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Evaluation of the Repellency and Larvicidal Efficiency of *Pulicaria undulata* Extracts against *Culex pipiens* (L.) Mosquito Larvae: Phytochemical Profile and In-Silico Studies

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ABSTRACT

Replacing synthetic insecticides with bioactive botanicals is essential for sustainable and eco-friendly mosquito control, addressing health risks from mosquito-borne diseases. This study evaluates the larvicidal, repellent, and biochemical effects of *Pulicaria undulata* extracts (water, ethanol, and petroleum ether) against *Culex pipiens*. The extracts were tested following WHO protocols, showing time- and concentration-dependent larvicidal activity. The petroleum ether extract exhibited the highest potency, with LC_{50} values of 221.60, 157.99, and 103.98 ppm at 24, 48, and 72 hours, respectively. Additionally, it demonstrated superior antifeedant and repellency effects, achieving 82% repellency at a concentration of 3.33 mg/cm². Biochemical analysis of larvae treated with LC_{50} doses of each extract revealed significant enzyme activity changes. Acetylcholinesterase, acid phosphatase, and alkaline phosphatase levels were reduced, with the petroleum ether extract showing the greatest effect. Glutathione S-transferase activity increased across all treatments, while protein, carbohydrate, and lipid contents declined, most prominently in larvae treated with petroleum ether extract. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed a distinct chemical profile for each extract. The water extract was rich in monoterpenes and sesquiterpenes, with linalool being the major compound. Ethanol extracts contained fatty acid esters and flavonoids, dominated by hexadecanoic acid. Petroleum ether extracts were abundant in fatty acid esters and phenylpropanoids, with eugenol as the principal component. Molecular docking studies of the major compounds in the petroleum ether extract revealed strong binding for Oleic acid and Eugenol to AChE and GST, supporting its role in the observed bioactivities. These findings highlight the potential of *Pulicaria undulata* extracts, particularly petroleum ether extract, as eco-friendly alternatives for sustainable mosquito control.

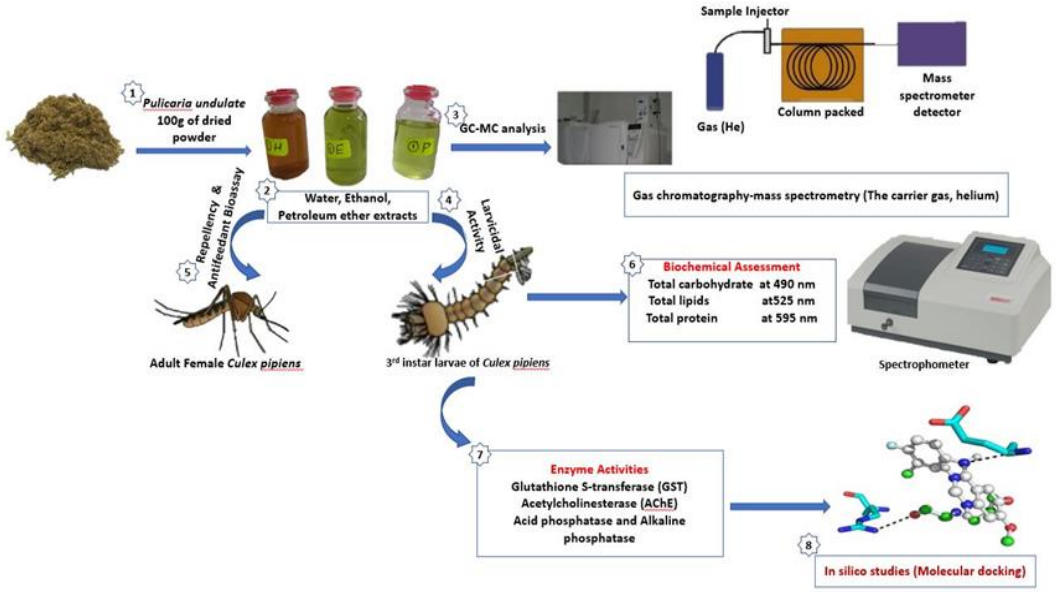

Fig. 1. A schematic illustration outlining the serial designing for the evaluation of the repellency and larvicidal effectiveness of *Pulicaria undulata* extracts (Water, ethanol, petroleum ether) against *Culex pipiens* mosquito, demonstrating the experimental arrangement of Phytochemical Profile and In-Silico Studies. The graphical investigation was planned according to one of the methodologies mentioned by **Jambor and Bornha (2024).**

INTRODUCTION

Global pandemic awareness is at risk due to the persistent threats posed by mosquito-borne diseases. The quick spread of viruses like dengue and chikungunya, combined with the disruption of mosquito control efforts following the onset of COVID-19 in 2019, global warming, and environmental changes caused by human activities, all contribute to the increasing risk of mosquito-borne virus spread (**Jing *et al*. 2024).** Also,global urbanization and population growth in the past few decades have significantly intensified the global challenge of mosquito-borne diseases. Vector-borne diseases account for over 17% of all transmitted diseases contributing to a high mortality rate annually, as reported in the World Health Organization's (WHO) most recent report **(World Malaria Report 2022).** These illnesses represent a long-standing public health concern as they threaten more than half of the world's population (**Engdahl *et al*. 2022**). Consequently, numerous front-line mosquito control strategies are universally implemented to resolve this issue.

Due to their high adaptability, relying on single mosquito control strategies is insufficient, increasing the prevalence of mosquito-borne diseases such as dengue fever, yellow fever, and malaria (**Mafra-Neto and Dekker 2019**). As a result, in 2004 the WHO introduced the concept of integrated strategies targeting vector control (**WHO 2021**). Mosquito-targeted procedures include the use of biopesticides and insect sterility

techniques (**Zheng *et al*. 2019; Isman 2020**). These biocontrol approaches offer an ecologically viable solution for mosquito management while safeguarding non-target valuable insect species.

Repellency plays a crucial role in preventing vector-borne diseases by minimizing human-vector interactions. However, the use of artificial chemicals and insecticides for vector control has raised significant environmental concerns, as many are non-biodegradable and cause irreversible damage to ecosystems. Additionally, synthetic repellents may lead to adverse effects, including skin irritation and dermal reactions **(Das et al., 2000)**. Studies have also highlighted that these chemical repellents may pose safety risks for public use **(Zadikoff, 1979; Ronald et al., 1985)**. Their unpleasant odor, greasy texture, potential toxicity **(Gryboski *et al*. 1961; Robbins and Cherniack 1986; Lipscomb *et al*. 1992),** and discomfort to some individuals **(Smith 1970; Skinner and Johnson 1980)** further limit their application. Medicinal plants represent a valuable source of natural compounds, offering promising alternatives to traditional mosquito repellents. Their diverse chemical constituents and repellent activity provide opportunities for developing ecological and sustainable alternatives to chemical insecticides **(Sharma *et al*. 1993; Sharma and Ansari 1994)**.

The use of botanical extracts is considered a sustainable and long-term solution to the problem of mosquito-borne diseases. These extracts contain a wide variety of chemicals that can target different mosquito life stages from larvae to adults. Various plant extracts have been proven to be effective in both killing and repelling mosquitoes. The successful integration of plant-based mosquito control methods into public health programs requires further research, formulation standardization, and active community involvement (**Tanmay *et al*. 2024)**. Phytochemicals extracted from plant sources have demonstrated various bioactivities, including insecticides, insect growth regulators, and repellent effects (**Babu and Murugan 1998; Venkatachalam and Jebanesan 2001 a, b**).

