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Larvicidal potency of four Egyptian herbs on *Culex pipiens* larvae: Phytochemical composition and molecular networking for most potent extracts

Mostafa A. Asmaey^{a,*}, Hanan Y. Aati^b, Mahmoud Emam^c, Nikolas Tsfantakis^d,
Nikolas Fokialakis^d, Mohamed seif^{e,*}, Ahmed I. Hasaballah^f

^a Department of Chemistry, Faculty of Science, Al-Azhar University, Assiut Branch, 71524 Assiut, Egypt

^b Pharmacognosy Department, College of Pharmacy, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia

^c Phytochemistry and Plant Systematics Department, National Research Centre, Dokki, Giza 12622, Egypt

^d Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, 15771 Athens, Greece

^e Food Toxicology and Contaminants Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Giza 12622, Egypt

^f Zoology and Entomology Department, Faculty of Science, Al-Azhar University, 11884 Cairo, Egypt

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ABSTRACT

Utilizing synthetic insecticides to control mosquito populations has many adverse side effects as they can cause environmental pollution and insecticide resistance. Therefore, the search for eco-friendly and effective mosquito control agents has led to the exploration of plant extracts with larvicidal efficacy. In this study, we aimed to explore the larvicidal activity of four Egyptian plant extracts: *Rosmarinus officinalis*, *Melissa officinalis*, *Cichorium intybus*, and *Beta vulgaris* var. *cicla* against *Culex pipiens* and to identify the chemical constituents present in the active extracts. The methanolic extracts of the four plants were prepared and tested against the third instar larvae of *C. pipiens* using a standard method. The lethal concentrations (LC₅₀ and LC₉₀) were calculated using probit analysis. The active extracts were subjected to ultra-performance liquid chromatography-mass spectroscopy (UPLC-MS/MS) analysis and the data were processed using the global natural products social molecular networking (GNPS) platform to generate molecular networks and identify the compounds based on spectral similarity and database matching. The methanolic extracts of *R. officinalis* L. and *M. officinalis* L. had the highest larvicidal activity, where LC₅₀ values were 9.795 and 26.505 µg/mL, respectively. Their exposure caused a high mortality rate and disrupted the biochemical and physiological parameters (total carbohydrates, total lipids, total protein, acetylcholinesterase (AChE), and glutathione S-transferase (GST) in the body of *C. pipiens* when compared with *Cichorium intybus* (Chicory) and *Beta vulgaris* var. *cicla* (Chard). The UPLC-MS/MS analysis with the aid of the GNPS platform, revealed the presence of 23 and 41 metabolites from *R. officinalis* L. and *M. officinalis* L., respectively. The identified metabolites may act as larvicidal agents by interfering with the physiological or biochemical processes of the mosquito larvae. Overall, the findings suggest that the methanolic extracts of *R. officinalis* and *M. officinalis* are potential sources of natural larvicides for mosquito control.

1. Introduction

Mosquitoes are vectors of many pathogens that affect millions of people and animals worldwide, There are more than 3500 mosquito species that have been identified worldwide (Robert, 2001). In 2019, several outbreaks of dengue fever were witnessed in Egypt, with over 12,000 cases reported (Gaber et al., 2022). Moreover, many arboviral

diseases, such as Zika virus and chikungunya are prevalent and their spread is rising (Tiwari et al., 2020).

Furthermore, mosquitoes can carry and spread the Rift Valley fever virus, West Nile virus, and filariasis (Conley et al., 2014), as well as other diseases such as dengue and malaria that result in significant mortality and economic loss, especially in developing countries where the resources for prevention and treatment are limited (Onen et al., 2023).

* Corresponding authors.

E-mail addresses: asmaey.mostafa.2142@azhar.edu.eg (M.A. Asmaey), seif.eg@gmail.com (M. seif).

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Culex spp. is one of the most widespread vectors in Egypt (Haba and McBride 2022). Moreover, *C. pipiens* may affect food safety particularly dairy and meat products, by transmitting and spreading microbial contamination. This can occur not only through mechanical methods but biologically as well (Adly et al., 2022; Abdel-Haleem et al., 2024).

