securely stored in airtight bags to prevent moisture absorption and degradation until it was used for further analytical procedures. For the extraction of essential oils, fine hairs containing the volatile essence of the plant were ground. This grinding process releases the essential oils, making them available for evaporation and extraction via a hydrodistillation apparatus. To prepare aqueous and methanolic solutions, a precise maceration process was performed using 100 mg of the finely powdered upper parts of wall rocket mixed with 1 mL of distilled water and MeOH, respectively. This mixture was incubated for 24 hours using an agitator to ensure thorough

interaction between the powdered plant material and the solvent. After this period, the samples were subjected to centrifugation at a speed of 3000 rpm for 10 minutes. The supernatant was then filtered to remove solid residues and subsequently stored in vials at a controlled temperature of +4°C until further analysis. The phytochemical constituents, specifically the phenolic and terpenic compounds present in samples of *D. erucoides*, were systematically identified and quantified using ultra-high-performance liquid chromatography coupled with a quadrupole-Orbitrap mass spectrometry analyzer (UHPLC-Q-Orbitrap-MS/MS). These methods provided a comprehensive profile of the bioactive compounds present in the samples, yielding valuable data for further research.

2.2. Chemicals and reagents

Aqueous and methanolic solutions of *D. erucoides* were at a concentration of 100 mg/mL were prepared using distilled water and methanol, respectively. These solutions were stored at 20°C to maintain their integrity and diluted as required for various experimental applications. Ascorbic acid, a potent antioxidant, and a phosphate buffer solution at a concentration of 0.1 M with a pH of 6.6, ideal for maintaining physiological conditions during reactions, were used. Additional critical reagents included butylhydroxytoluene (BHT), a well-known antioxidant; 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical used to assess the radical scavenging activity of various compounds; and antibiotics such as Cefoxitin 30 µg (FOX30), Cloxacillin 5 µg (COX5), Ticarcillin/Clavulanic Acid 85 µg (TCC85), Ceftazidime 10 µg (CAZ10), Levofloxacin 5 µg (LEV5), Ampicillin 10 µg (AM10), Vancomycin (VA), Timentin 85 µg (TIM85), and Piperacillin-tazobactam 36 µg (PIZ36), procured for testing purposes, Ferric Reducing Antioxidant Power (FRAP) solution, potassium ferricyanide ($K_3Fe(CN)_6$) at a 1% concentration along with ferric chloride ($FeCl_3$) prepared at 0.1% to facilitate colorimetric analysis, trichloroacetic acid (TCA) at 10% that served as a protein precipitation agent in various assays and a range of phenolic compounds, including quercetin, catechin and gallic acid, recognized for their bioactive properties and used as calibration curves were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. UHPLC-MS-Orbitrap operating conditions

Chromatographic analyses were conducted utilizing the advanced Transcend™ 600 LC system, developed by Thermo Fisher Scientific, based in San Jose, California, USA. This system was paired with a highly efficient Waters Acquity C18 column featuring dimensions of 2.1 × 100 mm, with a particle size of 1.9 µm, ideal for high-resolution separation of compounds. The mobile phase for the analyses was meticulously prepared, consisting of an aqueous solution of 0.1% formic acid, designated as eluent A, complemented by MeOH, termed eluent B. The flow rate during the chromatographic run was set at 0.5 mL/min, ensuring consistent and reproducible conditions. The gradient elution began with a composition of 90 % eluent A, which gradually decreased to 20 % over a period of 70 minutes. Following this phase, the solvent composition was quickly reverted back to 90 % eluent A in just 0.5 minutes. To ensure the stability and efficiency of the column for subsequent analyses, a re-equilibration period of 15 minutes was incorporated, resulting in a total running time of 85.5 minutes. Throughout the procedure, the column temperature was set at 30°C. The injection volume was 10 µL.

Mass spectrometry was performed using a Q-Exactive™ Plus Orbitrap instrument manufactured by Thermo Fisher Scientific, located in Bremen, Germany. This setup featured a heated electrospray ionization (HESI) source that operated efficiently in both positive and negative ionization modes. The ionization process was initiated with a spray voltage of 4.0 kV, while the sheath and auxiliary gases, predominantly nitrogen ($N_2 > 95\%$), were carefully regulated to flow rates of 35 and 10 arbitrary units, respectively. To optimize ionization conditions, the capillary temperature was maintained at 300°C and the heater was set at 305°C. The data acquisition process consisted of four sequential scan events, consisting of full MS and data dependent analysis for tandem mass spectrometry under both ESI^+ and ESI^- modes. The instrument achieved resolving powers of 25,000 (full width at half maximum, FWHM) for full scans and 10,000 (FWHM) for tandem mass spectrometry scans, allowing for precise differentiation of ions based on their mass-to-charge ratios. The mass ranges covered during the analysis were meticulously defined, with full scans encompassing m/z 100–1000 and MS/MS scans targeting the narrower range of m/z 70–700. A rapid polarity switching time of approximately 0.27 seconds was achieved during the experiments, which translated to a scan rate of 0.56 Hz, facilitating the capture of dynamic changes in the sample. To ensure the accuracy and precision of the results, external calibration procedures were implemented. Instrument performance was monitored on a daily basis using established reference standards, reinforcing the reliability of the data obtained. Data processing and interpretation were carried out using sophisticated software tools, specifically Xcalibur™ version 2.2.1 and ToxID™ version 2.1.1, both developed by Thermo Fisher Scientific, which provided robust analytical capabilities for comprehensive data analysis.

2.4. Quantification of phenolic content and screening of antioxidant activity

The total phenolic compounds, flavonoids, and condensed tannins were measured using established analytical methods. Total phenols were quantified according to the technique developed by Pérez et al. (2023), which uses a colorimetric measurement to determine the phenolic content of the extracts. Flavonoids were measured using the approach described by Dewanto et al. (2002), which employs specific reagents to form a complex that can be quantified spectrophotometrically. For the quantification of condensed tannins, the method outlined by Panzella and Napolitano (2022) was used, relying on the interaction between tannins and specific reagents that allows their measurement. The antioxidant capacity of the evaluated samples, encompassing both essential oils and extracts, was determined through the DPPH assay, as described by Girma et al. (2025), with some modifications. This method is based on the principle that DPPH, a stable free radical, changes color when it encounters antioxidants, with the degree of color change directly correlating with the sample's ability to scavenge free radicals. Additionally, the FRAP test, following Oyaizu's protocol (1986), was performed to assess the reducing power of the samples. This test involves monitoring the reduction of the $Fe^{3+/}$ferricyanide complex to Fe^{2+}, which is measured spectrophotometrically. Control samples of BHT and ascorbic acid were included as standards for comparison, and the absorbance of the resulting solutions was measured at 517 nm for the DPPH assay and 700 nm for the FRAP test using a Spectronic Genesys 8 spectrophotometer (Rochester, NY, USA). All experiments were conducted in triplicate to ensure the statistical validity and reliability of the results.

2.5. Bacteria

The bacterial strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 10876, *Acinetobacter* spp. 35865**)** are diluted in physiological saline solution. Through this process, a final concentration of 10^6 germs/mL was achieved, ensuring a consistent and reliable inoculum for our experiments. The prepared inoculum is then stored at a controlled temperature of 4°C to maintain its viability and prevent degradation over time. To validate the accuracy of the inoculum and confirm the absence of contaminants, the bacterial dilution solutions are cultivated. Samples are inoculated onto an appropriate culture medium, allowing for the observation of any growth that could indicate contamination.

2.6. Antimicrobial effects of D. erucoides

The study aimed to assess the antibacterial properties of *D. erucoides* using the disc diffusion method, a standard approach for evaluating bacterial sensitivity to different extracts and antibiotics. For this procedure, a specialized nutritive medium known as Mueller Hinton Agar was prepared, as it is optimal for the growth of various bacterial strains. To create the agar medium, 38 grams of agar powder were measured and mixed with a specific volume of distilled water. This mixture was then thoroughly homogenized and heated on a JENWAY 1000 hotplate and stirrer (Thermo Fisher Scientific, Waltham, MA, USA) while being continuously stirred to ensure complete dissolution of the agar. The resulting solution was brought to a boil and maintained at that temperature for about one minute to ensure sterilization. It was then placed in a Systec VE-95 autoclave (Labexchange-Die Laborgerätebörse GmbH, Bruckstr, Germany) and sterilized at 121.1°C for 15 minutes, effectively eliminating any potential microbial contaminants. Once sterilization was complete, the agar was poured into sterile petri dishes and allowed to cool and solidify in a sterile environment. After solidification, a well-isolated colony of the target bacteria was extracted using a sterile platinum loop and transferred into a sterilized test tube containing 5 mL of physiological saline solution. The concentration of bacterial cells in this suspension was carefully quantified using a densitometer to ensure accurate dosing for the experiment. Next, a specific volume of this calibrated bacterial suspension was uniformly distributed across the surface of Mueller Hinton agar using a sterile spreading stick, creating an even bacterial lawn. Sterilized filter paper discs (Whatman, 6 mm in diameter) were then impregnated with 1 μL of the plant extract and strategically placed on the surface of the agar using sterile forceps; four discs were applied to each petri dish to allow effective comparison of antibacterial activity. Following inoculation, the petri plates were carefully inverted to prevent condensation on the agar surface and incubated in a microbiological incubator J.P. SELECTA (Highway A-2, Abrera, Barcelona) set at 37°C for 24-hour to promote optimal bacterial growth. After incubation, the petri dishes were retrieved for analysis, and the diameters of the zones of inhibition around each disc were measured with precision. In this experimental design, DMSO was employed as the negative control to determine baseline bacterial growth, while well-established antibiotics were used as positive controls to validate and compare the effectiveness of the *D. erucoides* extracts against bacterial growth.