The genus *Pulicaria* (Asteraceae), comprising around 80 species, is widely dispersed across various regions, including North Africa, Europe, and Asia, with a significant presence in the Mediterranean basin **(Williams *et al*., 2003)**. *Pulicaria undulata* (L.) C.A. Mey. is a flowering plant, either annual or sometimes perennial, characterized by its small size, and yellowish blossoms. It is traditionally used as an herbal medicine in numerous countries, including Egypt, Kuwait, Iraq, Iran, Saudi Arabia, Afghanistan, India, Pakistan, and parts of West and North Tropical Africa (**Al-Rawi 1987; Boulos 2002**). Remarkably, the plant is valued for its antioxidant, antimicrobial, and anti-inflammatory properties in Saudi Arabia **(Mohammed *et al*., 2021)**. *Pulicaria undulata* (L.) C.A. Mey., one of the greatest prevalent desert plants in Egypt, is commonly known as “Dethdath”. Its flowering branches are traditionally used

to create a potent sneezing powder and also serve as an effective insect repellent **(Boulos, 2002)**.

In the present study, we aim to investigate a novel approach using plant extracts, which have proved efficient in repelling and killing disease vector mosquitoes. The study focuses on identifying the phytochemical profile of different extracts (water, ethanol, petroleum ether) of *Pulicaria undulata* and evaluating their larvicidal potential and repellency against *Culex pipiens* mosquito referring to the biochemical and enzymatic alternations induced by these extracts to explore alternative and sustainable methods for mosquito control.

MATERIALS AND METHODS

Plant collection, Identification, and Extraction

Collection and classification of the plant material were conducted during the blooming season, with samples of *Pulicaria undulata* gathered from the desert Wadi Hagol environment in the governorate of Egypt. The Department of Botany, Faculty of Science, Ain Shams University, verified the plant's identity. After cleaning and allowing the plant material to air dry at normal temperature in the shade, the flower heads and aerial components (leaves and stems) were ground into a powder using mechanical mills. Distilled water, ethyl alcohol, or petroleum ether were used to extract 100g of dried powder at 4°C. The extracts were filtered, and after 72 hours, the filtrates were concentrated at 30°C in a rotary evaporator under reduced pressure. To remove color from the crude concentrated extracts, active charcoal was used, followed by the addition of water to the final volume for phytochemical investigation (**Harborne, 1973**).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the extracts was analyzed using a Trace GC-TSQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TR–5MS (30 m × 0.25 µm × 0.25 µm film thickness) at the Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt. The column oven temperature was initially adjusted at 60°C, then raised by 5°C per minute to 200°C with a 2-minute holding period, followed by further increase to 300°C (10 °C per minute). The temperature of the injector was retained at 270°C. Helium was used as the carrier gas and was kept at a steady flow rate of 1 mL/min. Diluted samples of 1 µL were automatically injected using the Auto-sampler AS3000 combined with GC in split mode after a 4-minute solvent delay. EI mass spectra were obtained in full-scan mode at an ionization voltage of 70 eV, covering the m/z range of 50–750. The ion source temperature was set at 200°C, and the transfer line temperature was set at 250°C. The components were categorized by comparing their mass spectra and retention times with those in the WILEY 09 and NIST 14 mass spectral databases.

Insect Maintenance

A susceptible mosquito colony was established at the insectary of the Entomology Department, Faculty of Science, Ain Shams University, under laboratory conditions with a photoperiod of 12:12 hours (L/D cycle), a temperature of 27±2°C, and 75±5% relative humidity, following the method of **Kasap and Demirhan (1992)**. Newly hatched larvae were fed Tetramin, and upon reaching the pupal stage, they were transferred to screened wooden cages (25 by 25 by 25 cm). Adult mosquitoes were provided with 10% sucrose solution daily, and pigeons were used as the source of blood meal for female mosquitoes.

Larvicidal bioassay

The larvicidal activity of *Pulicaria undulata* was evaluated using the WHO immersion procedure (**WHO 2005).** Different extracts (water, ethanol, and petroleum ether) were applied to the 3rd instar of *Cx. pipiens* larvae at five different concentrations for each extract; (50,100, 150, 200, 250, and 300 ppm) for petroleum ether, (150, 200, 250, 300, 350, and 400 ppm) for ethanol extract, (350, 550, 750, 1000, 1250, and 1450 ppm) for water extract. Each concentration was diluted with distilled water to a final volume of 100 ml, with only distilled water used as a control. Twenty larvae were used for each extract and the control group, with three replications of each treatment and control. Mortality was recorded 24-, 48-, and 72-hours post-treatment. LC_{25}, LC_{50}, and LC_{90} values were calculated using LDP line software at a 95% confidence level (**Finney 1971**). Abbott's formula (**Abbott 1987**) was used to adjust for control mortality.

Biological activities

After treating the 3rd instar *Cx. pipiens* larvae with various concentrations of each extract, the larvae were observed daily till pupation and adult emergence. The mean larval period, pupal duration, and adult emergence were recorded for each concentration of each extract, as well as for the untreated control group. Laboratory-controlled conditions were maintained during the experiments.

Repellency and Antifeedant bioassay

To evaluate the repellent properties of the extracts, standard cages measuring 20 x 20 x 20 cm were utilized. Different concentrations were set by dissolving varying quantities of each extract in 2 milliliters of distilled water with the addition of a drop of Triton ×100. After removing the feathers from the abdomen, the concentration was immediately applied to 5 x 6 cm of the pigeon's ventral region. Pigeons were treated for 10 minutes before being put in cages with the unfed female *Cx. pipiens* lab strain. Water was used as a control, while, commercial repellent 15 % DEET (N, N-diethyl-meta-toluamide) (C12H17NO) (Johnson Wax Egypt) was used as a positive control. To determine the average repellent activity, each test was conducted three times. Following

treatment, the number of fed and unfed females were counted, and the Abbott formula was used to statistically record the repellency percentage, calculated as follows (**Abbott 1987**):

$$\text{Repellency\%} = (\%Y - \%Z / 100 - Z\%) \times 100$$

Where Y represents the proportion of treated unfed females, while Z represents the % unfed females % in the control group.

Biochemical Assessment.

The whole *Cx. pipiens* 3rd instar larvae, 48-hour post-treatment with LC_{50} of each extract, were homogenized in phosphate buffer saline (50 mg/1 ml). In a chilled centrifuge, homogenates were centrifugated for 15 minutes at 5°C at 8000 r.p.m. The supernatants were stored in a deep freezer at 20°C until they were used for biochemical analyses and enzyme activities. Total carbohydrate content was calculated following the methods of **Aly *et al.* (2023**). Briefly, 100 µL of the sample was mixed with 200 µL of sulfuric acid and 100 µL of phenol (5 g/100 mL), the absorbance at 490 nm was measured after 30 minutes of incubation. Total lipid content was estimated using the 20% phospho-vanillin reagent, according to **Knight *et al.* (1972**). A test tube containing 250 µL of the sample and 5 mL of sulfuric acid was heated in a boiling water bath for 10 minutes. The sample was placed into the phospho-vanillin reagent (6 mL) once it had cooled to room temperature. After forty-five minutes, the color's absorbance at 525 nm was measured. Total protein content was determined by mixing 100 µL of the sample with 5 mL of Bradford reagent, a solution of Coomassie Brilliant Blue diluted in 95% ethanol. The absorbance was then measured at 595 nm. **(Bradford 1976)**. The biochemical results were analyzed using one-way analysis of variance (ANOVA with a p-value of less than 0.05 considered statistically significant.