Controlling mosquito populations has become one of the most effective strategies to prevent the transmission of many vector-borne diseases and food contamination. Particularly, larviciding is a fruitful way of reducing the mosquito population in their breeding places before they emerge from the larval stage (Karunaratne and Surendran 2022).

Commonly, the traditional procedures of mosquito control, which mainly involve the use of synthetic insecticides, have several limitations due to their adverse effects on the environment, food chain, human and animal health, and insecticide resistance (Rani et al., 2021; Abbasi et al., 2022; Tantawy et al., 2022; Onen et al., 2023). Therefore, the search for safe and effective mosquito control agents became obligatory and led to the exploration of plant-based natural products that may substitute conventional pesticides and may act as insecticides, antifeedants, and repellents (Abdel-Haleem et al., 2024).

Plants have been used for centuries for insect control, and their insecticidal properties are well known. Recently, several plant species have been reported to have larvicidal activity against mosquitoes, including *Culex pipiens*, *Culex antennas*, and *Aedes aegypti* (Shehata, 2019; Shahat et al., 2020; Assemie and Gameda, 2023; Priya et al., 2023; Farag et al., 2024). Due to the variety of the Egyptian climate, the Egyptian flora is widely diverse and a rich source of natural molecules (Asmaey et al., 2024; Al-Tohamy et al., 2018; El-Seedi et al., 2021; Elsoghiar et al., 2024). For instance, previous studies have reported that the essential oils of *Melissa officinalis* and *Rosmarinus officinalis* have shown promising larvicidal activity against different mosquito species (Conti et al., 2010; Ebadollahi et al., 2020; Zeghib et al., 2020; Ramzi et al., 2022; Khater et al., 2023). However, the larvicidal activity of plant extracts grown in Egypt has not been extensively evaluated.

Acetylcholinesterase (AChE) and glutathione S-transferase (GST) are key enzymes in insect control strategies and are among the most common detoxification enzymes. Evaluating their role is important to understand the effect of tested materials. The AChE enzyme catalyzes the hydrolysis of the neurotransmitter in the nervous system which is impacted by synthetic and botanical insecticides (Zibae et al., 2009; Oni et al., 2019). The GST enzyme plays a crucial role in detoxification and cellular antioxidant defenses against oxidative stress, including insecticides, through its conjugation of reduced glutathione to the electrophilic sites of both natural and synthesized exogenous xenobiotics (Enayati et al., 2005; BK et al., 2022).

Therefore, this study aimed to explore and compare the efficiency of four plant extracts from Egypt as biopesticides to potentially replace synthetic insecticides for controlling *C. pipiens*, a common mosquito vector that can transmit several diseases. The tested plants namely; *Rosmarinus officinalis* (*Lamiaceae* family), also known as "alecrim," is a perennial herb native to the Mediterranean region. *Melissa officinalis* (lemon balm) is a crucial herb among the autochthonous medicinal plants of the genus *Melissa*. *Cichorium intybus* L., commonly known as chicory, is a medicinally important plant in Eurasia and parts of Africa. *Beta vulgaris* var. *cicla* (chard) was selected based on its wide application in traditional medicine and culinary purposes in many countries, including Egypt. Moreover, the study sheds light on the investigation of the metabolic profile of the most effective extracts against the larvae of *C. pipiens* using UPLC-MS/MS analysis with the aid of GNPS molecular networking to explain their effects as bio-insecticides.

2. Materials and methods

2.1. Plant material

The aerial parts of the tested plants (Table 1) were collected from Al-Azhar Agricultural Research Nursery for Trees and Plants, Al Walideyah,

Table 1

Plant used in the study.