2.7. Insect farming

The experimental procedures were conducted at the Biology Department of the Faculty of Sciences of Gafsa, Tunisia, from May 2024 to July 2024. During sample collection, fully grown adult specimens of *Tribolium confusum*, commonly known as the confused flour beetle, were extracted from stored wheat and corn supplies located in various warehouses. The collection used techniques designed to preserve the integrity and health of the specimens, ensuring they were neither damaged nor compromised. After collection, the insects were promptly transported to the laboratory for detailed analysis. Once in the laboratory, the insects were reared in insect breeding room. It is also important to note that the reddish-brown *T. confusum* species is flightless, which may affect its ecological interactions. The insect cultures were maintained by housing both adult beetles and larvae from the laboratory in separate cages, each containing untreated organic varieties of corn and wheat seeds. The wheat used was the Karim variety, scientifically called *Triticum durum* L., alongside barley from the *Hordeum vulgare* species, specifically chosen to support the growth and development of *T. confusum* larvae aged between one and three months. The rearing conditions were controlled in an air-conditioned chamber that maintained a temperature of 29 ± 5°C, a relative humidity of 50 ± 5%, and a light-dark cycle of 12 hours each. These conditions strictly followed the standard protocols established by the Entomology Section at Gafsa University, Tunisia, providing an optimal environment for studying these insects.

2.8. The efficacy of D. erucoides as an insect repellent

Recent research has examined the effectiveness of the DXe methanolic fraction, particularly in attracting and repelling the pest *T. confusum*. The study used Whatman filter paper (Thermo Fisher Scientific Inc., Waltham, MA, USA) N°2 disks to evaluate the repellent properties of the diluted *D. erucoides* at concentrations of 0.159 and 0.318 μL/cm^2, alongside a reference chemical, thymol, at a concentration of 0.362 μL/cm^2 in acetone. The experimental setup involved petri plates with a surface area of 50.24 cm^2, divided into two sections. One section was treated with the *D. erucoides* (0.5 mL) or thymol in acetone, while the other received acetone alone as a negative control. Each petri dish contained 10 adults or larvae of *T. confusum*, placed at the center of a disk containing powdered wheat seeds. The study aimed to assess the repellent effectiveness of the DXe methanolic fraction by calculating the percentage of repelled insects (PRI), the RI, and the 50% repellency concentration (RC50). The experiments were conducted in triplicate, providing a robust dataset for analysis. The PRI quantifies the effectiveness of the insect repellent by comparing the number of insects in the untreated (acetone) area to those in the treated area.

The PRI is calculated using the formula:

$$PRI\ (\%) = [(Nnc - Nt) / (Nnc + Nt)] \times 100,$$

Where Nnc and Nt are the number of insects in the untreated and treated area, respectively.

Based on the PRI values, repellent effectiveness is categorized into six levels, ranging from no repellency (PRI < 0.1%) to very high repellency (PRI > 80.1%). The RI provides further insight into treatment efficacy by comparing the attraction rates of insects to the treatment versus the control:

$$RI = 2IT/IC,$$

Where IT and IC represent the percentage of attracted insects to the treatment and to the control, respectively.

An RI of 1 indicates neutral treatment, greater than 1 suggests attractant effects, and less than 1 denotes a repellent effect. Additionally, RC50 was determined using Probit analysis.

2.9. Mortality test overview

The study aimed to evaluate the toxicity of the *D. erucoides* methanolic fraction against various developmental stages of the insect *T. confusum*. For the experiment, adult insects and larvae (20 per group) were placed in separate Petri dishes (90 mm diameter × 15 mm height) and maintained in dark conditions. They were provided with an unlimited supply of *Hordeum vulgare* and *Triticum* seeds. The insects were exposed to increasing concentrations of the *D. erucoides* methanolic extract, ranging from 0.159 to 0.477 μL/cm^2, as well as thymol at a concentration of 0.362 μL/cm^2, both diluted in acetone. The treatment was administered using filter paper discs (18 cm^2) to ensure even distribution. Acetone served as the negative control throughout the experiments. Mortality rates were monitored hourly following the initial exposure to the rocket extract until the insects displayed signs of death, characterized by immobility and a lack of movement in their antennae and legs (Guesmi et al., 2024). The percentage of mortality was calculated using Abbott's correction formula for natural mortality in the untreated control groups, as outlined in Abbott's (1925) study. The formula used is as follows:

$$PPM\ (\%) = 100\ (A-B)/A$$

Where PPM represents the percentage of pest mortality, A is the average number of pests before treatment, and B is the average number

of pests after treatment.

2.10. Molecular docking procedure

Molecular docking simulations were performed *via* Auto Dock 4.2 program package (Trott and Olson, 2010). The X-ray crystal structure of *Tribolium castaneum* Arylalkylamine N-acyltransferase in complex with acetyl-CoA (O'Flynn et al., 2020) were downloaded from the RSCB protein data bank (https://www.rcsb.org/). First, water molecules were removed, and missing hydrogens and Gasteiger charges were added to the system during the preparation of the receptor input file. Then, AutoDock Tools was used to prepare the ligand and protein files (PDBQT). Pre-calculation of grid maps was carried out with AutoGrid to save time during docking. Geometric optimization of all compounds was performed using ACD (3D viewer) software (http://www.filefacts.com/acd3d-viewer-freeware-info), and visualization and analysis of interactions were performed using Discovery Studio 2017R2 (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/) and PyMOL 0.99rc6 (De Lano, 2002).

2.10. Statistical analysis

In this study, all experimental procedures were performed at least three times to ensure the reliability and accuracy of the findings. Results are presented as mean values accompanied by the standard deviation (SD) to indicate data variability. For the assessment of antioxidant activity, the effective concentration required to achieve 50% inhibition (EC50) was log-transformed. This transformation was carried out using regression analysis, allowing clearer interpretation of the data, specifically representing the mean percentage of DPPH radical scavenging activity. To evaluate the statistical significance of differences between treated and control samples, a two-way analysis of variance (ANOVA) was conducted. Tukey's multiple comparison test was then applied to further clarify specific differences among the sample groups. A *P*-value of less than 0.05 was considered statistically significant, indicating that any observed differences were unlikely to be due to random chance.

3. Results

3.1. Nutritional benefits of rocket phytocompounds identified by UHPLC-Orbitrap-MS

The Gafsa region of Tunisia holds rocket leaves in high regard, often referring to them as a nutrient-dense food. This is due to their flavorful and versatile nature, which makes them a popular ingredient in various dishes, such as salads, couscous (known locally as seffa), and fresh rolls (Fig. 1). Rocket leaves are celebrated not only for their distinct, pungent flavor but also for their impressive nutritional profile. They are rich in essential nutrients, including fiber, calcium, iron, potassium, beta carotene, zeaxanthin, lutein, folic acid, and flavonoids. Additionally, they are low in calories, making them a healthy choice for those looking to enhance their diet.

The study involves the phytochemical analysis of extracts obtained from *D. erucoides*, aiming to identify and characterize the various bioactive compounds present in the plant species.

UHPLC-MS-Orbitrap was used to carry out a thorough analysis of the phytochemical profile derived from the aerial parts of *D. erucoides* (Fig. 1, Table 1,2), specifically examining both methanolic and aqueous extracts. This advanced analytical approach enabled the precise identification of several key phenolic acids and flavonoids present in the extracts. The use employment of high-resolution mass spectrometry yielded accurate mass measurements, significantly enhancing the reliability of the compound assignments. Mass errors were consistently maintained within an acceptable threshold of less than 5 ppm, underscoring the robustness and accuracy of the analytical method employed. This approach ensured a high degree of confidence in the chemical profile derived from the extracts. By assessing the various phytochemicals present in this plant, the research aims to highlight its potential health benefits and applications in traditional medicine. Chemical composition analysis of the extracts derived from the *Diplotaxis* genus revealed that the primary constituents were aromatic compounds and phenolics, which are known for their effective insecticidal properties. The compounds analyzed in this study demonstrated good precision in their mass measurements. For example, gluconapine ($C_{11}H_{19}NO_9S_2$) was identified with an observed [M-H]- ion at an *m/z* value of 372.04285,

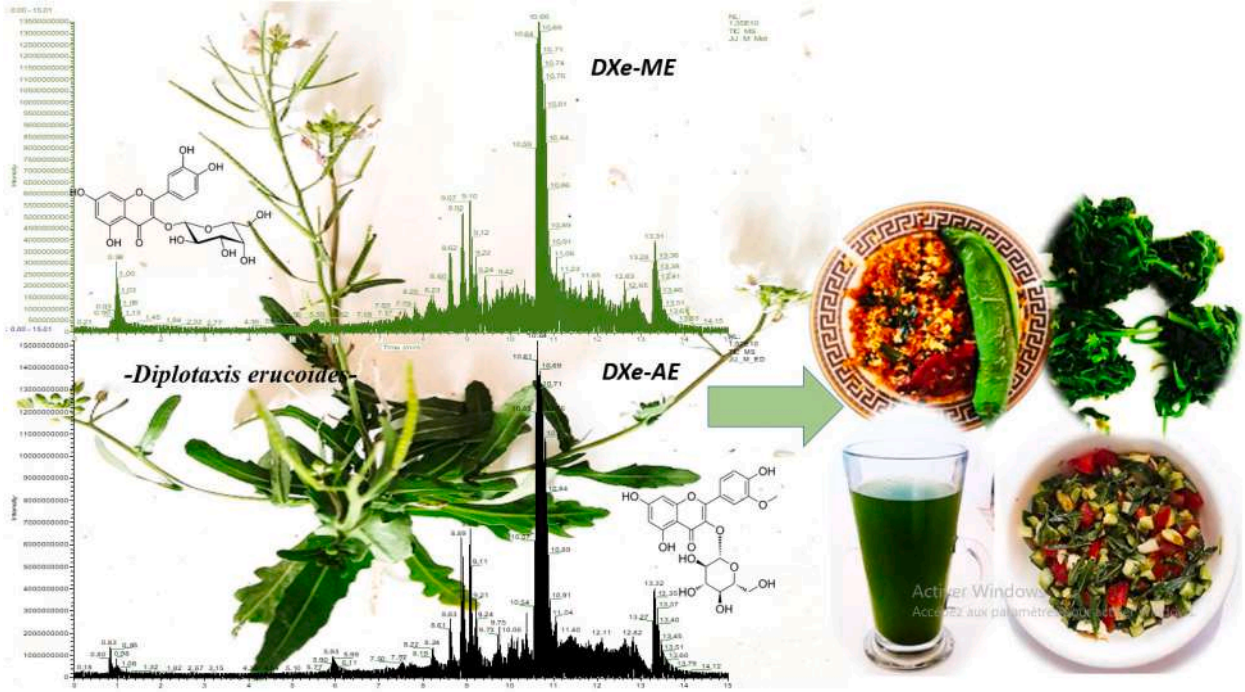


Fig. 1. LC/MS profiles of *D. erucoides* extracts. DXe-ME : *D. erucoides* methanolic extracts; DXe-AE : *D. erucoides* aqueous extracts.

