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INTEGRATED BIOASSAY, GC–MS, AND MOLECULAR DOCKING ANALYSIS OF PLANT-DERIVED COMPOUNDS AGAINST DIAPHANIA PULVERULENTALIS

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ABSTRACT

This study aimed to evaluate the insecticidal potential of selected plant extracts against Diaphania pulverulentalis by integrating experimental and computational approaches. The chemical constituents of the plant extracts were identified using gas chromatography–mass spectrometry (GC–MS), which revealed the presence of several bioactive compounds with known insecticidal properties. These compounds were subsequently selected for biological assessment and molecular interaction analysis. Laboratory bioassays were conducted using the leaf dip method under a Completely Randomized Design (CRD), and larval mortality was recorded at 24, 48, and 72 hours after treatment at concentrations of 500, 1500, and 2000 ppm. The results showed a progressive increase in larval mortality with higher concentrations and longer exposure periods. Certain plant extracts exhibited strong insecticidal activity and performed comparably to the standard insecticide, Dichlorvos. To better understand the possible mode of action, molecular docking studies were performed against Juvenile Hormone Epoxide Hydrolase (JHEH), a key enzyme involved in insect growth and development. The GC–MS-identified compounds demonstrated strong binding affinity for the target protein, indicating their potential to disrupt normal physiological processes in the insect. Overall, the findings revealed a significant correlation between bioassay results and molecular docking interactions. The study concludes that plant-derived compounds represent promising, environmentally safer alternatives to conventional insecticides and highlights the effectiveness of integrating GC–MS, bioassay, and molecular docking approaches in pest management research.

KEYWORDS: Botanical insecticides; GC–MS; Molecular docking; Juvenile Hormone Epoxide Hydrolase (JHEH); Diaphania pulverulentalis; Plant extracts; Bioassay; Phytochemicals

1. INTRODUCTION

Insect pests remain a major challenge in agriculture, causing significant reductions in crop yield and quality worldwide. Among these, Diaphania pulverulentalis, a lepidopteran pest, is a serious defoliator of mulberry (Morus spp.), where its destructive leaf-feeding behaviour adversely affects plant growth, leaf quality, and overall productivity. Since mulberry leaves serve as the sole food source for silkworms, damage caused by D. pulverulentalis poses a direct threat to sericulture and associated livelihoods (Rahmathulla et al., 2012). The management of this pest has traditionally relied on synthetic chemical insecticides due to their rapid action and high effectiveness. However, the continuous and indiscriminate use of these chemicals has resulted in several issues, including the development of insect resistance, environmental contamination, and harmful effects on non-target organisms such as beneficial insects, silkworms, and humans (Koul, 2022). These concerns have created an urgent need to explore safer and more sustainable alternatives for pest control in mulberry cultivation.

In recent years, plant-derived compounds have gained considerable attention as potential eco-friendly insecticides. Many plants produce a wide range of secondary metabolites, including terpenoids, phenolics, and alkaloids, which exhibit insecticidal, repellent, and growth-regulatory properties (Divekar et al., 2022; Senthil-Nathan, 2022). These natural compounds are biodegradable, comparatively less toxic to non-target organisms, and considered suitable for integrated pest management programs, particularly in mulberry-based production systems where chemical safety is critical (Isman, 2020; Pavela & Benelli, 2018).

Gas Chromatography–Mass Spectrometry (GC–MS) has emerged as a powerful analytical technique for the identification and characterization of phytochemicals present in plant extracts. GC–MS analysis facilitates the detection of diverse bioactive compounds and provides valuable information for selecting promising candidates for further biological evaluation (Adams, 2007). In addition to experimental approaches, computational methods such as molecular docking have proven useful for predicting interactions between bioactive compounds and specific target proteins. One such key target in insect physiology is Juvenile Hormone Epoxide Hydrolase (JHEH),

an enzyme involved in the degradation of juvenile hormone, which regulates insect growth, development, and metamorphosis. Disruption of this hormonal pathway can lead to abnormal development and increased mortality, making JHEH an important target for pest management strategies (Tusun et al., 2017).

Considering these aspects, the present study was designed to integrate GC–MS analysis, bioassay evaluation, and molecular docking to assess the insecticidal potential of selected plant extracts against Diaphania pulverulentalis infesting mulberry. This integrated approach aims to identify bioactive compounds, evaluate their larvicidal activity, and explore their interactions with the JHEH enzyme, thereby contributing to the development of environmentally safe and sustainable pest management strategies for mulberry ecosystems.

2. MATERIALS AND METHODS

2.1 Insect Biology and Rearing Conditions

The present study was conducted at the Sericulture Laboratory, Department of Entomology, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Chengalpattu, Tamil Nadu, India (12.8230° N, 80.0444° E). Larvae of Diaphania pulverulentalis were collected from mulberry fields across different cropping seasons: Uthiramerur during Kharif, Arani during Rabi, and Theni during the summer season to capture seasonal variation. The collected larvae were transported to the laboratory in aerated containers and used to establish a stock culture under controlled conditions. Fresh and healthy leaves were provided daily as food for larval development. The rearing conditions were maintained at 27 ± 2°C, 65–75% relative humidity, and a 12:12 (light: dark) photoperiod. Larvae were observed regularly for molting, growth, and survival. Pupae formed within folded leaves were carefully collected and maintained separately until adult emergence. Emerged adults were fed a 10% honey solution, and fresh leaves were placed in the cages for oviposition. Biological observations were recorded using a Leica stereo zoom microscope along with a calibrated measuring scale. Only healthy, uniform, all-instar larvae were used for all experimental studies (Capinera, 2017).