Enzyme Activities

The activity of Glutathione S-transferase (GST) was assessed using the procedure of **Kao *et al.* (2016**). 1-Chloro-2,4-dinitrobenzene (CDNB) was tested as a substrate in the toxicity studies for measuring the enzyme activity of GST via the –SH group of glutathione. Acetylcholine bromide (AChBr) and methyl butyrate (MeB) were used as substrates to assess the activity of acetylcholinesterase (AChE), respectively according to **Simpson (1964)**. For the estimation of the levels of acid phosphatase and alkaline phosphatase, the methodology of **Powell and Smith (1954)** was applied using phenyl phosphate as a substrate, the reaction mixture included 0.1 ml of sample, 1 ml of 0.01 M disodium and 1 ml of carbonate buffer (pH 10.4) was used for alkaline phosphatase, while 1 ml of citric buffer (pH 4.9) was used for acid phosphatase. The biological data means were compared using SPSS statistics (Version 26 for Windows) and one-way

analysis of variance (ANOVA). A significant difference in the data was indicated by a $P < 0.05$.

In silico studies (Molecular docking)

The proteins AChE (PDB ID: 6XYU) and GST (PDB ID: 1M0U) have their 3D crystalline structures available for download from the protein data bank website (www.rcsb.org). The major compounds in the petroleum ether extract docked at the GST and AChE receptors' active pockets (**Nachon *et al*., 2020**). Docking calculations will be performed using Discovery Studio 2.5.5, and the results were displayed using Discovery Studio Visualizer 2016.

RESULTS

Gas chromatography-mass spectrometry

GC-MS analysis was performed to analyze the biological active constituents in the different extracts of *Pulicaria undulata*. Results revealed the identification of 16, 16, and 24 chemical compounds in the water, ethanol, and petroleum ether extracts of *Pulicaria undulata*, respectively. Various chemical compounds with varying concentrations were obtained from the tested extracts. The most prominent chemical compounds in the water extracts are linalool (47.60%) followed by cis-α-Bergamotene (9.59%), tau-Cadinol (6.88%), Germacrene D (6.28%), γ-Muurolene (4.81). Most of the compounds were Terpenes, specifically sesquiterpenes, and a few fatty acids as represented in Table 1. In the ethanolic extract, most compounds were fatty acid esters and a few phenylpropanoids, terpenes, and a flavonoid (Table 2). The highly prominent conistituent in the ethanolic extract was Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (69.1%), then Glycidyl oleate (4.83%,), 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-(4.7%), Ethyl iso-allocholate (1.12%) and (6-Hydroxymethyl-2,3-dimethylphenyl) methanol (1.05%). For the petroleum ether extracts several fatty acid esters were detected besides some terpenes and a few phenylpropanoids. Eugenol is the most prominent compound in the petroleum ether extract and accounted for (24.85%), followed by Oleic Acid (6.59%), 1,2 Benzenedicarboxylic acid, butyloctyl ester (6.54%), 13-Heptadecyn-1-ol (5.5%) (Table 3).

Table 1: The chemical composition of *Pulicaria undulata* water extracts by GC-MS

Peak	R.t*	Name	Area %	M.Wt*	Molecular formula	Chemical class
1	5.40	Eucalyptol	3.79	154	$C_{10}H_{18}O$	monoterpenes
2	6.93	Linalool	47.60	154	$C_{10}H_{18}O$	monoterpenes
3	11.54	α-Fenchyl acetate	2.08	196	$C_{12}H_{20}O_2$	monoterpenes
4	13.45	Phenol, 2-methoxy-5-(1propenyl)-, (E)-	2.72	164	$C_{10}H_{12}O_2$	Phenylpropanoid
5	14.24	β-Elemene	3.85	204	$C_{15}H_{24}$	Sesquiterpene
6	15.32	cis-α-Bergamotene	9.59	204	$C_{15}H_{24}$	Sesquiterpene
7	15.84	a-Humulene	1.47	204	C15H24	Sesquiterpene
8	16.47	Germacrene D	6.38	204	$C_{15}H_{24}$	Sesquiterpene
9	16.82	γ-Elemene	1.28	204	$C_{15}H_{24}$	Sesquiterpene
10	16.98	α-Guaiene	2.32	204	$C_{15}H_{24}$	Sesquiterpene
11	17.25	γ-Muurolene	4.81	204	$C_{15}H_{24}$	Sesquiterpene
12	18.75	(-)-Spathulenol	1.74	220	$C_{15}H_{24}O$	Sesquiterpene alcohol
13	19.63	Epi-cubenol	1.52	222	$C_{15}H_{26}O$	Sesquiterpene alcohol
14	20.24	tau-Cadinol	6.88	222	$C_{15}H_{26}O$	Sesquiterpene alcohol
15	29.53	10-Octadecenoic acid, methyl ester	1.93	296	$C_{19}H_{36}O_2$	Fatty acid ester
16	36.68	Cyclopropanedecanoic acid, α-(acetoxy)-2-hexyl-, methyl ester	2.02	368	$C_{15}H_{28}O_2$	Fatty acid ester

*RT: retention time *Molecular Weight

Table 2: The chemical composition of *Pulicaria undulata* ethanol extracts by GC-MS

Peak	R.t*	Name	Area %	M.Wt*	Molecular formula	Chemical class
1	9.64	(6-Hydroxymethyl-2,3-dimethylphenyl) methanol	1.05	166	$C_{10}H_{14}O_2$	Phenylpropanoid
2	14.63	Benzene,1,2-dimethoxy-4-(1-propenyl)-	0.56	178	$C_{11}H_{14}O_2$	Phenylpropanoid
3	14.98	Caryophyllene	0.76	204	$C_{15}H_{24}$	Sesquiterpene
4	24.44	7-Methyl-Z,Z-8,10-hexadecadien-1-ol acetate	0.39	294	$C_{19}H_{34}O_2$	Fatty acid ester
5	26.27	Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis-	0.89	312	$C_{19}H_{36}O_3$	Fatty acid ester
6	28.69	9,12-Octadecadienoic acid (Z,Z)-,TMS derivative	2.72	352	$C_{21}H_{40}O_2Si$	Fatty acid
7	29.53	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.77	356	$C_{21}H_{40}O_4$	Fatty acid ester
8	30.73	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1[(trimethylsilyl)oxy] methyl ester, (Z,Z,Z)	0.95	496	$C_{27}H_{52}O_4Si_2$	Fatty acid
9	30.84	7,10,13-Eicosatrienoic acid, methyl ester	0.43	320	$C_{21}H_{36}O_2$	Fatty acid ester
10	31.60	Hexadecadienoic acid, methyl ester	0.68	266	$C_{17}H_{30}O_2$	Fatty acid ester
11	35.14	Linoleic acid ethyl ester	0.84	308	$C_{20}H_{36}O_2$	Fatty acid ester
12	39.35	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	4.70	344	$C_{18}H_{16}O_7$	Flavonoid
13	39.50	Palmitic acid, 2-(tetradecyloxy)ethyl ester	0.95	496	$C_{32}H_{64}O_3$	Fatty acid ester
14	41.05	Ethyl iso-allocholate	1.12	436	$C_{26}H_{44}O_5$	Steroid derivative
15	41.27	Glycidyl oleate	4.83	338	$C_{21}H_{38}O_3$	Fatty acid ester
16	42.52	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	69.10	568	$C_{35}H_{68}O_5$	Fatty acid ester

*RT: retention time *Molecular Weight

Table 3: The chemical composition of *Pulicaria undulata* Petroleum ether extracts by GC-MS