Table 1
Phenolic compounds identified in *D. erucoides* methanolic extract by UHPLC-Q-Orbitrap-MS/MS.

Compound Name	Formula	Adduct	RT (min)	Measured Area	m/z (Expected)	% Relative
Gluconapine	$C_{11}H_{19}NO_9S_2$	[M-H]-	2.1	9968930.964	372.04285	0.21
Hydroxybenzoic acid -4	$C_7H_6O_3$	[M-H]-	5.79	417047825.7	137.02442	8.90
p-Coumaric acid	$C_9H_8O_3$	[M-H]-	6.76	68376368.89	163.04007	1.46
Chlorogenic acid	$C_{16}H_{18}O_9$	[M-H]-	6.99	284325002.6	353.08781	6.07
Caffeic acid	$C_9H_8O_4$	[M-H]-	7.25	331442234.3	179.03498	7.07
Sinapic acid	$C_{11}H_{12}O_5$	[M-H]-	7.43	6623559.996	223.0612	0.14
Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	[M-H]-	7.66	101275865.9	593.15119	2.16
Delphin	$C_{27}H_{30}O_{17}$	[M+H]+	8.15	15850343.21	627.15558	0.34
K-7-O-glucoside/ Quercetin-3-O-rhamnoside (quercitrin)/ Luteolin-4′-O-glucoside/ Luteolin-7-O-glucoside	$C_{21}H_{20}O_{11}$	[M-H]-	8.54	135891750.5	447.09328	2.90
Quercetin-3-O-rutinoside (rutin)	$C_{27}H_{30}O_{16}$	[M-H]-	8.61	121465429.2	609.14611	2.59
Isorhamnetin-3-O-glucoside/ petinidin-3-O-glucoside	$C_{22}H_{22}O_{12}$	[M+H]+	8.65	51817130.94	479.1184	1.11
Quercetin 3-O-glucoside/ Quercetin-3-O-galactoside (hyperoside)/ Quercetin-3-O-glucopyranoside	$C_{21}H_{20}O_{12}$	[M-H]-	8.66	21852916.76	463.0882	0.47
Ferulic acid	$C_{10}H_{10}O_4$	[M-H]-	8.68	385344401.6	193.05063	8.22
Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	[M-H]-	8.96	12153498.53	623.16176	0.26
Eriodictyol	$C_{15}H_{12}O_6$	[M-H]-	8.99	1061130.588	287.05611	0.02
Fisetin/ Luteolin	$C_{15}H_{10}O_6$	[M-H]-	9.21	19714496.2	285.04046	0.42
Glycitin	$C_{22}H_{22}O_{10}$	[M+H]+	9.72	2459281200	447.12857	52.48
Isorhamnetin	$C_{16}H_{12}O_7$	[M-H]-	9.73	38196537.04	315.05103	0.82
Sakuranetin	$C_{16}H_{14}O_5$	[M-H]-	9.41	16863757.53	285.07685	0.36
			Sum	4686261262		

Table 2
Phenolic compounds identified in *D. erucoides* aqueous extract by UHPLC-Q-orbitrap-MS/MS.

Compound Name	Formula	Adduct	RT (min)	Measured Area	m/z (Expected)	% Relative
Chlorogenic acid	$C_{16}H_{18}O_9$	[M-H]-	6.99	84394597.04	353.08781	28.97
Glycitin	$C_{22}H_{22}O_{10}$	[M+H]+	9.75	21118794.01	447.12857	7.25
Hydroxybenzoic acid -4	$C_7H_6O_3$	[M-H]-	8.65	34366087.8	137.02442	11.80
Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	[M-H]-	8.55	2744294.319	623.16176	0.94
Isorhamnetin	$C_{16}H_{12}O_7$	[M-H]-	9.72	41046473.44	315.05103	14.09
K-7-O-glucoside/ Luteolin-4′-O-glucoside/ Luteolin-6-C-glucoside (homoorientin)/ Luteolin-7-O-glucoside/ Luteolin-8-C-glucoside (orientin)/ Quercetin-3-O-rhamnoside (quercitrin)	$C_{21}H_{20}O_{11}$	[M-H]-	8.55	2450786.441	447.09328	0.84
Quercetin 3-O-glucoside/ Quercetin-3-O-galactoside (hyperoside)/ Quercetin-3-O-glucopyranoside	$C_{21}H_{20}O_{12}$	[M-H]-	8.67	2832951.388	463.0882	0.97
Quercetin-3-O-rutinoside (rutin)	$C_{27}H_{30}O_{16}$	[M-H]-	8.62	14668662,59	609.14611	5.04
Sinapic acid	$C_{11}H_{12}O_5$	[M-H]-	8.06	3116493.26	223.0612	1.07
Vanillic acid	$C_8H_8O_4$	[M-H]-	9.13	84554591.28	167.03498	29.03
			Sum	291293731,6		

which aligned well with its theoretical mass of 372.04291 (Table 1). This resulted in a minimal mass error of 0.15513 ppm, underscoring the high level of analytical accuracy achieved. Similarly, quercetin 3-O-glucoside ($C_{21}H_{20}O_{12}$) showed a measured *m/z* of 463.0882, closely matching its theoretical mass of 463.08844 Da (Tables 1 and 2). The phytochemical profiles exhibited considerable variation depending on the type of solvent used for extraction. Research has shown that the methanolic extract exhibits the highest diversity of bioactive phytochemicals. Among these, significant amounts of caffeic acid, chlorogenic acid, delphin, eriodictyol, fisetin, gluconapin, glycitin, isorhamnetin, kaempferol, luteolin, petinidin, quercetin, sakuranetin and sinapic acid, compared to the aqueous sample, which includes vanillic acid, luteolin-6-C-glucoside (homoorientin), and luteolin-8-C-glucoside (orientin) have been identified. These compounds are particularly valued for their beneficial properties, which include insecticidal, antioxidant and antimicrobial potential. The profile of the aqueous extract was similar to that of the methanolic extract, containing many of the glycosyloxyflavones identified in the leaves. Additionally, it uniquely included chlorogenic acid and orientin, suggesting their involvement in plant defence mechanisms and indicating potential health benefits.

The detailed analysis presented here uncovers a broader and more intricate variety of aliphatic glucosinolates, including the gluconapin compound, than has been recorded in prior studies.

3.2. Total phenolic content of D. erucoides fractions

The summary of total bioactive molecules from *D. erucoides* extracts is shown in Fig. 2**A**.

The methanolic extract derived from *D. erucoides* demonstrated a total phenolic content of 73.6 mg GAE/g DM. Additionally, the extract exhibited a flavonoid content of 24 mg QE/g DM. The findings of this study highlight a notable variation in the condensed tannin levels found in extracts from the aerial parts of *D. erucoides*. Specifically, the methanolic extract of *D. erucoides* contains approximately 15. mg CE/g DM.

These measurements highlight the extract's high concentrations of total phenolic, flavonoid, and condensed tannins compounds, which are known for their antioxidant properties and potential health benefits.

3.3. Antioxidant activity of D. erucoides

Research on various fractions of *D. erucoides* reveals a sigmoidal increase in antioxidant activity that correlates with the concentration of the aqueous and methanolic extracts. This investigation employed a scavenging assay to evaluate the $DPPH^{\bullet}$ free radical scavenging capacity of both natural antioxidants and synthetic reference compounds.

Fig. 2**B** shows that the percentage of inhibition increases as extract concentrations rise, highlighting the dose-dependent nature of their