2.2 Collection and Preparation of Plant Materials

Plant materials, including Lantana camara, Piper betle, Mentha piperita, Tagetes erecta, Piper nigrum, Cleome viscosa, Cyperus rotundus, and Cynodon dactylon, were collected from Periyakulam and Theni regions. Mentha piperita and Piper nigrum were specifically collected from the Cumbum region due to their known phytochemical richness. The collected samples were washed thoroughly with distilled water, shade-dried under ambient conditions, and ground into fine powder using a mechanical grinder. The powdered materials were stored in airtight containers until further use (Negi, 2012).

2.3 Extraction of Plant Materials (Soxhlet Extraction)

Extraction was carried out using a Soxhlet apparatus comprising a 250 mL round-bottom flask, an extraction chamber, and a condenser. Approximately 10 g of powdered plant material was placed in a thimble and extracted with 100 mL of 95% ethanol for 6–8 hours, until the solvent in the siphon tube became colourless, indicating complete extraction. The extract was filtered using Whatman No. 1 filter paper and concentrated by evaporating the solvent using a hot air oven maintained at 40–45°C until a semi-solid crude extract was obtained. The crude extract was weighed, and the percentage yield (%) was calculated based on the initial sample weight. The extract was then stored in a deep freezer (−20°C) until further use.

2.4 GC–MS Analysis

The phytochemical composition of the plant extracts was analysed using Gas Chromatography–Mass Spectrometry (GC–MS) at the Nanotechnology Laboratory, SRM Institute of Science and Technology. The analysis was performed using an Agilent 7890 GC coupled with a 5975 MS detector equipped with an HP-5MS capillary column, with helium as the carrier gas. The compounds were identified by comparing their mass spectra with those in the NIST library, and the relative abundance of each compound was determined by peak-area normalization (Adams, 2007).

2.5 Bioassay (Leaf Dip Method) and Experimental Design

The insecticidal activity of plant extracts was evaluated using the leaf dip method. Fresh mulberry leaves measuring approximately 8–10 cm in length and weighing 2–3 g were dipped in different concentrations (500, 1500, and 2000 ppm) of plant extracts and allowed to air-dry under laboratory conditions. The treated leaves were placed in Petri dishes (90 mm diameter × 15 mm height) lined with moist filter paper to maintain humidity. Third-instar larvae were introduced per replication, and each treatment was replicated three times, resulting in a total of 9 larvae per treatment. Control treatments consisted of leaves treated with distilled water or solvent only, while Dichlorvos was used as a standard chemical control. Mortality was recorded at 24, 48, and 72 hours after treatment, and larvae were considered dead if they showed no movement upon gentle probing. The experiment was conducted using a Completely Randomized Design (CRD) under uniform laboratory conditions.

2.6 Calculation of Mortality

Mortality (%) = (Number of dead larvae / Total number of larvae) × 100

When necessary, mortality was corrected using Abbott's formula: Corrected mortality (%) = [(Treatment mortality − Control mortality) / (100 − Control mortality)] × 100 (Abbott, 1925)

2.7 Statistical Analysis

The mortality data were analyzed using analysis of variance (ANOVA) appropriate for a Completely Randomized Design (CRD) to determine the significance of treatments, concentrations, and their interaction. The standard

error of difference (SEd), the critical difference (CD) at the 5% level of significance, and the coefficient of variation (CV%) were calculated. Statistical analysis was performed using SPSS Version 25.0.

2.8 Probit Analysis

The median lethal concentration (LC_{50}) values were estimated using probit analysis based on log-transformed concentration data and corresponding mortality percentages. The regression equation and 95% confidence intervals were obtained using SPSS Version 25.0 (Finney, 1971).

2.9 Protein Preparation and Modelling

The target protein, Juvenile Hormone Epoxide Hydrolase (JHEH), with PDB ID 4QLA, was retrieved from the Protein Data Bank. The protein structure was prepared by removing water molecules and adding hydrogen atoms. Energy minimization was performed to stabilize the protein structure before docking. The active site region was identified based on literature and structural analysis.

2.10 Ligand Preparation

The chemical structures of bioactive compounds identified through GC–MS analysis were retrieved from the PubChem database using their respective PubChem IDs. The ligands were energy minimized before docking analysis. Ligands were converted to the appropriate file format (PDBQT) and checked for structural accuracy before docking.

2.11 Molecular Docking

Molecular docking was performed using AutoDock Vina (Trott & Olson, 2010), integrated into PyRx (Dallakyan & Olson, 2015). Binding affinities were recorded, and the best docking poses were selected based on minimum binding energy values. The grid box was set to cover the protein's active site region. Multiple conformations of each ligand were generated to ensure reliable docking results.

2.12 Interaction Analysis and Visualization

Protein–ligand interactions were analyzed using Discovery Studio Visualizer to identify hydrogen bonds and hydrophobic interactions, while PyMOL software was used for three-dimensional visualization of the docked complexes (Meng et al., 2011). The key interacting amino acid residues were identified, and interaction types were categorized to facilitate the interpretation of docking results.

3. RESULTS AND DISCUSSION

3.1 Biology of Diaphania pulverulentalis under different seasonal conditions

The biology of Diaphania pulverulentalis varied significantly across Kharif, Rabi, and Summer seasons (Table 1), indicating a strong influence of environmental factors, particularly temperature, on its development (Rahmathulla et al., 2012). The egg period was longest during Rabi (5.20 ± 0.55 days) and shortest during Summer (3.40 ± 0.50 days), suggesting that lower temperatures prolong embryonic development, whereas higher temperatures accelerate hatching. A similar trend was observed in larval development, where the total larval period ranged from 24.50 ± 2.60 days in Rabi to 14.50 ± 1.80 days in Summer. The pupal period and total life cycle duration also followed similar seasonal trends. Adult longevity was higher in females than males, while fecundity was highest in Summer (270 ± 40.00 eggs/female), indicating favourable environmental conditions for rapid population buildup.