Peak	R.t*	Name	Area %	M.Wt*	Molecular formula	Chemical class
1	13.14	Eugenol	24.85	164	$C_{10}H_{12}O_2$	Phenylpropanoid
2	14.98	10-Heptadecen-8-ynoic acid, methylester, (E)-	2.71	278	$C_{18}H_{30}O_2$	Fatty acid ester
3	16.41	α-Longipinene	3.72	204	$C_{15}H_{24}$	Sesquiterpene
4	17.35	Phenol, 2-methoxy-5-(1-propenyl)-,(E)-	2.01	164	$C_{10}H_{12}O_2$	Phenylpropanoid
5	17.57	2,15-Heptadecadiene, 9-(ethoxymethyl)-	2.25	294	$C_{20}H_{38}O$	Hydrocarbon derivative
6	18.75	Tricyclo[5.2.2.0(1,6)]undecan-3-ol,2-methylene-6,8,8-trimethyl-	1.16	220	$C_{15}H_{24}O$	Terpene derivative
7	20.82	Patchouli alcohol	1.24	222	$C_{15}H_{26}O$	Sesquiterpene alcohol
8	25.54	13-Heptadecyn-1-ol	5.50	252	$C_{17}H_{32}O$	Fatty alcohol
9	25.73	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1.97	276	$C_{17}H_{24}O_3$	Organic peroxide derivative
10	26.26	Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis-	3.14	312	$C_{19}H_{36}O_3$	Fatty acid ester
11	26.74	1,2-Benzenedicarboxylic acid, butyloctyl ester	6.54	334	$C_{20}H_{30}O_4$	Phthalate ester
12	28.69	α-D-Glucofuranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,5-bis(butylboronate)	4.12	384	$C_{17}H_{34}B_2O_6Si$	Sugar derivative
13	29.44	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	2.44	268	$C_{16}H_{28}O_3$	Epoxide ester
14	29.53	Oleic Acid	6.59	282	$C_{18}H_{34}O_2$	Fatty acid
15	35.84	Glycidyl oleate	1.59	338	$C_{21}H_{38}O_3$	Fatty acid ester
16	37.20	Ethyl iso-allocholate	4.30	436	$C_{26}H_{44}O_5$	Steroid derivative
17	37.62	2HNAPHTHO [2',1':4,5] INDENO[1,2-D][1,3]DIOXOL-ONE,4A,4B,5,6,6A,6B,9A,10,10A,10B,11,12-DODECAHYDRO-5-HYDROXY-6B-(HYDROXYACETYL)-4A,6ADIMETHYL-8-PROPYL-,(4AR,4BS,5S,6AS,6BS,9AR,10AS,10BS)-	1.75	430	$C_{25}H_{34}O_6$	Fatty acid
18	39.77	9,12-OCTADECADIENOIC ACID(Z,Z)-,2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER	2.10	498	$C_{27}H_{54}O_4Si_2$	Fatty acid ester
19	39.96	9,12,15OCTADECATRIENOICACID,2,3BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER, (Z,Z,Z)-	1.35	496	$C_{27}H_{52}O_4Si_2$	Fatty acid ester
20	41.41	Oleic acid, 3-(octadecyloxy)propyl ester	4.40	592	$C_{39}H_{76}O_3$	Fatty acid ester
21	43.31	Trilinolein	1.38	878	$C_{57}H_{98}O_6$	Fatty acid triester
22	43.65	1,25-Dihydroxyvitamin D3, TMS derivative	5.32	488	$C_{30}H_{52}O_3Si$	Vitamin D derivative
23	44.14	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	5.09	884	$C_{57}H_{10}4O_6$	Fatty acid triester
24	45.24	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	2.80	600	$C_{42}H_{64}O_2$	Carotenoid (terpene)

*RT: retention time
*Molecular Weight

Larvicidal bioassay

The larvicidal activity of *Pulicaria undulata* (Asteraceae) extracted from the aerial parts (leaves and stem) and flower heads was tested against the 3rd instar larvae of *Culex pipiens* at 24, 48, and 72 hours post-treatment, with the data presented in Table 4. The mortality level of the larvae augmented with both the exposure time and the concentration of the extracts. The results showed that *Pulicaria undulata* extracts in petroleum ether, ethanol, and distilled water caused varying levels of larval mortality, with median lethal concentration values (LC_{50}) of 221.60, 379.55, and 1096.66 ppm at 24 hours; 157.99, 256.54, and 699.54 ppm at 48 hours; and 103.98, 196.13, and 432.31 ppm at 72 hours, respectively. Among the extracts, petroleum ether was the most effective (Table 4).

Table 4: Susceptibility of the third instar larvae of *Culex pipiens* to three different extracts of *Pulicaria undulata* at different time intervals (24, 48, and 72 hrs post-treatment), coefficient limits, (χ2) Chi-square value and slope of the concentration-inhibition regression line ± standard error.

Extract ppm	Petroleum Ether Extract			Ethanol Extract			Water Extract		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
LC25 (ppm) (co. limit)	125.54 (109.9-139.4)	88.57 (75.2-100.4)	60.05 (49.5-69.6)	281.07 (265.0-296.4)	191.79 (177.1-204.3)	139.09 (120.7-154.1)	675.20 (512.6-787. 6)	441.98 (383.9-492.6)	266.58 (211.9-314.4)
LC50 (ppm) (co. limit)	221.60 (201.2-248.3)	157.99 (143.6-173.6)	103.98 (92.7-114.8)	379.55 (356.2-412.7)	256.54 (244.1-269.1)	196.13 (181.1-209.3)	1096.66 (932.5-1400.5)	699.54 (644.2-754.5)	432.31 (376.5-481.2)
LC90 (ppm) (co. limit)	652.34 (519.2-907.8)	474.46 (395.2-610.2)	295.09 (257.8-351.5)	671.65 (581.3-831.8)	445.86 (409.9-498.2)	376.80 (344.5-425.7)	2756.11 (2312.6-5255.9)	1673.83 (1474.1-1981.9)	1083.35 (972.7-1245.4)
Slope±SE	2.73±0.27	2.68±0.24	2.82±0.23	5.17±0.53	5.33±0.43	4.51±0.43	3.20±0.30	3.38±0.28	3.21±0.29
χ2	9.49	9.22	9.48	9.24	4.75	6.92	9.69	9.10	3.96

Biological activities

Data from Tables (5, 6, and 7) highlight the latent effects of *Pulicaria undulata* extracted in three different solvents on various biological aspects of *Cx. pipiens* larvae. The growth index, adult emergence, pupation rates, and developmental durations were calculated for each concentration in the three separate extracts. The petroleum ether extract of *Pulicaria undulata* significantly prolonged both the larval and pupal developmental times. At concentrations of 250 and 300 ppm, the larval duration increased to 4.7 and 4.8 days, respectively, compared to 4.3 days in the control group (Table 5). The pupal duration was extended to 2.7 days at 300 ppm, compared to 1.9 days in the control group. The ethanolic and water extracts had no impact on larval duration at lower concentrations. However, at the highest concentrations (400 and 1450 ppm), the duration was significantly prolonged to 4.6 and 4.9 days, respectively, compared to 4.1

and 4.3 days for the control group (Tables 6 and 7). Regarding the number of emerging adults after larval treatment at different concentrations of the three extracts, there were no significant effects on adult emergence, except for the distilled water extract, which had a 69% emergence rate at a concentration of 1450 ppm.