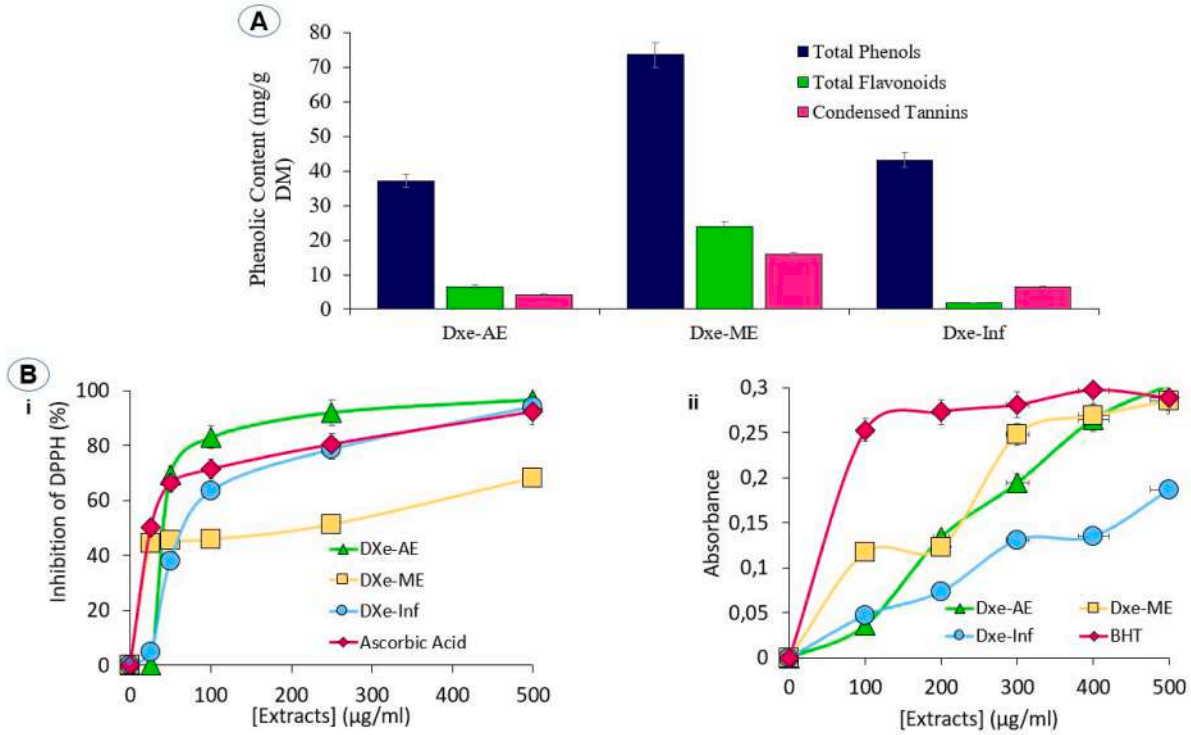


Fig. 2. A. Phenolic compounds of *D. erucoides*. **B.** Antioxidant potential of *D. erucoides* extracts by DPPH **(i)** and FRAP (**ii**) assays. DXe-ME : *D. erucoides* methanolic extracts; DXe-AE : *D. erucoides* aqueous extracts; DXe-Inf : *D. erucoides* infusion. These are representative results of three independent experiments.

antiradical effects. Among the samples tested, the methanolic extract derived from rocket showed notable antioxidant activity, particularly evident from the DPPH assay and reducing power tests. The IC50 values obtained (Fig. 2**Bi**) for the extract were lower than those of commercial reference antioxidants, indicating strong efficacy. However, when compared with the positive control, all samples showed a reduced antiradical effect relative to this synthetic antioxidant. Remarkably, the methanolic extract at a concentration of 500 µg/mL exhibited the highest antiradical power at 68.25%.

The FRAP method is used to assess the ability of extracts to reduce

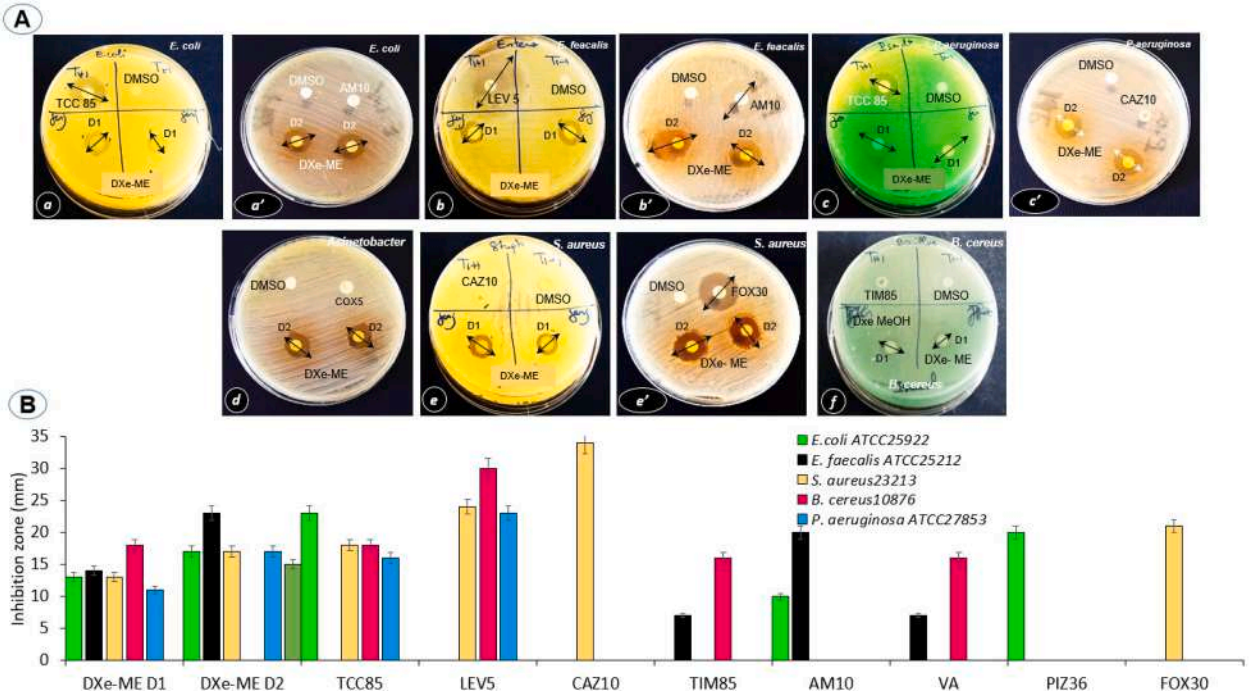


Fig. 3. Antibacterial effects of *D. erucoides* methanolic extracts. a-f: bacterial strains (Gram- and Gram+ bacteria); DXe-ME : *D. erucoides* methanolic extracts; D1: dose 1 (1µL); D2: dose 2 (10 µL); DMSO: dimethyl sulfoxide; TCC85: Ticarcillin/Clavulanic Acid 85 µg; LEV5: Levofloxacin 5 µg; TIM85: Timentin 85 µg; AM10: Ampicillin (10 µg/µL); CAZ10: Ceftazidim (10 µg/µL); COX5 : Cloxacillin 5 µg; VA: Vancomycin; PIZ36: Piperacillin-tazobactam 36 µg; FOX30: Cefoxitin 30 µg; *E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; E. faecalis: Enterococcus faecalis;* P.a : *Pseudomonas eruginosa.*

Fe^{3+}/ferricyanide to Fe^{2+}. The antioxidant capacity of *D. erucoides* was evaluated, as illustrated in Fig. 2**Bii**. This test involves ferric iron, which undergoes reduction to form a ferrous salt. The results indicate a significant increase in absorbance for the *Diplotaxis* fractions compared with BHT. Notably, the plant extract derived from maceration with water from the aerial parts of *D. erucoides* exhibited the lowest antioxidant potential, whereas the methanolic extract demonstrated a stronger effect at a concentration of 500 µg/mL, comparable to that of BHT.

Recent findings indicate that *D. erucoides* demonstrates significant antioxidant properties, as evidenced by its ability to inhibit DPPH and reduce ferric ions, both of which play a role in inducing oxidative stress. The investigation revealed that *Diplotaxis* species commonly exhibit antioxidant activities, primarily attributed to their phenolic and volatile compounds. Furthermore, it was found that solvent extraction methods are effective in obtaining these compounds, with the optimal harvesting period for this species occurring after the flowering stage.

3.4. Antibacterial effect of DXe

The research findings highlighted the potential of Brassicaceae extracts, particularly those derived from rocket, as innovative antimicrobial agents and nutrient-rich foods. Specifically, the study revealed that *D. erucoides* at concentration of 100 mg/mL was highly effective in inhibiting the growth of various pathogenic bacteria (Fig. 3**A**). Further analysis indicated that maceration extracts created a significant zone of inhibition in different bacterial strains such as *E. coli, P. aeruginosa, S. aureus*, and *E. faecalis*, with an effect comparable to TIM85, VA, and AM10, effectively preventing the growth of these harmful bacteria (Fig. 3**B**). Additionally, the results indicated that *D. erucoides* also displayed a moderate level of antimicrobial activity against the tested bacterial strains, showcasing its potential as a healthful option for combating bacterial infections.

3.5. Insecticidal activity of D. erucoides methanolic fraction

Recent findings indicate that the *D. erucoides* extract exhibits notable insecticidal properties, particularly as a repellent for both larvae and adults of *Tribolium confusum* (Fig. 4, Table 3). The effectiveness of this extract is influenced by all the concentrations applied and the duration of exposure. At a concentration of 0.159 µL/cm², *D. erucoides* demonstrated an initial repellent effect for both larvae and adults within the first few minutes of treatment, achieving 85.1 % and 70 % repellency, respectively, after 30 minutes of exposure.

Furthermore, even at lower concentration (0.159 µL/cm²), specifically at 10 minutes of exposure, the plant displayed significant attractant action (PRI<0.1) against *T. confusum* adults (RI>1). Increasing the concentration to 0.318 µL/cm² resulted in a pronounced attractive effect (RI>1), reaching nearly -60% and -40 % effectiveness after 30 minutes of application for larvae and adults, respectively. Interestingly, at the highest concentration, the extract shifted from a repellent to an attractive effect on larvae and adults between 10 and 30 minutes of treatment. In comparison, thymol, another insecticidal agent, exhibited effectiveness after 30 minutes of exposure. These results highlight the potential of false rocket extract as a viable option for managing *T. confusum* populations, showcasing its versatility in repelling and attracting these insects depending on concentration and exposure time.