Scientific name	Common name	Time of collection	Herbarium Code
<i>Rosmarinus officinalis</i> L.	Rosemary	June 2022	CHM103
<i>Melissa officinalis</i> L.	Lemon balm	July 2022	CHM104
<i>Cichorium intybus</i>	Chicory	August 2022	CHM105
<i>Beta vulgaris</i> var. <i>cicla</i>	Chard	June 2022	CHM106

Asyut 2, (27°11'55.4"N 31°10'26.1"E), Assiut Governorate, Upper Egypt in 2022. The plants were identified (Belhumeur et al., 2008) and stored at the chemistry department of the Faculty of Science, Al-Azhar University (with serial numbers CHM103, CHM104, CHM105, and CHM106).

2.2. Plant extraction

The collected plants (1000 gm from each) were cleaned, dried, and ground to a fine powder (250 gm as the ground yield of each plant) and kept in a dark place at room temperature. A 200 g portion of the fine powder of rosemary and lemon balm was hydrodistilled over 120 min by using a Clevenger apparatus resulting in the isolation of 0.230 gm and 0.620 gm of essential oils, respectively. Then the dried residue remaining after hydrodistillation was extracted three times with 2 L of methanol for 60 min in an ultrasonic bath (Elsoghiar et al., 2024); the solvent was evaporated completely under reduced pressure until dryness to afford 37.8 and 40.6 gm of extract, respectively.

For chicory, and chard (stems and leaves), 200 g of the fine powder were directly extracted three times with 2 L of methanol for 60 min in an ultrasonic bath. The resulting solvent was evaporated under reduced pressure until dryness. The yield of the chicory extract was 25.4 gm, whereas the chard extracts from stems and leaves were 31.4 and 27.2 gm, respectively. The crude extracts of the four plants were subjected to larvicidal evaluation. To evaluate the phytochemical analysis, the extracts were further fractionated and defatted with n-hexane. Then, the polar and non-polar fractions were kept at 4 °C until use.

2.3. Larvicidal activity evaluation of the different extracts

2.3.1. Mosquito rearing

The mosquito, *C. pipiens* larvae were acquired from the Insectary of Medical Entomology, Animal House, Al-Azhar University, Cairo, Egypt, where it was established there since 2018. Then, we reared it for several generations to obtain enough progeny for current experimental studies. The colony was reared under controlled conditions of temperature of 27 ± 2 °C, and relative humidity of 75 ± 5 % with a (light: dark = 14 h:10 h) regimen. The emerged adults were transferred to wooden cages (30 × 30 × 30 cm) supplied with a 10 % sucrose solution for development and 500 mL plastic cups containing distilled water for hatching. The females' blood meal source was the pigeons' blood used to stimulate reproductive and developmental purposes.

2.3.2. Larvicidal activity of the tested herbal extracts

The larvicidal activity of the tested extracts was assessed against the mosquito vector, *C. pipiens* using the procedures of the World Health Organization bioassay (WHO, 2005). Briefly, larvae were reared under laboratory conditions of a temperature of 27 ± 2 °C, and relative humidity of 75 ± 5 % with a constant photoperiod (light:dark = 14 h:10 h). Then, 25 early 3rd instar larvae were transferred to polyethylene disposable cups (500 mL) containing 249 mL distilled water + 1 mL of test concentration solubilized in dimethyl sulphoxide (DMSO). The larvae were then exposed to different ranges of concentrations of the tested extracts, 5, 10, 20, 40, and 80 µg/mL for the rosemary and lemon balm, while at 25, 50, 100, 200, and 400 µg/mL for the chicory, stem chard, and leaves chard extracts.

The negative control was tested under the same conditions without

toxicants with the same amount of DMSO, while temephos (1 µg/mL) was used as a positive control. The mortality was recorded 24 h after exposure, and each concentration was tested in five replicates.

2.4. Biochemical assays

For biochemical assays, a hundred first-instar *C. pipiens* larvae were collected 3 days post-treatment with the LC₅₀ and LC₉₀ concentrations of each tested extract alongside the untreated (control) group. Treatment was done in triplicates. The collected larvae were then homogenized in distilled water using a Teflon homogenizer that had been dipped in a jacket of crushed ice. The homogenization process was carried out to ensure a thorough mixture. Subsequently, the samples were centrifuged in a cooling centrifuge at 4427 RCF for 10 min, and the supernatant was collected for further biochemical assays.