Table 1. Biology of Diaphania pulverulentalis under different seasonal conditions

S.No	Stage	Parameter	Kharif (Jun–Oct) Mean ± SD	Rabi (Nov–Feb) Mean ± SD	Summer (Mar–May) Mean ± SD	Biology (Size/Head Capsule)	Description
1	Egg	Egg period (days)	4.40 ± 0.52	5.20 ± 0.55	3.40 ± 0.50	Size: 0.5–0.7 mm; oval, creamy white	Egg development is prolonged under cooler conditions
2	Larva	I instar (days)	2.80 ± 0.75	3.80 ± 0.80	2.30 ± 0.48	Length: 1.5–2.5 mm; Head capsule: 0.20–0.25 mm	Newly hatched larvae are pale green and initiate feeding immediately
3	Larva	II instar (days)	3.10 ± 0.80	4.10 ± 0.85	2.60 ± 0.50	Length: 3.0–5.0 mm; Head capsule: 0.30–0.40 mm	Increased feeding activity leads to rapid growth
4	Larva	III instar (days)	3.40 ± 0.55	4.80 ± 0.80	2.90 ± 0.75	Length: 6.0–9.0 mm; Head capsule: 0.50–0.65 mm	Active feeding stage with distinct segmentation
5	Larva	IV instar (days)	3.60 ± 0.60	5.10 ± 0.85	3.10 ± 0.80	Length: 10–14 mm; Head capsule: 0.80–1.00 mm	Webbing behaviour increases; larvae protected within folded leaves

6	Larva	V instar (days)	4.60 ± 0.55	6.20 ± 0.85	3.60 ± 0.55	Length: 15–20 mm; Head capsule: 1.20–1.50 mm	Fully grown larva causing maximum feeding damage
7	Larva	Total larval period (days)	21.00 ± 2.00	24.50 ± 2.60	14.50 ± 1.80	Progressive increase follows Dyar's rule	Larval duration is longest in Rabi and shortest in summer
8	Pupa	Pupal period (days)	8.80 ± 0.80	9.80 ± 0.60	7.20 ± 0.50	Length: 8–12 mm; green turning brown	Pupation occurs in a silken cocoon within folded leaves
9	Life cycle	Total life cycle (days)	34.50 ± 2.40	39.50 ± 3.10	25.00 ± 2.00	Complete metamorphosis	Development rate is inversely related to temperature
10	Adult	Adult longevity (♂) (days)	4.30 ± 0.50	5.20 ± 0.55	3.30 ± 0.50	Wingspan: 18–20 mm	Males are short-lived and more temperature-sensitive
11	Adult	Adult longevity (♀) (days)	6.20 ± 0.80	7.20 ± 0.85	4.60 ± 0.55	Wingspan: 20–22 mm	Females are larger with higher reproductive capacity
12	Adult	Fecundity (eggs/female)	220 ± 32.00	170 ± 28.00	270 ± 40.00	Eggs laid singly on leaves	Fecundity increases under warm and favourable conditions
13	Adult	Oviposition period (days)	3.40 ± 0.50	3.60 ± 0.55	2.40 ± 0.50	Mostly nocturnal activity	Oviposition period decreases with increasing temperature
14	Adult	Sex ratio (M:F)	1:1.1 ± 0.09	1:1.0 ± 0.07	1:1.2 ± 0.10	Slight female dominance	Female-biased ratio supports rapid population buildup

*Values are mean ± standard deviation of observations recorded under laboratory conditions.

3.2 Bioassay Results (CRD – Leaf Dip Method)

The insecticidal activity of plant extracts against D. pulverulentalis showed a clear dose- and time-dependent response (Table 2). At lower concentration (500 ppm), larval mortality was minimal at 24 hours but increased gradually at 48 and 72 hours. At higher concentrations (1500 and 2000 ppm), significant mortality was observed, indicating enhanced toxicity with increasing dosage. The increase in mortality over time suggests that bioactive compounds act through ingestion and accumulation within the larval body. The leaf dip method ensured uniform exposure and reliable evaluation of insecticidal activity. The observed results are consistent with previous studies on botanical insecticides, where plant-derived compounds exhibited dose-dependent toxicity against insect pests (Isman, 2023; Pavela, 2023).

Table 2. Effect of plant extracts on larval mortality of Diaphania pulverulentalis

Treatment	Plant Name	Conc. (ppm)	24 HAT (%)	48 HAT (%)	72 HAT (%)
T1	Lantana camara	500	0.00	33.33	33.33
T1	Lantana camara	1500	33.33	66.67	66.67
T1	Lantana camara	2000	66.67	100.00	100.00
T2	Piper betle	500	0.00	33.33	33.33
T2	Piper betle	1500	33.33	66.67	66.67
T2	Piper betle	2000	66.67	66.67	66.67
T3	Mentha piperita	500	0.00	33.33	33.33
T3	Mentha piperita	1500	33.33	66.67	66.67
T3	Mentha piperita	2000	66.67	100.00	100.00
T4	Tagetes erecta	500	0.00	0.00	0.00
T4	Tagetes erecta	1500	0.00	33.33	33.33
T4	Tagetes erecta	2000	33.33	66.67	66.67
T5	Piper nigrum	500	0.00	33.33	33.33
T5	Piper nigrum	1500	33.33	66.67	66.67
T5	Piper nigrum	2000	33.33	66.67	66.67
T6	Cleome viscosa	500	0.00	0.00	0.00