Table 5: Biological alterations in *Culex pipiens* after Petroleum Ether extract treatment of *Pulicaria undulata*

Conc. (ppm)	Mean larval period (days) ±SD	Mean pupal duration (days) ±SD	Adult emergence (%)(A)	Developmental duration (B) (days)±SD	Growth index*
untreated	4.25±0.008[a]	1.87±0.11[a]	100±0.00	6.11±0.33[a]	16.36±0.33[a]
50	4.40±0.31[b]	2.14±0.33[b]	100±0.00	6.54±0.07[b]	15.29±0.52[b]
100	4.50±0.33[b]	2.29±0.06[b]	100±0.00	6.79±0.08[b]	14.72±0.15[b]
150	4.58±0.04[b]	2.47±0.08[b]	100±0.00	7.05±0.08[b]	14.18±0.16[b]
200	4.67±0.31[b]	2.52±0.07[b]	100±0.00	7.19±0.08[b]	13.90±0.16[b]
250	4.73±0.15[b]	2.56±0.07[b]	100±0.00	7.29±0.08[b]	13.72±0.14[b]
300	4.78±0.05[b]	2.66±0.09[b]	100±0.00	7.44±0.07[b]	13.44±0.13[b]

Each value represents the mean of 3 replicates ± SD; Means with the same letters are not (significantly different).
*Growth index = A/B; A is the percentage of adult emergence and B is the developmental duration (days).

Table 6: Biological alterations in *Culex pipiens* after Ethanol extract treatment of *Pulicaria undulata*

conc. (ppm)	Mean larval period (days) ±SD	Mean pupal duration (days) ±SD	Adult emergence (%) (A)	Developmental duration (B) (days)±SD	Growth index*
untreated	4.10±0.13[a]	2.06±0.12[a]	100±0.00	6.17±0.33[a]	16.21±0.34[a]
150	4.25±0.04[b]	2.25±0.04[b]	100±0.00	6.50±0.08[b]	15.38±0.12[a]
200	4.36±0.02[b]	2.35±0.11[b]	100±0.00	6.71±0.08[b]	14.90±0.14[a]
250	4.42±0.02[b]	2.40±0.06[b]	100±0.00	6.82±0.05[b]	14.66±0.15[a]
300	4.51±0.03[b]	2.45±0.07[b]	100±0.00	6.95±0.06[b]	14.38±0.09[a]
350	4.58±0.02[b]	2.55±0.08[b]	81.90±3.44	7.12±0.03[b]	11.50±0.45[b]
400	4.65±0.03[b]	2.72±0.09[b]	77.78±20.41	7.30±0.04[b]	10.65±3.67[c]

Each value represents the mean of 3 replicates ± SD; Means with the same letters are not (significantly different).
*Growth index = A/B; A is the percentage of adult emergence and B is developmental duration (days).

Table 7: Biological alterations in *Culex pipiens* after distilled water extract treatment of *Pulicaria undulata*

conc. (ppm)	Mean larval period (days) ±SD	Mean pupal duration (days) ±SD	Adult emergence (%) (A)	Developmental duration (B) (days)±SD	Growth index*
untreated	4.35±0.33	2.08±0.34[a]	100±0.00	6.44±0.07[a]	15.52±0.13[a]
350	4.49±0.02	2.19±0.03[a]	100±0.00	6.68±0.33[b]	14.97±0.06[a]
550	4.62±0.03	2.23±0.02[b]	100±0.00	6.88±0.03[b]	14.53±0.03[b]
750	4.72±0.03	2.35±0.05[c]	100±0.00	7.06±0.02[b]	14.16±0.04[c]
1000	4.79±0.05	2.47±0.10[d]	100±0.00	7.26±0.03[b]	13.77±0.06[c]
1250	4.84±0.02	2.58±0.04[d]	82.22±2.41	7.41±0.03[b]	11.09±0.31[c]
1450	4.96±0.03	2.71±0.06[d]	69.45±4.76	7.67±0.07[b]	9.05±0.42[c]

Each value represents the mean of 3 replicates ± SD; Means with the same letters are not (significantly different).
*Growth index = A/B; A is the percentage of adult emergence and B is the developmental duration (days).

Repellency/antifeedant action against female *Culex pipiens*

Overall, the repellency and antifeedant efficacy of the three tested extracts of *Pulicaria undulata* and DEET demonstrate a variable degree of repellency (Table 8). Potent repellency (100%) was obtained by DEET at a dose (1.8 mg/cm^2). From the three tested extracts, petroleum ether extract exhibited the highest repellency percentage of 81.98 % at a dose 3.33 mg/ cm^2, The lowest repellency percentage was observed with the distilled water extract, achieving 43.3% at a dose of 0.83 mg/cm². However, the relative repellency was increased as the dose increased in each extract.

Table 8: Repellency/antifeedant effect of *Pulicaria undulata* in three different extracts on females *Culex pipiens*

Extracts	Dose (mg/cm^2)	No. of tested females	Fed females		Unfed females		Repellency (%)
			No.	%	No.	%	
Petroleum Ether Extract	Control	56	53	94.6	3	5.3	--------
	0.83	49	18	37.5	31	60.4	58.2
	1.67	42	13	31	29	69.1	67.4
	3.33	46	8	17.4	38	82.6	81.6
Ethanol Extract	Control	45	43	95.5	2	4.4	-----
	0.83	53	25	47.2	28	52.8	50.6
	1.67	46	19	41.3	27	58.7	56.8
	3.33	53	14	26.4	39	73.6	72.4
Water Extract	Control	50	47	94	3	6	----
	0.83	48	26	54.2	22	45.8	42.3
	1.67	50	20	40	30	60	57.4
	3.33	56	17	30.4	39	69.4	67.4
DEET	1.8	55	0.0	0.0	55	100	100

Biochemical studies

Biochemical analysis of *Culex pipiens* third instar larvae, 48 hours post-treatment with LC_{50} of water, ethanol, and petroleum ether extracts of *Pulicaria undulata*, revealed significant changes in enzyme activities (acetylcholinesterase, glutathione S transferase, acid phosphatase, and alkaline Phosphatase) and biochemical constituents (total protein, carbohydrates, and lipids) when compared to the untreated larvae Table (9), Figures (2). A significant reduction ($P< 0.05$) in acetylcholinesterase (AChE) activity was observed in all treated groups, with the highest inhibition observed in the petroleum ether extract group (22.05%). In contrast, glutathione-S-transferase (GST) activity showed a significant increase ($P<0.05$), with percent increases of 96.15%, 92.40%, and 88.46% for the water, ethanol, and petroleum ether extracts, respectively. The activities of acid phosphatase and alkaline phosphatase were significantly reduced ($P<0.05$) in the treated groups. The greatest reduction in acid phosphatase activity occurred in the petroleum ether-treated larvae (43.34%). Similarly, alkaline phosphatase activity was significantly reduced, with the highest reduction observed in the petroleum ether group (42.86%). The biochemical constituents (protein, carbohydrates, and lipids) were significantly decreased ($P<0.05$) across all treated groups. The maximum percent decrease was observed in the petroleum ether extract with a reduction of 41.94%, 50%, and 69.93% for the total protein, carbohydrates, and lipids, respectively.

Table 9: Enzyme Activities of *Culex pipiens* 3rd instar larvae 48-hour post-treatment with LC_{50} *Pulicaria undulata* Extracts

Enzyme activity	Control (Mean ± S.E)	Water Extract (Mean ± S.E) (% change)	Ethanol Extract (Mean ± S.E) (% change)	Petroleum Ether Extract (Mean ± S.E) (% change)
Acetylcholinesterase (AChE) (µg AchBr/min/g b.wt)	2.54 ± 0.005^{a}	2.09 ± 0.003^{b} (-17.72%)	2.02 ± 0.003^{c} (-20.48%)	1.98 ± 0.005^{d} (-22.05%)
GlutathioneS-transferase (GST) (m mole substrate conjugated/min/g b.wt)	0.26 ± 0.005^{a}	0.51 ± 0.000^{b} (96.15%)	0.50 ± 0.000^{bc} (92.40%)	0.49 ± 0.005^{c} (88.46%)
Acid Phosphatase (µg phenol/ml/min)	0.30 ± 0.003^{a}	0.23 ± 0.003^{b} (-23.34%)	0.20 ± 0.003^{c} (-33.34%)	0.17 ± 0.003^{d} (-43.34%)
Alkaline Phosphatase (µg phenol/ml/min)	0.28 ± 0.003^{a}	0.20 ± 0.006^{b} (-28.58%)	0.18 ± 0.003^{c} (-35.72%)	0.16 ± 0.003^{d} (-42.86%)

*Means with different letters in the same row are significantly different.