3.6. T. confusum mortality rate

The mortality rate of insects refers to the percentage of an insect population that dies within a specific period or under certain conditions. This rate can be influenced by various factors, including environmental conditions, food availability, predation, disease, and exposure to pesticides. Understanding insect mortality rates is crucial for studying ecosystem dynamics, pest management, and conservation efforts. The report analyzed the effects of *D. erucoides* on mortality rates of the pest

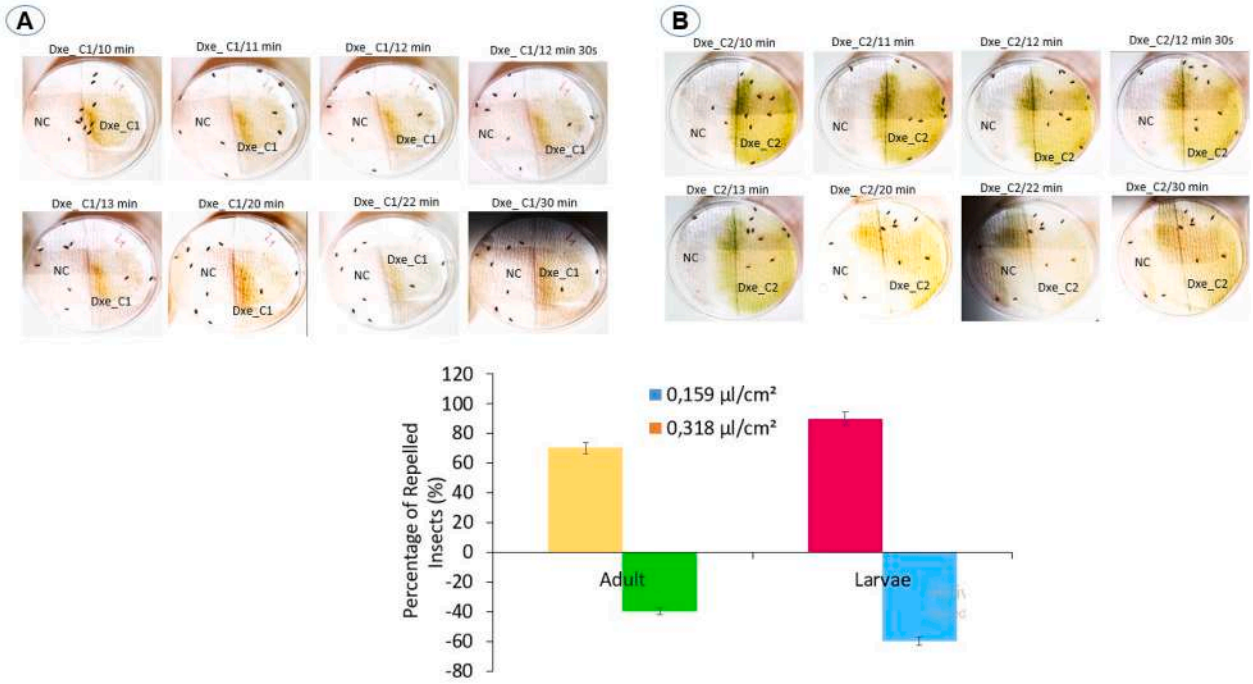


Fig. 4. *D. erucoides* repellency for *T. confusum* at different concentrations. The photographs provide a visual representation of the walking patterns exhibited by *T. confusum* pests over a 30-minute observation period on filter paper impregnated with extracts of *D. erucoides*. The experimental design includes three specific treatment conditions for comparison. NC (Negative Control): This group uses acetone as the solvent, serving as a baseline to assess the effects of the *D. erucoides* extract. DXe_C1 (Low Concentration Treatment): This condition involves a low concentration of *D. erucoides* at 0.159 µL/cm², allowing analysis of the pests' behavior at minimal exposure. DXe_C2 (High Concentration Treatment): In this case, a higher concentration of *D. erucoides* is applied at 0.318 µL/cm², enabling evaluation of the effects when pests are exposed to a more potent dose.

Table 3
% Repellency response of *T. confusum* to *D. erucoides* methanolic extract.

EOs/Biochemical	[] µL/cm^2	Repellency (%) after 30 min		Repellency class		Repellency Index (RI)		Response
		T. confusum larvae	*T. confusum* adult	*T. confusum* larvae	*T. confusum* adult	*T. confusum* larvae	*T. confusum* adult	
***D. erucoides* -ME**	0.159	85.1 (2.1)^{*a}	70.0 (3.0)^{*b}	5	4	RI<1	RI<1	Repellent
	0.318	-60.0 (0.0)^{*c}	-40.0 (0.0)^{*c}	0	0	RI>1	RI>1	Attractant
Thymol	0.368	36.7 (0.5)^{*d}	29.3 (0.0)^{*d}	2	2	RI<1	RI<1	Repellent
Untreated control		0.0 (0.0)^{e}	0.0 (0.0)^{e}	1	1	RI=1	RI=1	Neutral

D. erucoides -ME: *D. erucoides* methanolic extract; the same letter (a,b,c,d,e) following the % repellency in each column denotes a non-significant difference ($P \leq 0.05$). *Statisically different from untreated control group. Level 0: no effect; Level 1: very low effectiveness; Level 2: low effectiveness; Level 3: repellent; Level 4: high effectiveness; Level 5: very high effectiveness.

T. confusum. As shown in Fig. 5, the methanolic extract demonstrated a significant level of efficacy against both larval and adult stages of the insect. Specifically, a dosage of 0.318 µL/cm^2 resulted in 75% mortality for adult *T. confusum* within 72 hours and for larvae within 48 hours. In contrast, larvae exposed to a lower concentration of 0.159 µL/cm^2 exhibited 30 % mortality after 24 hours, which increased to 63.157 % after 48 hours at the same concentration. Furthermore, probit analysis indicated distinct levels of susceptibility between developmental stages: the LD50 value for adults was 0.29 µL/cm^2 after 48 hours and the LT50 was 44 hours at a concentration of 0.389 µL/cm^2, while larvae exhibited higher susceptibility with an LT50 of 48 hours at a dosage of 0.238 µl/cm^2. In comparison, thymol showed a milder mortality effect, with an LD50 of 0.76 µL/cm^2 for *T. confusum*. The negative control group demonstrated minimal lethality, highlighting the significant impact of *D. erucoides* extract on *T. confusum* populations. Mortality rates among both adult beetles and larvae of the grain borer showed a significant dependence on the concentrationof plant extract and duration of exposure compared to an untreated control group.

3.7. Molecular docking analysis

Drug discovery is a complex and multifaceted process in which selecting the right lead compound is crucial to the overall success of the project. Since the early 1980s, molecular docking has been extensively employed as a computational approach in structure-based drug design (Stanzione, Giangreco, Cole, 2021; Kuntz et al., 1982). In this context, molecular docking was used in the present study to propose a potential mechanism of action consistent with the recorded insecticidal potential of *D. erucoides* extract by modeling the major phytoconstituents (4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, ferulic acid and glycitin) against '*Tribolium castaneum* arylalkylamine N-acyltransferase'(pdb: 6v3t).

The results in Table 4 show that all docked phytomolecules had a more significant docking score than the co-crystallized ligand. More specifically the most bioactive compound is 'glycitin', which exhibited the best docking score (binding energy value = −8.3 kcal/mol). The 3D model in Fig. 6 shows that this ligand fits well in the binding cavity of the target enzyme. The interactions shown in Fig. 6 demonstrate the formation of three conventional H-bonds with Asn21, Asp24 and

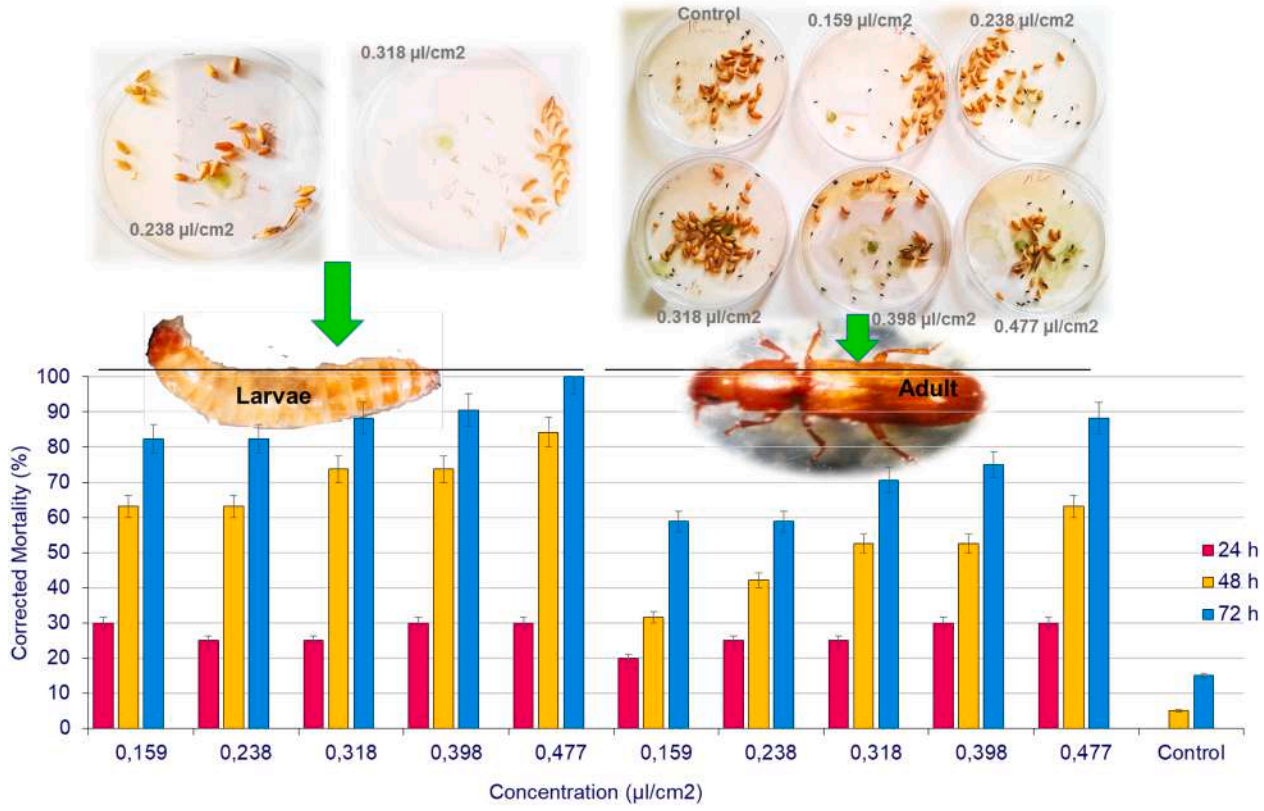
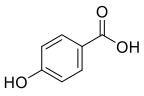
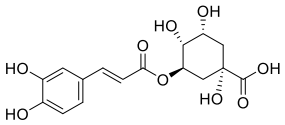
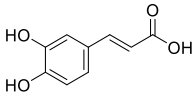
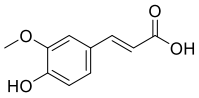
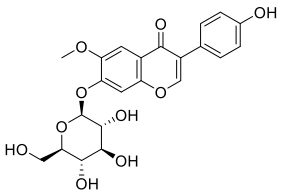
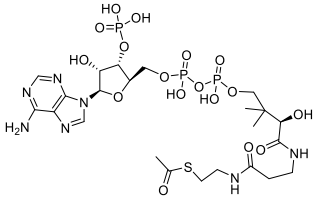


Fig. 5. Corrected mortality (%) at different concentrations and exposure times of *D. erucoides* methanolic extract against *T. confusum* larvae and adults. These are representative results of three independent experiments.