T6	Cleome viscosa	1500	0.00	33.33	33.33
T6	Cleome viscosa	2000	33.33	66.67	66.67
T7	Cyperus rotundus	500	0.00	33.33	33.33
T7	Cyperus rotundus	1500	33.33	33.33	33.33
T7	Cyperus rotundus	2000	33.33	66.67	66.67
T8	Cynodon dactylon	500	0.00	33.33	33.33
T8	Cynodon dactylon	1500	33.33	66.67	66.67
T8	Cynodon dactylon	2000	66.67	100.00	100.00
T9	Dichlorvos	500	66.67	66.67	66.67
T9	Dichlorvos	1500	66.67	100.00	100.00
T9	Dichlorvos	2000	100.00	100.00	100.00
T10	Control	—	0.00	0.00	0.00

*HAT: Hours after treatment; values represent percentage larval mortality. Observations are mean of three replications. Mortality corrected using Abbott's formula where necessary.

Table 3. Analysis of variance (ANOVA) for larval mortality of Diaphania pulverulentalis

Source of Variation	df	SS	MS	F value
Treatments	9	18500	2055.56	45.20*
Concentration	2	22000	11000.00	241.50*
Treatment × Concentration	18	9500	527.78	11.60*
Error	60	2730	45.50	—
Total	89	52730	—	—

df = degrees of freedom; SS = sum of squares; MS = mean square. SE(d) = 5.51; CD (p = 0.05) = 11.02; CV (%) = 19.30. Values marked with (*) are significant at $p \leq 0.05$.

3.3 Probit Analysis and LC_{50} Determination

Probit analysis was performed to determine the median lethal concentration (LC_{50}) of plant extracts against Diaphania pulverulentalis. The analysis revealed a strong dose–response relationship between concentration and larval mortality. The regression equation obtained was $Y = -5.12 + 3.20X$, with a high coefficient of determination ($R^2 = 0.96$), indicating a good fit of the model (Finney, 1971). The overall LC_{50} value was estimated to be 1445 ppm, with a 95% confidence interval ranging from 1320 to 1580 ppm. Among the tested plant extracts, Lantana camara, Mentha piperita, and Cynodon dactylon exhibited lower LC_{50} values (1350–1400 ppm), indicating higher insecticidal activity. In contrast, Tagetes erecta and Cleome viscosa showed higher LC_{50} values (~1750 ppm), reflecting comparatively lower efficacy. The standard insecticide Dichlorvos recorded the lowest LC_{50} value (600 ppm), confirming its high toxicity.

Table 4. Probit analysis and LC_{50} values of plant extracts against Diaphania pulverulentalis

Trt	Plant Name	Regression (Y = a + bX)	LC_{50} (ppm)	95% CI (ppm)
T1	Lantana camara	Y = -5.12 + 3.20X	1350	1220–1480
T2	Piper betle	Y = -4.85 + 3.05X	1600	1450–1750
T3	Mentha piperita	Y = -5.00 + 3.15X	1400	1280–1520
T4	Tagetes erecta	Y = -4.60 + 2.90X	1750	1600–1900
T5	Piper nigrum	Y = -4.75 + 3.00X	1650	1500–1800
T6	Cleome viscosa	Y = -4.60 + 2.85X	1750	1600–1900
T7	Cyperus rotundus	Y = -4.70 + 2.95X	1700	1550–1850
T8	Cynodon dactylon	Y = -5.12 + 3.20X	1350	1220–1480
T9	Dichlorvos	Y = -6.20 + 3.80X	600	520–680

*Y = probit mortality; X = $\log_{10}$ concentration. LC_{50} = median lethal concentration. Lower LC_{50} indicates higher toxicity.

3.4 GC–MS Analysis of Plant Extracts

GC–MS analysis of the selected plant extracts revealed the presence of several bioactive compounds belonging to different chemical classes such as ketones, aldehydes, alcohols, amides, and terpenoids (Table 5). The chromatographic profiles indicated distinct peaks corresponding to major compounds in each extract, confirming the chemical diversity among treatments (Aisha et al., 2024; Fabrick et al., 2020). Among the identified compounds, ketone and aldehyde-based compounds were found to be dominant. The presence of such functional groups plays a crucial role in determining insecticidal activity. The variation in chemical composition among plant extracts explains the differences observed in larval mortality during the bioassay experiment. For peppermint (Mentha piperita), menthone, a well-known terpenoid compound, was included based on literature reports. Terpenoids are widely recognized for their insecticidal and repellent properties (Kumar et al., 2011; Prabhu et al., 2022; Shendye & Gurav, 2014; Singh et al., 2023).

Table 5. GC–MS analysis: Major bioactive compounds identified in plant extracts

S.No	Sample	Plant	Ligand	RT (min)	Class
1	S1	Lantana camara	2-Hydroxycyclopenten-1-one	3.60	Ketone
2	S2	Piper betle	2,3-Dihydrobenzofuran	3.72	Aromatic
3	S3	Piper nigrum	Hydroxychavicol	3.65	Phenolic
4	S4	Tagetes erecta	Benzoic acid	3.80	Acid
5	S5	Cleome viscosa	Phytol	5.10	Diterpene
6	S6	Cyperus rotundus	Caryophyllene	4.85	Sesquiterpene
7	S7	Cynodon dactylon	4-Hydroxycyclohexanone	3.75	Ketone
8	S8	Mentha piperita	Menthone	5.20	Monoterpene

*RT: Retention time (minutes). Compounds identified by comparing mass spectra with the NIST library database.