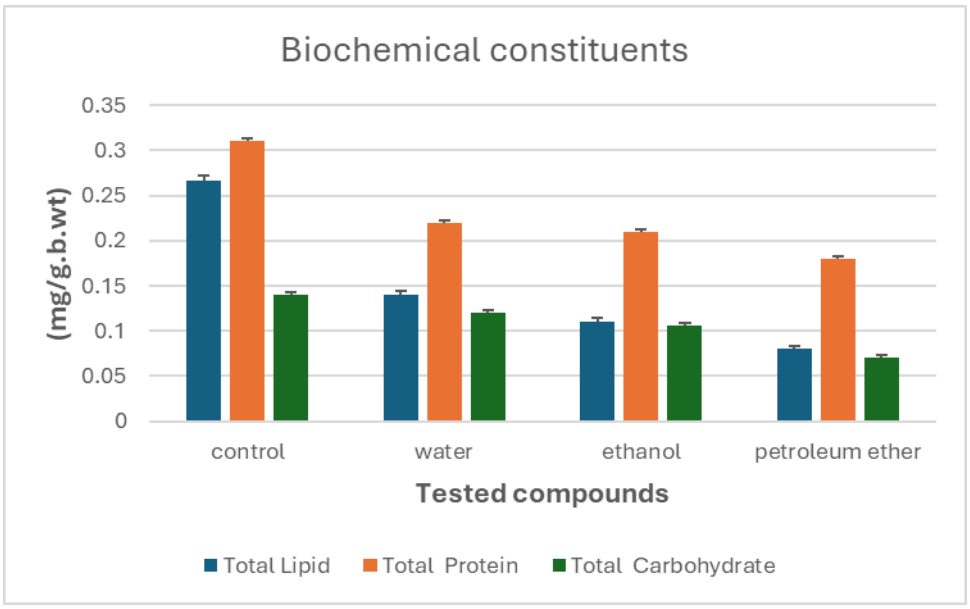

Fig. ٢: Biochemical Constituents of *Culex pipiens* 3rd instar larvae 48-hour post-treatment with LC_{50} *Pulicaria undulata* Extracts

In silico studies

The interactions of the most prominent compounds in the petroleum ether extracts, Eugenol (24.85%) and Oleic acid (6.59%), were separately docked into the active pockets of AChE (PDB ID: 6XYU) and GST (PDB ID: 1M0U) to investigate their binding nature with the enzymes provided intuitions into the mode of action of the petroleum ether extract (Fig. 3,4 and Table 10). For each compound, 10 poses were formed and the pose with the highest – CDOCKER energy score was selected. Oleic acid showed the highest – CDOCKER energy score of 38.13 forming a conventional hydrogen bond with Alanine 239, a Pi-anion interaction and attractive charge with Histidine 480 and Tryptophan 271, and an additional hydrogen bond with Glycine 151. Eugenol formed a Pi-alkyl interaction with Tyrosine 71, the aromatic ring of the compound formed a Pi-Pi stacked interaction with Tryptophan 83, and a Pi-doner hydrogen bond with Tyrosine 370. The – CDOCKER energy score of Eugenol with AChE is 14.97.

In the binding interactions with GST, Oleic acid also showed the highest – CDOCKER energy score of 37.47 forming a salt bridge with Arginine 112. The energy binding interaction of Eugenol with GST had a – CDOCKER energy score of 13.35 forming a Pi cation interaction with Arginine 112, and alkyl and Pi alkyl bonds with Cysteine 115 and Leucine 36.

Table 10: The -CDOCKER Energy score for major compounds identified in Petroleum ether extract with AChE and GST active sites

Enzyme	Compound	-CDOCKER Energy
AChE	Oleic Acid	38.18
	Eugenol	14.97
GST	Oleic Acid	37.47
	Eugenol	13.35

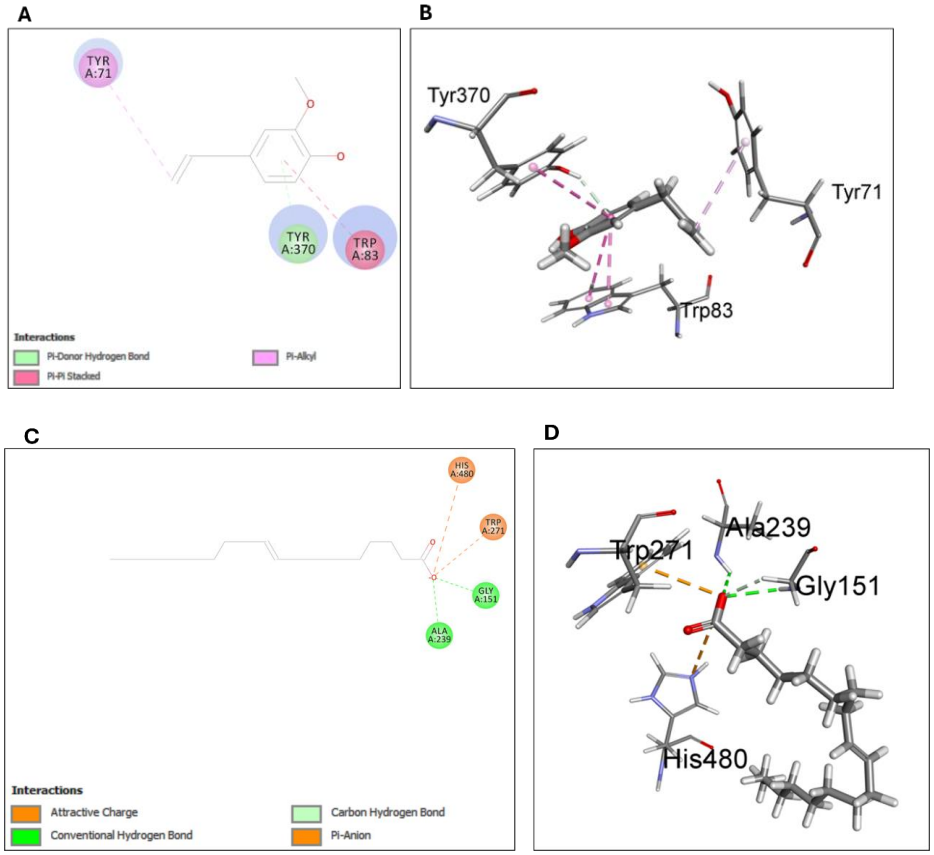

Fig. 3 (A and C) 2D-binding interaction profile for Eugenol and Oleic acid, respectively with the active pocket of the AChE enzyme. (B and D) In-depth 3D ligand-AChE interaction mode for Eugenol and Oleic acid, respectively.