Table 4
Docking results of the major phytocompounds with the binding cavity of '*Tribolium castaneum* Arylalkylamine N-acyltransferase' (pdb: 6v3t).

Docked compounds	Interacting Residues	Binding Energy (kcal/mol)
4-Hydroxybenzoic acid 	**van der Waals:** Phe22, Glu25, Pro26, Ile126, Leu127, Asp161, Ser164; **H bond:** Arg125, Thr167; **Pi-Donor Hydrogen Bond :** Thr163	-5
Chlorogenic acid 	**van der Waals:** Phe22, Pro23, Pro26, Ser128, Val129, Arg134, Gly135, Lys140, Ser164, Phe166, Arg169, Ala170, His202; **H bond:** Asn21, Asp24, Glu25, Leu127 ; **Alkyl/Pi–Alkyl:** Ala139	-7.8
Caffeic acid 	**van der Waals:** Phe22, Glu25, Leu124, Ile126, Leu127, Val160, Ser164, Phe166; **H bond:** Arg125, Asp161, Thr167; **Pi-Donor Hydrogen Bond :** Thr163	-5.8
Ferulic acid 	**van der Waals:** Phe22, Glu25, Leu127, Arg134,Asp161, Ala162, Phe166, Thr167, His202; **H bond:** Thr163, Ser164; **Amide-Pi Stacked :** Asp24 ; **Alkyl/Pi–Alkyl:** Pro26	-6.3
Glycitin 	**van der Waals:** Phe22, Pro23, Glu25, Leu127, Ser128, Gly135, Arg136, Gly137, Ile138, Phe166, Thr167, Arg169; **H bond:** Asn21, Asp24, Val129; **Carbon Hydrogen Bond :** Ser164 ; **Pi-Cation :** Arg134 ; **Pi-Sigma :** Ala139 ; **Alkyl/Pi–Alkyl:** Val129, Arg134, Ala139	-8.3
Co-crystallized ligand 	**van der Waals:** Phe22, Asp24, Glu25, Pro26, Leu127, Val129, Arg134, Arg136, Gly137, Ile138, Ala139, Lys140, Phe166, Ala170 ; **H bond:** Thr167, Arg169 ; **Carbon Hydrogen Bond :** Gly135 ; **Pi-Donor Hydrogen Bond :** Ser164	-4.8

Val129, which reduce the binding energy and thereby stabilize the docking complex.

In addition, other interactions are involved such as carbon-hydrogen bonding (Ser164), Pi-Cation (Arg134), Pi-Sigma (Ala139), as well as Alkyl/Pi–Alkyl (Val129, Arg134, Ala139).

On the other hand, the tabulated data show that 'chlorogenic acid' (−7.8 kcal/mol) was the second most active phytocompound. This docked ligand forms four H-bonds with the amino acid sequence: Asn21, Asp24, Glu25 and Leu127 besides to a Pi–alkyl with Ala139. 'Ferulic acid' (−6.3 kcal/mol) was identified as the third most effective ligand forming two H-bonds with Thr163 and Ser164, as well as an amide -Pi stacked interaction with Asp24 and alkyl/Pi–alkyl interactions with Pro26. 'Caffeic acid' (−5.8 kcal/mol) and '4-hydroxybenzoic acid' (−5 kcal/mol) were found to be the least bioactive ligands, displaying interactions detailed in Fig. 7. Based on the docking results described above, these five major phytocompounds evaluated *in silico* have significant capabilities to inhibit '*Tribolium castaneum* arylalkylamine N-acyltransferase'.

4. Discussion

The rate of loss induced by *T. confusum* due to poor storage is more than 50 % of the crop, especially in developing countries, as a result of inadequate or absent safe storage procedures in regions with hot climates and among retailers. This is of particular concern because these crops are a major daily component of nutrition for both humans and animals (Pasha and Abd-Eltawab, 2022). Brassicaceae include several species used in habitat manipulation studies, but few species in the

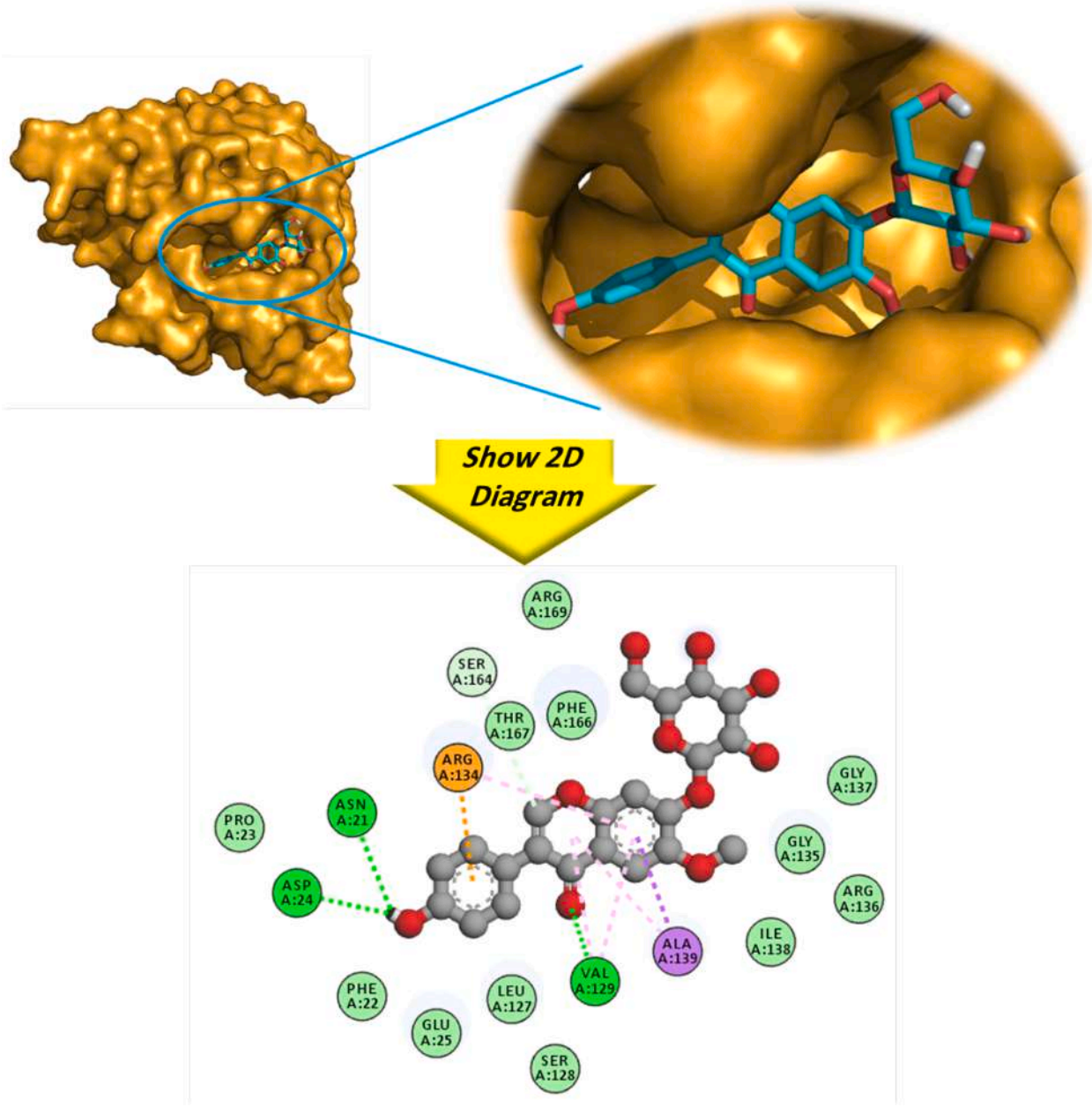


Fig. 6. 2D model of interactions formed by the most active compound 'glycitin' within the active site of '*Tribolium castaneum* arylalkylamine N-acyltransferase' (pdb: 6v3t).

family are known to have insecticidal properties (Amoabeng et al., 2019).

Diplotaxis plants are rich sources of both antiradical phenols and sulfur-containing defense molecules. The Brassicaceae family, renowned for its richness in bioactive compounds, includes glucosinolates (gluconapin, glucoraphanin, glucobrassicin, glucoiberin) that serve as precursors to toxic compounds known as isothiocyanates. These compounds have been associated with numerous health benefits and target the nervous and respiratory systems, disrupt metabolism, stunt growth and reduce feeding rates of pests. Glucoraphanin (4-methylsulfinylbutyl glucosinolate) is converted into the isothiocyanate sulforaphane, which imparts a pungent, slightly bitter flavour commonly found in vegetables like kale and broccoli (Ressurreição et al., 2025). The organosulfur compounds accumulated by cruciferous vegetables, glucosinolates -sulfur-containing glycosides (Ramirez et al., 2020), are the primary biochemical weapon of the Diplotaxis genus. Glucosinolates appear to be effective defenses against generalist lepidopteran herbivores during most stages of larval development. When an insect bites into the leaf, **myrosinase** (β-thioglucoside glucohydrolases) (Jeschke et al., 2017) hydrolyzes these compounds into volatile, highly toxic **ITCs** and nitriles. Additionally, flavonoids, including flavonols, act as bitter feeding deterrents. When combined with glucosinolates, they form a toxic cocktail that significantly reduces the survival and fertility of invading herbivore populations. Furthermore, chlorogenic acid, as a phenolic acid, is a potent ester of caffeic acid. When ingested by insects, these acids oxidize in the insect gut, inducing the accumulation of reactive oxygen species that reduce the activity of digestive enzymes and decrease nutrient absorption (Pratyusha, 2022).