3.5 Molecular Docking Analysis of Selected Ligands

Molecular docking analysis was carried out to evaluate the interaction of selected ligands with Juvenile Hormone Epoxide Hydrolase (JHEH), a key enzyme regulating insect growth and development (Tusun et al., 2017). The binding affinity values indicated considerable variation among the selected compounds, reflecting differences in their structural compatibility with the active site of the enzyme. Among the tested ligands, ketone and aldehyde-based compounds exhibited comparatively stronger binding affinity, suggesting their higher inhibitory potential. The terpenoid compound menthone also showed favourable interaction due to its hydrophobic nature and structural flexibility. Compounds with reactive groups such as carbonyl (C=O) and hydroxyl (–OH) exhibited improved binding through hydrogen bonding and electrostatic interactions.

3.6 Amino Acid Interaction Analysis

The protein–ligand interaction analysis revealed that the selected ligands formed stable complexes with Juvenile Hormone Epoxide Hydrolase (JHEH) through hydrogen bonding and hydrophobic interactions. Key amino acid residues such as SER, ASP, TYR, and ARG were involved in ligand binding, indicating their critical role in maintaining enzyme activity (Tusun et al., 2017). Ligands containing functional groups such as hydroxyl (–OH) and carbonyl (C=O) exhibited stronger interactions due to their ability to form hydrogen bonds with active site residues.

Table 6. Protein–ligand interaction profile of selected compounds with Juvenile Hormone Epoxide Hydrolase (JHEH)

S.No	Ligand	Residues Involved	H-bonds (No.)	Other Interactions
1	2-Hydroxycyclopenten-1-one	SER234, ASP227, GLU180	2	Electrostatic, van der Waals
2	2,3-Dihydrobenzofuran	TYR88, PHE221, LEU145	0	π–π stacking, Hydrophobic, van der Waals
3	Hydroxychavicol	SER234, ARG215, TYR88	2	π–interaction, π–cation
4	Benzoic acid	TYR88, ASP227, LYS73	2	π–anion, Electrostatic
5	Phytol	LEU145, VAL150, ILE152	0	Alkyl, Hydrophobic, van der Waals
6	Caryophyllene	ILE152, PHE221, LEU145	0	π–alkyl, Hydrophobic, van der Waals
7	4-Hydroxycyclohexanone	ASP227, GLU180, SER234	2	Electrostatic, van der Waals
8	Menthone	SER234, TYR88, LEU145	1	Hydrophobic, van der Waals

*H-bonds: number of hydrogen bonds formed between ligand and protein residues.

3.7 Drug-likeness and ADME Analysis

The drug-likeness properties of the selected ligands were evaluated using SwissADME (Daina et al., 2017) based on Lipinski's Rule of Five (Table 7). The parameters analyzed included molecular weight, lipophilicity (LogP), and hydrogen-bond donors and acceptors. Most of the selected ligands complied with Lipinski's criteria, with molecular weights below 500 Da and acceptable hydrogen-bonding capacity. Overall, the results indicate that the selected phytochemicals possess favourable physicochemical properties and good drug-like characteristics, supporting their potential biological activity (Lipinski et al., 2001).

Table 7. Drug-likeness and ADME properties of selected ligands

S.No	Ligand	MW (g/mol)	LogP	HBD	HBA	Viol.	QED
1	2-Hydroxycyclopenten-1-one	98.10	-0.2	1	2	0	0.45

2	2,3-Dihydrobenzofuran	120.15	2.1	0	1	0	0.62
3	Hydroxychavicol	150.17	2.3	2	2	0	0.68
4	Benzoic acid	122.12	1.9	1	2	0	0.70
5	Phytol	296.54	7.8	1	1	1	0.30
6	Caryophyllene	204.36	4.4	0	0	0	0.50
7	4-Hydroxycyclohexanone	114.14	0.3	1	2	0	0.55
8	Menthone	154.25	2.3	0	1	0	0.64

*MW: Molecular weight (g/mol); LogP: lipophilicity; HBD: hydrogen bond donors; HBA: hydrogen bond acceptors; Viol.: Lipinski violations.

3.8 Integrated Discussion

The integration of bioassay, GC–MS, and molecular docking results provides a comprehensive understanding of the insecticidal potential of the plant extracts studied. The bioassay results demonstrated significant larval mortality, while GC–MS analysis identified key bioactive compounds responsible for this activity. Molecular docking further confirmed that these compounds exhibit strong binding affinity toward JHEH, suggesting a mechanism of action through enzyme inhibition. The correlation between higher larval mortality and stronger ligand–protein interaction indicates that disruption of juvenile hormone regulation plays a crucial role in insect control. The presence of multiple phytochemicals in plant extracts suggests a synergistic mode of action, enhancing insecticidal efficiency and reducing the likelihood of resistance development (Benelli et al., 2023; Raveau et al., 2022). Overall, the study establishes a clear linkage between chemical composition, biological activity, and molecular interactions, supporting the use of plant-derived compounds as sustainable pest management agents (Benelli, 2024; Pavela & Benelli, 2018).

4. CONCLUSION

The present study demonstrated the effectiveness of integrating bioassay, GC–MS analysis, and molecular docking in evaluating the insecticidal potential of selected plant extracts against Diaphania pulverulentalis. The bioassay results clearly indicated a dose- and time-dependent increase in larval mortality, with certain plant extracts exhibiting promising activity comparable to the standard insecticide, Dichlorvos. GC–MS analysis revealed the presence of diverse bioactive compounds, particularly ketones, terpenoids, and phenolics, known for their insecticidal properties. Molecular docking analysis further supported these findings by demonstrating strong binding affinities of the selected ligands for Juvenile Hormone Epoxide Hydrolase (JHEH), a key enzyme involved in insect growth and development. The interaction analysis confirmed the involvement of important amino acid residues and suggested that these compounds may disrupt normal hormonal regulation, leading to impaired development and mortality in the target insect. Overall, the study establishes a clear relationship between phytochemical composition, biological efficacy, and molecular interaction, highlighting the potential of plant-derived compounds as eco-friendly alternatives to synthetic insecticides.