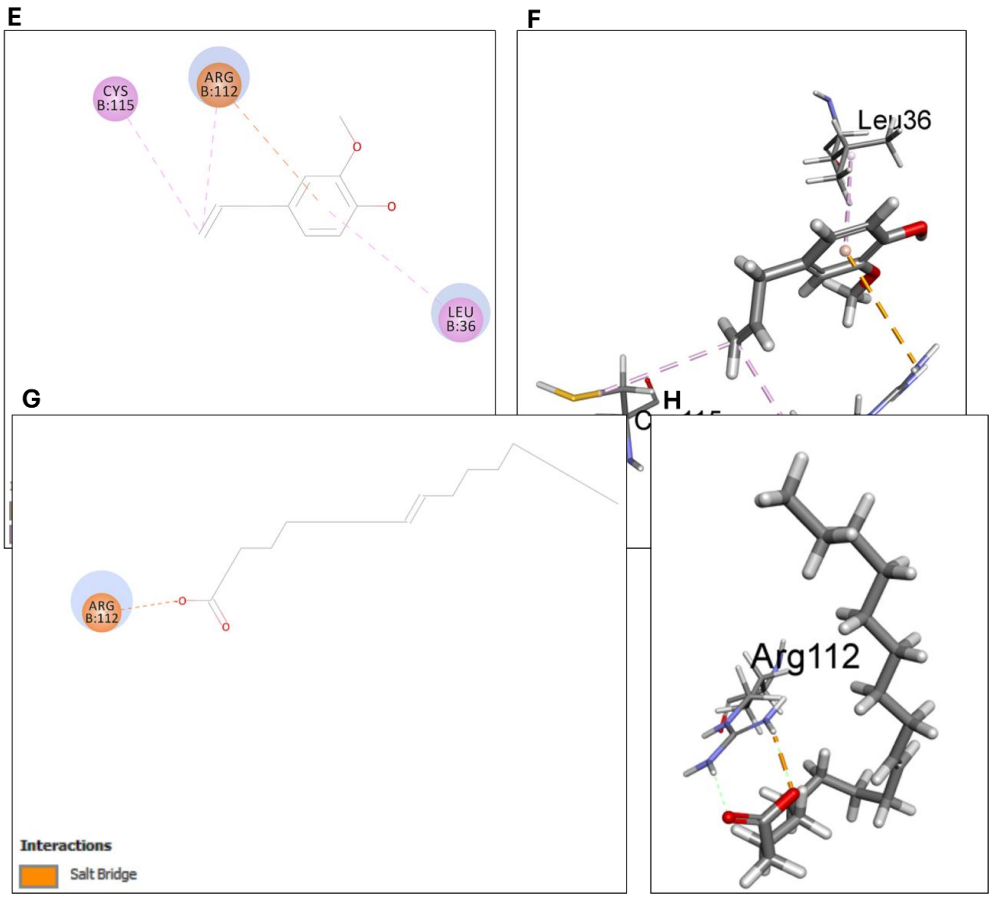

Fig. 4 (E and G) 2D-binding interaction profile for Eugenol and Oleic acid, respectively with the active pocket of the GST enzyme. (F and H) In-depth 3D ligand-GST interaction mode for Eugenol and Oleic acid, respectively.

DISCUSSION

Pulicaria undulata is one of the wild medicinal desert plants growing in Egypt belonging to the family Asteraceae. This family is one of the largest angiosperm families, featuring many aromatic species that comprise approximately 100 genera and 2300 species. The genus *Pulicaria* is one of these genera, with about 100 species distributed worldwide **(Hutchinson, J. The Families of Flowering Plants, 2nd edition, Oxford University Press, Oxford, 1959)**. *Pulicaria undulata* is a resilient flowering plant with small, bright yellow flowers that has been used as a traditional herbal remedy in Egypt.

As mentioned before, *Pulicaria* sp. is one of the worlds-widely distributed plant in North Africa. Because of its insecticidal and repellent properties, farmers in Morocco have long utilized this plant to manage stored grain pests, such as Tribolium castaneum and Sitophilus oryzae (L.). (**Lougraimzi *et al.*, 2022**). In Sudan, different *Pulicaria species* have been used traditionally to repels insects (**Mohamed *et al.*, 2020**). In addition to,

Algeria has approved its importance as new botanical insecticides against different insect species (**Ammar *et al*., 2020**). In Egypt, numerous studies have investigated the effectiveness of *Pulicaria* from the pharmacological point of view as anti-cancer, anti-microbial. This study is considered as the first study that concerns to demonstrate the insecticidal power and the potential of repellency of *P. undulata* against mosquito vector borne diseases , i.e., *Culex pipiens*. All plant extracts at different concentrations exhibited a positive correlation of larvicidal activity and repellency action towards the tested 3rd instar larvae and adults of *Culex pipiens*, respectively. The petroleum ether extract was more effective demonstrating an adult repellency action of approximately 82% at a concentration of 3.33 mg/cm². Our findings on *P. undulata* are in accordance with results reported by other researchers using *Pulicaria* species, such as *arabica* against *Cx. quinquefasiatus* in Algeria **(Ammar *et al*., 2020)** and biocidal activities of *Pulicaria Jaubertii* against *Aedes aegypti* through its different life stages **(Shehata *et al* 2020)**; besides its effectiveness of species *incisa* against stored grains pests such as *Sitophilus oryzae* and *Tribolium castaneum* in Morocco (**Lougramizi *et al*., 2022**).

Despite the observed susceptibility of *Cx. pipiens* larvae to various *P. undulata* plant extracts, there was a significant negative impact on both larval and pupal development, as well as on reproductive parameters in adults. The petroleum ether extract of *P. undulata* significantly prolonged the larval developmental time, extending it to 4.7 and 4.8 days at 250 and 300 ppm, respectively, compared to 4.3 days for the control group. Pupal duration was also extended to 2.7 days at 300 ppm, compared to 1.9 days for the control group. Our results are in agreement with findings reported by **Shehata *et al*. (2020)**. This observation aligns with the phytoextract-induced lengthening of mosquito larval periods, likely owing to its interference with normal hormonal activity **(Arivoli and Tennyson, 2011)**. The failure of adults to emerge, particularly at high concentrations of ethanolic and water extracts (77.8% and 69.5%, respectively), may be attributed to the limited availability of chitin during metamorphosis, leading to death of larvae and pupae trapped in the weak integument. This explanation is in accordance with the findings in **Arivoli and Tennyson (2011) and Shehata *et al*., 2020**. Overall, by the increase of the concentration of each extract, the growth index tended to decline.

The existence of biologically active conistiuents in the three extracts of *P. undulata*, such as linalool, sesquiterpenes, and fatty acid esters, highlights their potential as natural larvicidal agents against *Culex pipiens*. This aligns with findings from other research exploring the efficacy of plant-derived essential oils (EOs) against mosquito larvae. These compounds have been identified in multiple studies as having significant larvicidal properties. Monoterpenes, like linalool identified in *P. undulata* (47.60%), are cytotoxic to insect tissues. **Tripathi *et al*. (2009)** demonstrated that monoterpenes disrupt cellular functions by impairing mitochondrial respiration, decreasing cell membrane permeability, and reducing Golgi body integrity. Additionally, monoterpenes exert neurotoxic effects,

targeting acetylcholinesterase (AChE), sodium channels, nicotinic acetylcholine receptors, and GABA-gated chloride channels **(Priestley *et al.*, 2003 and Mossa, 2016)**. These effects were further corroborated by **Tang *et al.* (2024)**, who reported that citronellol, a key component of lemongrass oil, demonstrated potent larvicidal effects against *Anopheles sinensis* larvae through AChE inhibition.

Fatty acids, also detected in *P. undulata* extracts, target AChE and octopaminergic receptors **(Perumalsamy *et al.*, 2015)**. Their larvicidal activity has been validated in multiple studies, where exposure resulted in prolonged larval development, decreased larval weight, and histological alterations in midgut epithelial cells **(Moustafa *et al.*, 2018)**. The histological damage observed aligns with findings from **Sebak *et al.* (2024)**, who reported destructive changes larval midgut of *Cx. pipiens* treated with various EOs.