Within this family, the genus *Diplotaxis* includes several edible species commonly used in culinary practices. The leaves of these plants are particularly popular, often enjoyed raw in salads or incorporated into a variety of dishes (Ressurreição et al., 2024; Nascimento et al., 2024; Palumbo et al., 2024; Khoramizadeh et al., 2024; Raffo et al., 2024; Kilibarda et al., 2025). When examining the various aromas found

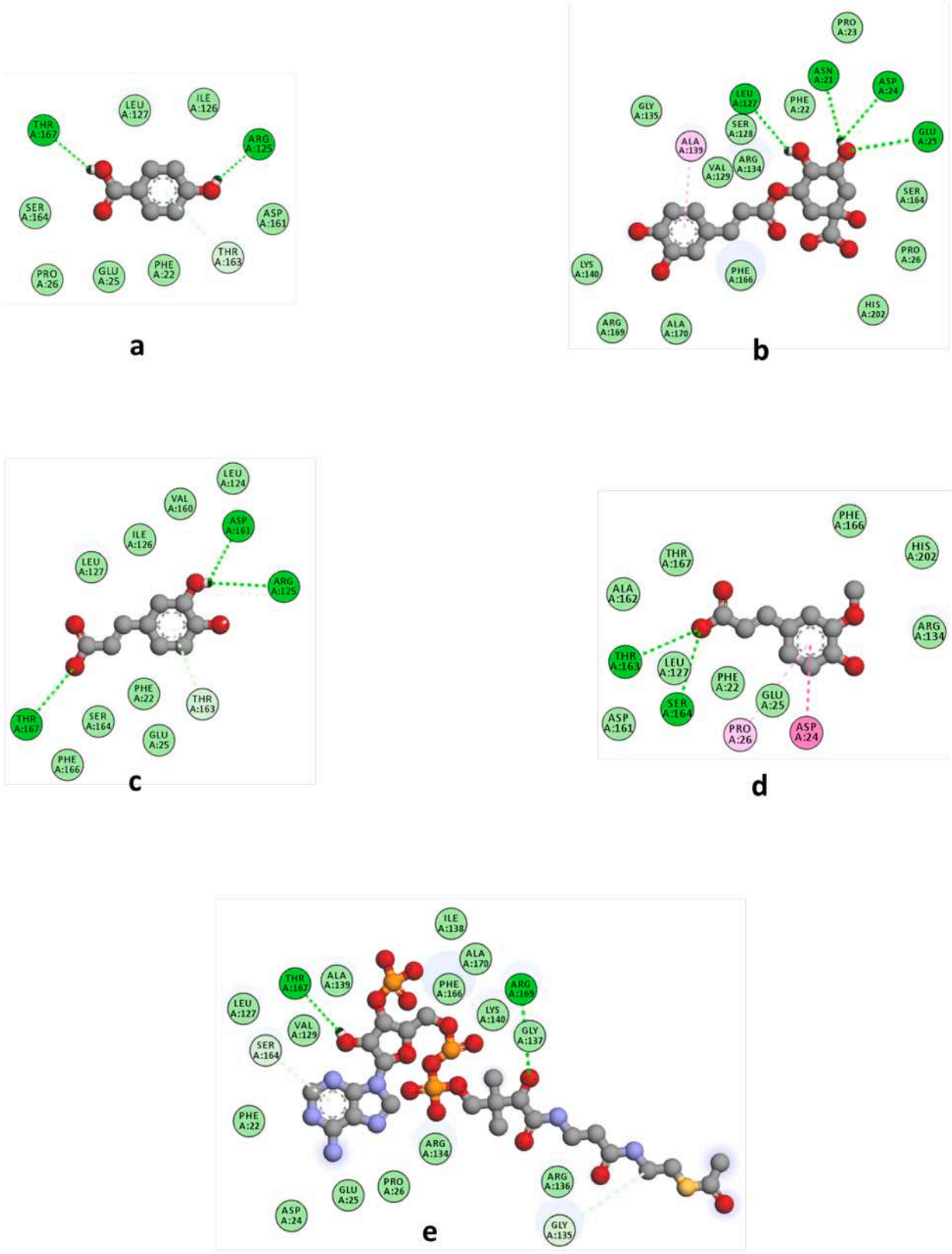


Fig. 7. 2D model of different interactions of : 4-hydroxybenzoic acid (a), chlorogenic acid (b), caffeic acid (c), ferulic acid (d) and the co-crystallized ligand (e) within the active site of '*Tribolium castaneum* arylalkylamine N-acyltransferase' (pdb: 6v3t).

within this genus, it is essential to highlight the significant influence of compounds derived from glucosinolates and their hydrolysis products. These sulfur-containing compounds, when broken down, contribute to the distinctive aroma profiles and the characteristic pungent flavors often associated with this group of plants. This chemical transformation not only shapes the sensory attributes of the plants but also plays a vital role in their overall culinary appeal, enriching dishes with complex tastes and fragrances that are both robust and memorable. In addition, some Brassicaceae plants are consumed raw, while others are cooked; this affects not only the hydrolysis of glucosinolates but also the generation and release of sulfides (Bell et al., 2018). The sharp, piquant flavors found in certain foods can largely be attributed to the presence of glucosinolates In the case of rocket salad, specific chemical compounds such as dimeric glucosativin, progoitrin and epiprogoitrin significantly contribute to its unique flavor profile. Dimeric glucosativin provides an intense bitterness, while progoitrin adds a subtle earthiness, and epiprogoitrin enhances the peppery kick that characterizes this leafy green. This combination of flavors creates a complex, bold taste experience that is highly valued by consumers and plays a crucial role in the growing popularity of rocket salad as a vibrant addition to various culinary creations (Ressurreição et al., 2024). Environmental factors play a crucial role in shaping the development of secondary metabolites and nitrate levels in various crops. Consequently, the quality of wall rocket, particularly its bioactive compounds and nitrate content, can be significantly influenced by these external conditions (Guijarro-Real et al., 2019b). The different parts of the *Diplotaxis* plant contain distinct glucosinolate profiles. The seeds and roots are predominantly rich in 4-methylthiobutylglucosinolate, whereas the leaves are characterized by high levels of 4-mercaptobutylglucosinolate, together with lower concentrations of 4-methylthiobutylglucosinolate and 4-methylsulfinylbutylglucosinolate. The flowers primarily contain 4-methylsulfinylbutylglucosinolate. In addition, the roots of *Diplotaxis* species also contain 4-hydroxybenzylglucosinolate. Both seeds and seedlings contain sinapin but they lack the N-heterocycles typically found in other parts of the plant. Furthermore, all tissues of rocket, except the roots, have significant levels of polyglycosylated flavonoids, which may or may not include hydroxycinnamoyl acylation. The main aglycones identified in these tissues are kaempferol, quercetin and isorhamnetin (Bennett et al., 2006). Furthermore, the ethanolic extract obtained from the aerial parts of *D. erucoides* has been analyzed and found to contain a variety of bioactive compounds. Notably, this extract includes derivatives of hydroxycinnamic acids, specifically caffeic and coumaric acid derivatives. Additionally, flavonol glycosides derived from quercetin, kaempferol, and isorhamnetin have also been identified. Furthermore, the analysis revealed the presence of glucosinolates, as well as oxylipins numbered 59 and 60, contributing to the extract's bioactivity (Ressurreição et al., 2024). Our results showed that the antioxidant activities of different extracts of the false rocket vary according to the solvent used for extraction of the aerial parts of *D. erucoides*. The extracts of *D. erucoides*, including the ethanol extract, various fractions such as ethyl acetate and n-butanol, as well as the isolated compound rhamnetin-3,3′-di-O-β-D-glucopyranoside, exhibited impressive DPPH radical scavenging activity compared with the synthetic antioxidant BHT. The observed activity levels ranged between 18.00 ± 0.01 μg/mL and 27.02 ± 1.53 μg/mL, indicating strong potential for these natural extracts in antioxidant applications. Notably, the crude ethanolic extract derived from the non-flowering aerial parts of *D. erucoides* displayed the highest activity at 27.04 ± 1.53 μg/mL, outperforming both its fractions and the tested compound, which had a scavenging activity of 19.04 ± 0.02 μg/mL. In fact, the tested compound demonstrated more than twice the antioxidant activity of BHT. However, it remained less potent than quercetin, which exhibited an impressive DPPH scavenging capability at 6.91 ± 0.42 μg/mL (Ben Salah et al., 2015). Additionally, the ethanolic extract obtained from the pre-flowering aerial parts of *D. erucoides* demonstrated significant antioxidant activity across multiple assays, highlighting its potential health benefits. In the ABTS assay, the extract exhibited an IC50 value of 97.87 μg/mL indicating a moderate capacity to scavenge free radicals. The DPPH assay further supported this finding, yielding an IC50 value of 135.13 μg/mL, which reflects the extract's ability to neutralize free radicals in solution. In the FRAP assay, the extract showed an IC50 of 16.88 μM Fe(II) g^{-1}, suggesting that it can effectively reduce ferric ions to ferrous ions, a critical mechanism of antioxidant action. Furthermore, in the β-carotene linoleate model, the extract provided remarkable protection against lipid peroxidation, with an IC50 value of just 10.32 μg/mL after 60 minutes. This result highlights the extract's potential to preserve lipid stability, which is vital for maintaining the integrity of biological membranes. Positive controls used in these assays included well-known antioxidants. Ascorbic acid, a potent antioxidant, showed superior activity with IC50 values of 5.0 ± 0.82 μg/mL in the DPPH assay and 1.7 ± 0.06 μg/mL in the ABTS assay, illustrating its strong free radical scavenging ability. BHT, another common antioxidant, recorded an IC50 of 63.2 ± 2.30 μM Fe(II)/g in the FRAP assay, while propyl gallate demonstrated exceptional efficacy in the β-carotene assay with an IC50 of 0.09 ± 0.04 μg/mL. In comparison with these positive controls, the *D. erucoides* extract exhibited appreciable antioxidant activity (Loizzo et al., 2021). The results obtained in the present report revealed that the methanolic extract of the upper parts of *D. erucoides* at the flowering stage produced the highest growth inhibition of Gram-negative bacteria, exceeding that of antibiotics. A comprehensive evaluation of a panel of Gram-positive and Gram-negative bacteria showed varying sensitivity levels to the n-butanol extract derived from the aerial parts of *D. erucoides*. The Gram-positive bacteria included *L. monocytogenes* and *S. aureus*, while the Gram-negative bacteria encompassed *A. hydrophila, P. aeruginosa, S. enteritidis, E. coli*, and *K. pneumoniae*. The n-butanolextract demonstrated notable antibacterial activity, with IZ ranging from 16.00 ± 0.01 mm to 21.07 ± 0.05 mm, reflecting its effectiveness against the tested bacterial strains. Interestingly, *E. coli* showed slightly higher sensitivity to rhamnetin-3,3'-di-O-β-D-glucopyranoside, yielding an IZ of 17.60 ± 0.04 mm, thus highlighting the differences in sensitivity among the bacterial strains tested and emphasizing the potential of *D. erucoides* as a source of antibacterial agents. This difference in sensitivity underscores the importance of exploring various extracts and compounds for their antimicrobial properties (Ben Salah et al., 2015). Furthermore, an ethanolic extract derived from the plant *D. erucoides* was subjected to a comprehensive evaluation to determine its effectiveness in inhibiting carbohydrate-hydrolyzing enzymes. The results indicated that this extract demonstrated slightly superior inhibitory activity against α-glucosidase, with an IC50 value of 85.18 μg/mL, compared with its action against α-amylase, which had an IC50 value of 92.36 ± 4.31 μg/mL. In both enzymatic assays, acarbose was used as a positive control, achieving impressive IC50 values of 35.5 ± 1.22 μg/mL for α-glucosidase and 50.0 ± 0.93 μg/mL for α-amylase, as reported in the study by Loizzo et al. (2021). Additionally, the ethanolic extract displayed notable inhibitory effects on pancreatic lipase, with an IC50 value of 61.27 μg/mL. In comparison, the standard drug orlistat, which is recognized for its potency in inhibiting this enzyme, yielded an IC50 value of 37.1 ± 1.30 μg/mL. These findings suggest the potential of *D. erucoides* extract as a natural inhibitor of enzymes involved in carbohydrate digestion, which could have implications for managing conditions such as diabetes and obesity (Loizzo et al., 2021).