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Figures

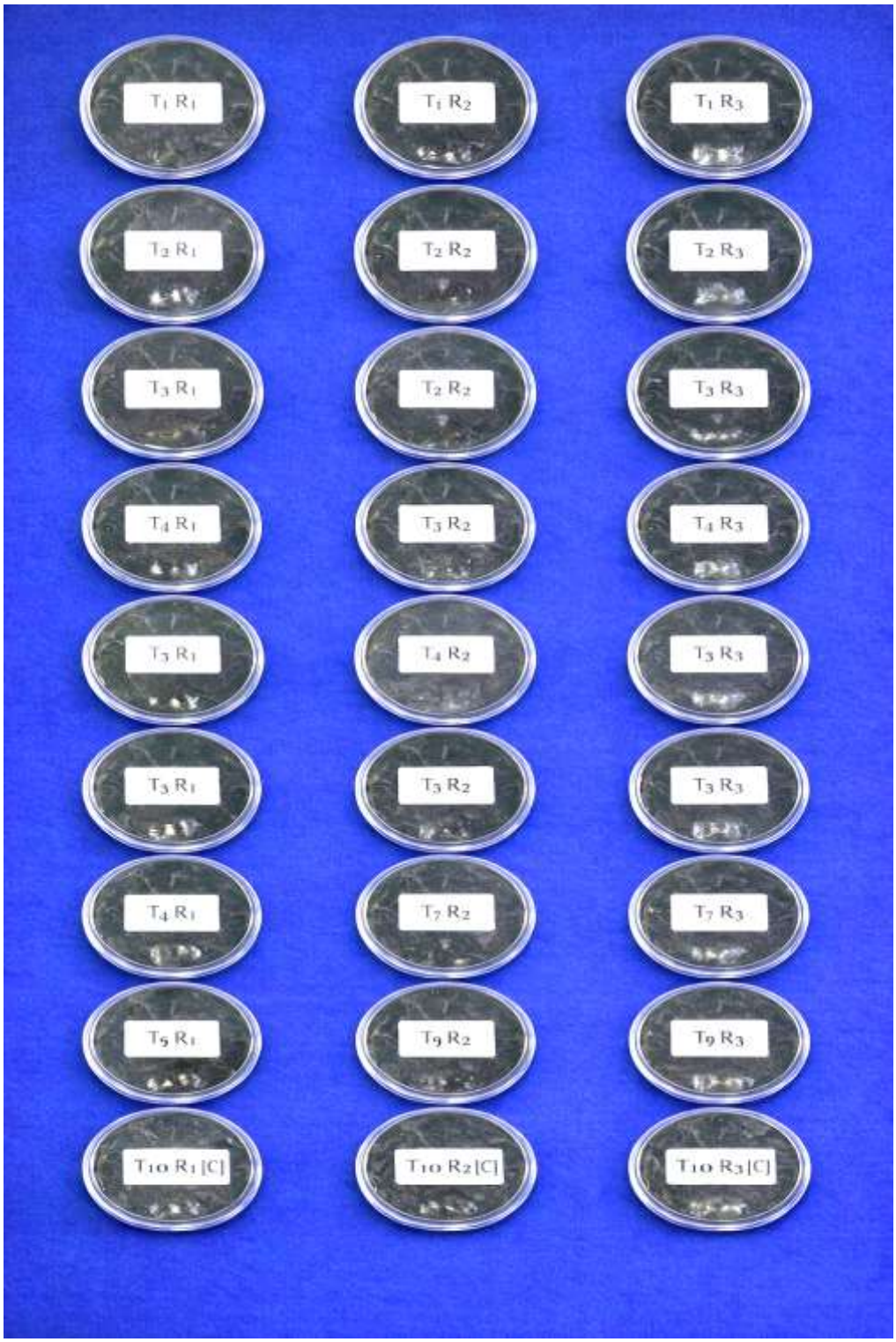

Figure 1. Bioassay leaf-dip plates arranged by treatment and replication.