The petroleum ether extract of *P. undulata* exhibited notable efficacy, with eugenol as its major component (24.85%). Eugenol's action mechanisms, including interference with respiratory processes, further enhance its larvicidal potential. Similar findings from the study by **Sebak *et al.* (2024)** noted that EOs, particularly orange oil, had LC_{50} values as low as 117.69 ppm and induced midgut damage, effectively reducing larval viability and adult fecundity.

This study contributes to the expanding body of evidence supporting the use of plant-based larvicidal agents, offering a sustainable and eco-friendly alternative to synthetic insecticides. The chemical diversity of *P. undulata* extracts, with compounds such as linalool, eugenol, and fatty acid esters, offers a multifaceted approach to targeting mosquito larvae, with potential applications in integrated vector management programs.

Despite the potential susceptibility of *Cx. pipiens* larvae to different *Pulicaria undulata* plant extracts, no significant reduction in larval and pupal duration was observed and this result contracts with other authors findings (**Farag *et al.*, 2021a**) and this may return to the usage of another plant extract with different side effects.

Botanical extracts may act as appropriate substitutes to synthetic repellents in the future due to their safety, cost-effectiveness, and availability in numerous regions around the world.As the plant extracts and essential oils are natural sources of bioactive molecules that are effective in controlling mosquito vectors and consequently transmitted diseases. Understanding the specific mechanisms through which these botanicals interact with the physiology of target insects is essential for developing effective insecticides **(Sofi *et al.*, 2022)**. In this study, the biochemical effects of *P. undulata* extracts on the third instar larvae *Culex pipiens* were evaluated by assessing enzyme activities and biochemical constituents 48 hours post-treatment with water, ethanol, and petroleum ether extracts. Results exhibited a significant reduction in the activities of acetylcholinesterase, acid phosphatase, and alkaline phosphatase with the highest percent

reduction observed in the petroleum ether extract followed by ethanol, and water extracts. Acetylcholinesterase is an esterase that is responsible for breaking down the neurotransmitter acetylcholine, terminating nerve impulses. Inhibition of AChE beyond a certain threshold leads to paralysis and eventual death in insects **(Fournier and Mutero 1994)**. Several reports have demonstrated that plant extracts can act as AChE inhibitors, suggesting that the molecules found in the extracts may disrupt cholinergic synapses, impairing nerve signal transmission **(Sofi *et al*., 2022)**. The observed lethal effects are likely due to the accumulation of acetylcholine at synaptic junctions, which disrupts the coordination between the nervous and muscular systems **(Farag *et al*., 2021b)**. In contrast, the activity of Glutathione S transferase was significantly increased in all treated larval groups with the maximum increase in water and ethanol groups. GST is a key enzyme involved in the detoxification of exogenous compounds, including insecticides, and plays a defensive role for the cells from oxidative damage **(Koirala *et al*., 2022)**. The increase in GST activity detected in this research suggests an upregulation of the larvae's detoxification system in response to treatment, aligning with findings from other studies on plant-based insecticides **(Zeghib *et al*., 2020 and Asmaey *et al*., 2024)**. In the current study, acid phosphatase - a lysosomal enzyme- showed a marked decrease in activity across all treated groups. The decrease in acid phosphatase could be refereed to the ingestion of toxic compounds from the extracts, which may interfere with lysosomal function. Our results are accordance with **Farag *et al*. (2021b)**, who reported similar inhibitory effects after treatment with pomegranate peel extract. Additionally, the observed decrease in alkaline phosphatase activity could be due to a decrease in enzyme synthesis or the binding of the insecticidal compounds to the enzyme's active site, as suggested by **Farag *et al*., 2021b**.

Moreover, treatment with the LC_{50} of each extract resulted in a significant decline in the total protein, carbohydrate, and lipid content of the larvae. The decline in protein levels may be due to interference with hormone regulation of protein synthesis **(Chaudhary *et al*., 2017 and Ahmed *et al*., 2023)**. Carbohydrates, the primary source of glucose necessary for various physiological activities **(Hammama *et al*., 2022)**, were also reduced, presumably due to the utilization of stored carbohydrates as a response to phytochemical-induced stress. The reduction in lipid content in treated larvae suggests a metabolic shift towards lipid catabolism, likely triggered by the stress exerted by the active compounds in the plant extracts. This energy reserve consumption is a defense mechanism that enables the larvae to cope with the toxicity of the extracts. This was in accordance with previous results reported the decrease in total lipid contents in *Culex pipiens* third instar following treatment with plant extracts **(Farag *et al*., 2021b; Asmaey *et al*., 2024)**.

Docking studies were employed to improve perceptions into the interaction of Eugenol and Oleic acid, the main compounds in petroleum ether extract, with the active

pockets of AChE and GST enzymes. Oleic acid revealed the highest affinity for AChE and GST with a – CDOCKER energy score of 38.13 and 37.47, respectively. In the interaction with AChE, Oleic acid formed a conventional hydrogen bond with Tyrosine 71, a Pi-Pi stacking interaction with Tryptophan 83, and a Pi-donor hydrogen bond with Tyrosine 370. The interaction of Oleic acid with GST formed by a salt bridge with Arginine 112. **Gurunathan *et al*.; 2016** recorded the larvicidal property of Oleic acid against *Aedes aegypti* and *Cx. quinquefasciatus*. Also, **Mossa *et al*., 2022** attributed the larvicidal activity in the neem oil to the presence of the fatty acid, Oleic acid that altered the activity of AChE and GST in the *Cx. pipiens* mosquito larvae. The interaction of Eugenol with AChE and GST active pockets has a – CDOCKER energy score of 14.97 and 13.35, respectively. In AChE, it formed a Pi-Pi stacked interaction with Tryptophan 83, a Pi-alkyl interaction with Tyrosine 71, and a Pi-donor hydrogen bond with Tyrosine 370. Results were inconsistent with **Murugan *et al*., 2024** who reported a high binding affinity of Eugenol in the methanolic extract of *Ocimum canum* with the AChE active pocket, causing a knockdown effect and mortality in three mosquito vectors. When interacting with GST, Eugenol formed a Pi-cation interaction with Arginine 112, along with alkyl and Pi-alkyl bonds with Cysteine 115 and Leucine 36. **Adhikari *et al*., 2022a** reported the susceptibility of *Aedes aegypti* to eugenol with noticeable alterations in GST enzyme activity of the treated larvae. This was further supported by in-silico studies of Eugenol with AChE and GST in *Aedes aegypti* (**Adhikari *et al*., 2022b)** showing a high binding affinity of the ligands with the enzyme active sites suggesting that eugenol might block metabolic detoxification enzymes.

CONCLUSION

This study emphasizes the significant bioefficacy of *Pulicaria undulata* extracts, particularly petroleum ether extract, as a sustainable biopesticide against *Culex pipiens*. The extract exhibited intense larvicidal and repellent activities, driven by its phytochemical constituents, notably eugenol and linalool. Biochemical assays revealed substantial disruptions in enzymatic activities, including acetylcholinesterase inhibition and metabolic derangements in protein, carbohydrate, and lipid reserves. The molecular docking studies demonstrated strong binding affinities of oleic acid and eugenol, the main compounds in the petroleum ether extract, to AChE and GST, further supporting their potential roles in the observed bioactivities. These findings underscore the promise of *P. undulata* as a pivotal component of integrated vector management strategies, drawing attention to the value of botanicals as sustainable alternatives to synthetic insecticides.

ETHICAL APPROVAL

The current paper was approved by the Institutional Ethical Committee of the Faculty of Science at Ain Shams University provided ethical approval. Code: ASU- SCI / ENTO/2024/2/1.

Conflict of interest

On behalf of all authors, there is no conflict of interest.

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