The species in question exhibited a high concentration of ITC, a compound known for its distinctive flavor profile. However, it is noteworthy that the baby leaves of the plant contained markedly low levels of this compound. Among the various forms of the plant, allyl ITC was the predominant type identified in both wall rocket and its seedlings, as well as in the tender, young baby leaves. This variation in ITC concentration is likely to play a significant role in shaping the flavor similarities observed across these different growth stages of the plant (Guijarro-Real et al., 2020). The compounds and aromas of these plants endow them with considerable potential. However, the choice of approach depends on the stability of the compounds during processing, consumer

acceptance, the desired health benefits, with regulatory and market considerations. Despite its various uses and economic interest, an integrated review of the current knowledge about the *Diplotaxis* genus is lacking, especially in terms of its chemical composition and nutritional and biological properties (Ressurreição et al., 2024).

This comprehensive report examines the attractancy, repellency and lethal effects of extracts derived from Tunisian *D. erucoides*, commonly known as false rocket, on both life stage (larvae and adult) of the confused flour beetle. The hardy plant, *D. erucoides*, has already been reported to attract a variety of beneficial insects, including essential pollinators such as bees and butterflies. By providing nectar and pollen, it fosters a thriving environment for these insects, which in turn promotes efficient pollination. This not only supports the growth of other flowering plants but also contributes to enhanced biodiversity, creating a vibrant ecosystem with diverse species and interdependent relationships. As shown by Tüfekçi (2025), the highest toxicity against *Sitophilus granarius* was observed with flowers of *Diplotaxis tenuifolia* extracted with acetone, which caused 86.6 % mortality. Flowers of *D. tenuifolia* extracted with ethyl acetate also showed a significant effect, resulting in 83.3 % mortality in *S. granarius* and 90 % in *Rhizopertha dominica*. Leaves and flowers of *D. tenuifolia* extracted with n-butanol showed negligible contact toxicity against the adult stages of both *S. granarius* and *R. dominica*. After 48 hours, leaves of *D. tenuifolia* extracted with acetone showed superior insecticidal activity, causing 56.6 % mortality in *R. dominica* and 60 % mortality in *S. granarius*. Research on wild species such as *D. muralis* and *D. tenuifolia* has identified genes coding for protease inhibitors that actively target gut proteases in herbivorous insect larvae (such as *Helicoverpa zea*) (Volpicella et al., 2009). Recent studies have also explored the bioinsecticidal effect of some specie of Brassicaceae family. Brassica juncea seeds cause high larval mortality and decrease the overall larval and pupal growth indices of Spodoptera litura (Gupta et al., 2017). Additionally, the findings of a study conducted by Selvakumaran et al. (2024), demonstrated that the essential oil extracted from black mustard seeds notably effective in controlling mosquito populations, exhibiting potent larvicidal, ovicidal, and pupicidal properties against various species, including *Aedes, Culex*, and *Anopheles*. The analysis revealed that this essential oil achieved a complete mortality rate of 100% within hours for the eggs of all mosquito species examined, underscoring its significant impact on the initial stages of mosquito development. Furthermore, the essential oil showcased impressive larvicidal and pupicidal effects, with specific mortality rates noted for each mosquito genus: *Aedes* suffered 92 % mortality in larvae and 84 % in pupae; *Culex* experienced 96 % mortality in larvae and 86% in pupae; and *Anopheles* recorded 98 % larval mortality alongside 90 % pupal mortality, all within the same 24-hour timeframe. Additionally, the seeds of *Brassica carinata* (A.) Braun and *Camelina sativa* (L.) Cranz possess complex and diverse chemical compositions that make them ideal candidates for the development of biopesticides. These natural substances can harness a wide range of mechanisms of action against pests, providing an eco-friendly alternative to synthetic pesticides. The rich variety of phytonutrients and bioactive compounds found in these seeds not only enhances their efficacy in pest management but also supports sustainable agricultural practices by minimizing environmental impact (Rzyska et al., 2024). Several seed meals from the Brassicaceae family, commonly known as the mustard family, have been investigated as potential sources of plant-derived isothiocyanates. These compounds are generated through the enzymatic hydrolysis of inactive glucosinolates and may be effective in controlling *Aedes aegypti*, a mosquito species known for transmitting diseases. In this study, five defatted seed meals were evaluated: *Brassica juncea* (L) Czern., *Lepidium sativum* L., *Sinapis alba* L., *Thlaspi arvense* L., and a heat-inactivated variant of *Thlaspi arvense*. Additionally, three key chemical products resulting from enzymatic degradation, namely, ITC, benzyl isothiocyanate, and 4-hydroxybenzyl isothiocyanate, were tested for their toxicity to *Aedes aegypti* larvae. The results indicated that all seed meals, except for heat-inactivated *Thlaspi arvense*, were toxic to the mosquito larvae. Notably, the seed meal from *Lepidium sativum* was identified as the most toxic treatment, with a lethal concentration (LC50) of 0.04 g/120 mL of deionized water after a 24-hour exposure. After 72 hours, the LC50 values for *Brassica juncea, Sinapis alba*, and *T. arvense* were determined to be 0.05, 0.08, and 0.1 g/120 mL of deionized water, respectively. Among the synthetic compounds tested, benzyl isothiocyanate demonstrated higher toxicity to larvae at 24 hours post-treatment (LC50 = 5.29 mg/L), compared to allyl ITC (LC50 = 19.35 mg/L) and 4-hydroxybenzyl isothiocyanate (LC50 = 55.41 mg/L). These findings suggest the potential of using plant-derived isothiocyanates for mosquito control (Flor-Weiler et al., 2023). Docking analyses indicate that the five major phytocompounds assessed *in silico* have significant potential to inhibit '*Tribolium castaneum* Arylalkylamine N-acyltransferase'.

5. Conclusion

The detailed profiling presented here reveals a broader and more intricate diversity of glucosinolates than has been documented in previous studies. These data highlight the noteworthy antioxidant properties of *D. erucoides* extracts, suggesting their potential for further exploration in health-related applications. Extracts of the aerial parts of *D. erucoides* act as a bioinsecticidal agent, free radical scavengers, and a bacterial growth inhibitor. This observation has important implications for upcoming breeding programs targeting wall rocket. A better understanding of the specific morphological traits that are underrepresented can guide breeders in selecting desired characteristics, potentially enhancing the genetic variability needed to develop new cultivars. Moving forward, it will be crucial to explore different genetic resources and employ innovative breeding techniques to enrich the diversity of wall rocket, ultimately improving the resilience and adaptability of the crop.

CRediT authorship contribution statement

Fatma Guesmi: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Mabrouk Horchani:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Wiem Tahri:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Sameh Ben Chaaban:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Mabrouka Saidani:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Mohamed Ali Borgi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Angelo Maria Giuffrè:** Writing – review & editing, Visualization, Supervision, Software, Project administration. **Rosalía López Ruiz:** Writing – review & editing, Methodology, Data curation. **Roberto Romero González:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation. **Antonia Garrido Frenich:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation. **Ahmed Landoulsi:** Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tunisia and Prof. Angelo Maria Giuffrè (Department AGRARIA, University of Studies *Mediterranea* of Reggio Calabria, Italy).

Data availability

All data generated or analysed during this study is included in this article.

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