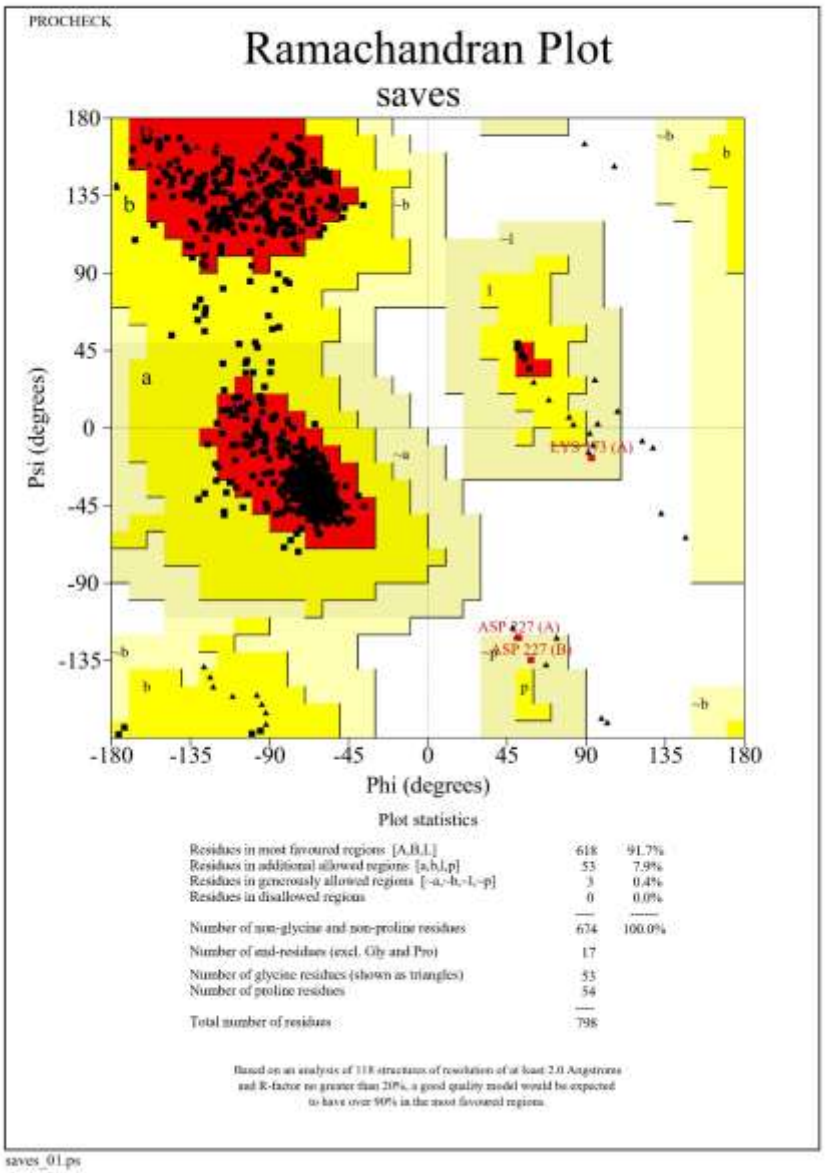

Figure 2. Ramachandran plot used for validation of the modeled target protein structure.

Plate 1. Molecular interaction images of selected ligands with JHEH.

Plate 1a. 2D image of 2-Hydroxy-2-cyclopenten-1-one

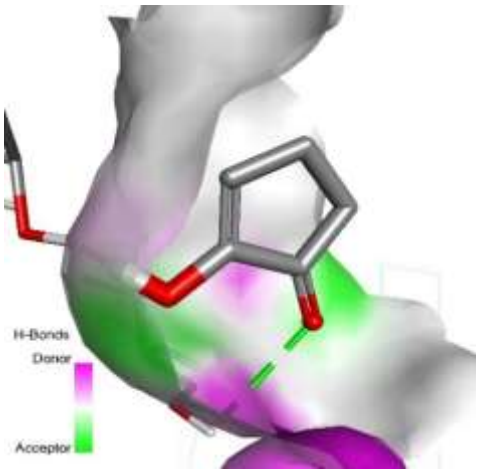

Plate 1b. 3D image of 2-Hydroxy-2-cyclopenten-1-one

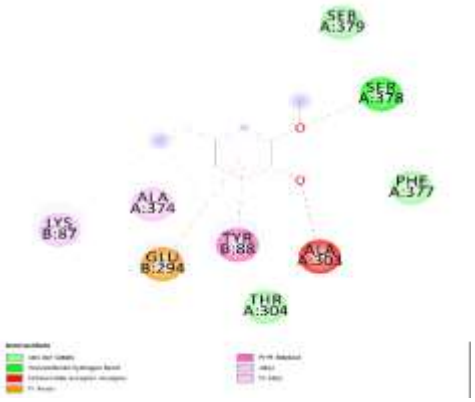

Plate 1c. 2D image of 2,3-Dihydrobenzofuran

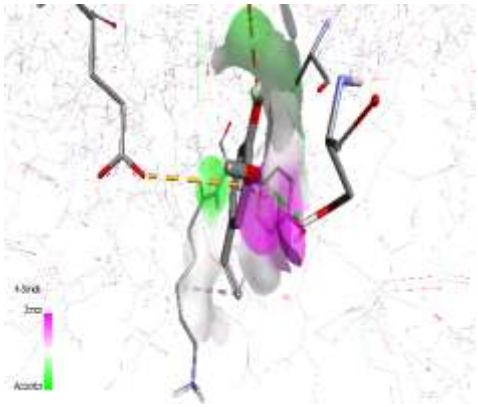

Plate 1d. 3D image of 2,3-Dihydrobenzofuran

Plate 1e. 2D image of Hydroxychavicol

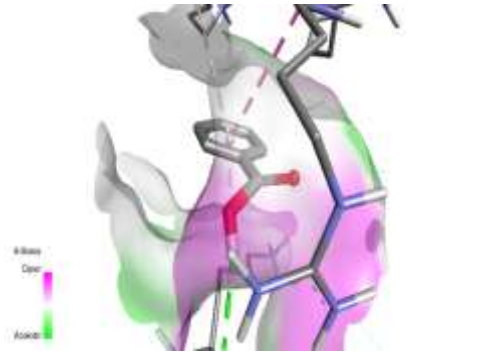

Plate 1f. 3D image of Hydroxychavicol

Note: Each plate contains the corresponding 2D interaction map on the left and the 3D docking pose on the right.

Plate 2. Molecular interaction images of Benzoic acid, Phytol, and 4-Hydroxycyclohexanone.

Plate 2a. 2D image of Benzoic acid

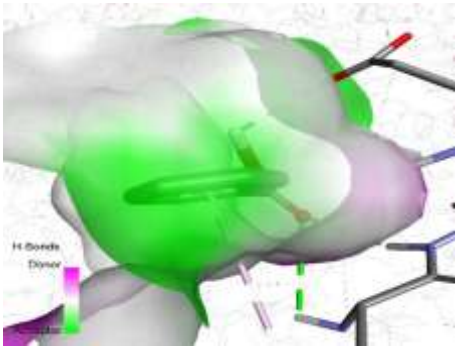

Plate 2b. 3D image of Benzoic acid

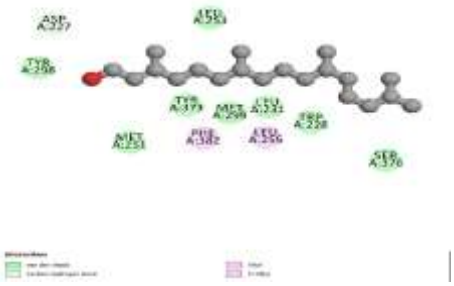

Plate 2c. 2D image of Phytol

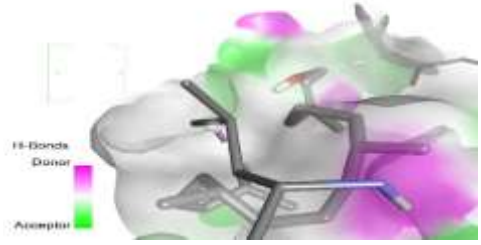

Plate 2d. 3D image of Phytol

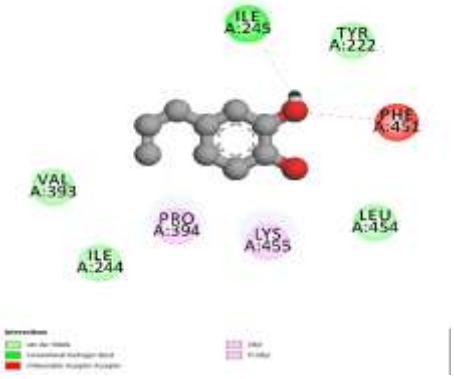

Plate 2e. 2D image of 4-Hydroxycyclohexanone

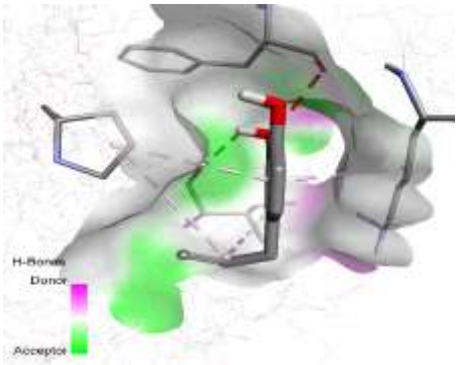

Plate 2f. 3D image of 4-Hydroxycyclohexanone

Note: Each plate contains the corresponding 2D interaction map on the left and the 3D docking pose on the right.

Plate 3. Molecular interaction images of Caryophyllene and Menthone.

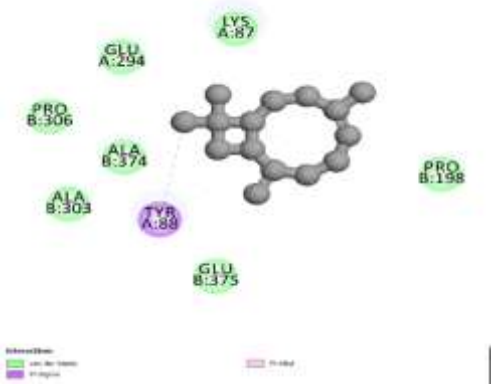

Plate 3a. 2D image of Caryophyllene

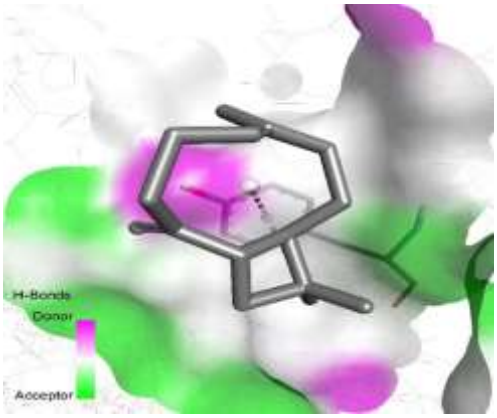

Plate 3b. 3D image of Caryophyllene

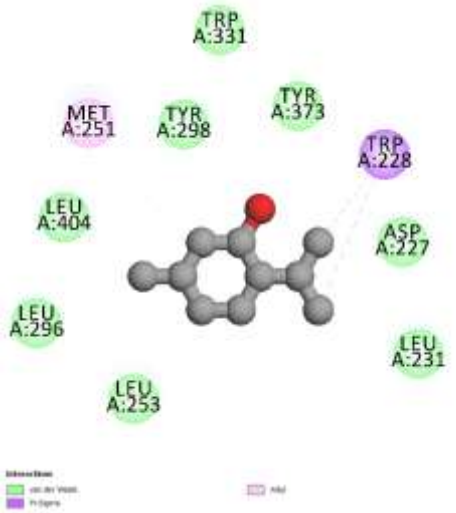

Plate 3c. 2D image of Menthone

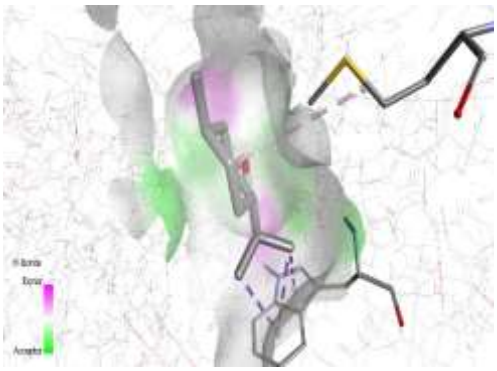

Plate 3d. 3D image of Menthone

Note: Each plate contains the corresponding 2D interaction map on the left and the 3D docking pose on the right.

Figure 3. Binding affinity values of selected compounds with JHEH.