2.4.1. Determination of total carbohydrates

Total carbohydrate content was determined using the phenol-sulfuric acid reaction method (DuBois et al., 1956). Total carbohydrates were then prepared for bioassay following the procedures outlined by (Crompton and Birt, 1967).

2.4.2. Determination of total lipids

Total lipid content was determined by the scheme of using phosphovanillin reagent (Knight et al., 1972) (prepared by dissolving 0.6 g pure vanillin in 10 mL ethanol and filled to 100 mL with distilled water), then 400 mL concentrated phosphoric acid was added.

2.4.3. Determination of total proteins

Total protein content was determined according to the method of Bradford M. (Bradford 1976).

2.4.4. Activity of the acetylcholinesterase (AChE) and glutathione S-transferase (GST) enzymes

Acetylcholinesterase activity was evaluated using acetylcholine bromide as a substrate according to the method briefly described by Simpson D (Simpson et al., 1964). The activity of glutathione S-transferase was evaluated using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the method briefly described (Kao et al., 1989).

2.5. Phytochemical analysis of only the most effective polar extracts

2.5.1. UPLC-MS/MS analysis

UPLC is accomplished through the use of a narrower column filled with particles typically less than 2 µm in diameter. To gain quick insight into the components, recording a mass spectrum of each component as it elutes from the LC column proves highly beneficial. For efficient online LC/MS, ESI-MS and LC are usually interfaced.

In this study, the defatted extract of rosemary and lemon balm was prepared and injected into the UPLC/ESI-MS apparatus after being dissolved in H₂O/MeOH mixtures. The UPLC analysis was carried out using a Dionex Ultimate 3000 UPLC system (Sunnyvale, CA, USA: Thermo Scientific™Dionex™), and a reverse-phased Hypersil Gold UPLC C18 column (2.1 × 150 mm, 1.9 µm) was employed, following a method previously reported (Asmaey et al., 2023; Nazeh et al., 2024).

2.5.1.1. Molecular networking (MNW). The online workflow (<https://ccms-ucsd.github.io/GNPSDocumentation>) on the GNPS website (<http://gnps.ucsd.edu>) was used to design a molecular network. All MS/MS fragment ions within ±17 Da of the precursor *m/z* were removed from the data. MS/MS spectra were window-filtered throughout the spectrum by selecting only the top 6 fragment ions in the ±50 Da window. The MS/MS fragment ion tolerance was set at 0.5 Da and the precursor ion mass tolerance was set to 2.0 Da. The edges of the network were then filtered to have a cosine score greater than 0.7 and more than

6 matching peaks. Edges between two nodes were also preserved in the network if and only if both nodes featured in each other's top 10 most similar nodes.

The MNW of our work is (<ProteoSAFe/status.jsp?task=354d7a818fc849e5882b48b50c553788>) for rosemary and (<ProteoSAFe/status.jsp?task=3fc656c21ad54cd6811238ce033debb8>) for lemon balm scanning. To improve chemical structural data within the molecular network, information from *in silico* structure annotations from GNPS Library Search was integrated into the network using the GNPS Mol-NetEnhancer workflow (<https://ccms-ucsd.github.io/GNPSDocumentation/molnetenhancer>) on the GNPS website (<http://gnps.ucsd.edu>).

Chemical class interpretations were performed using the ClassyFire chemical ontology (Djombou Feunang et al., 2016; Ernst et al., 2019). The GNPS spectral libraries and molecular network visualization software (Cytoscape, version 3.9.1) were also used.

2.6. Statistical analysis

Descriptive analysis including Mean ± Standard Error (SE) was calculated for each treatment. Larval mortality was subjected to probit analysis to determine, LC₅₀, and LC₉₀ with confidence limits of 95 %. Analysis of variance, lower and upper confidence limits, and chi-squared values were performed using SPSS (IBM SPSS ver. 25). The LSD post-hoc method was used for pairwise comparisons, while, the *P* value was considered significant at *p* < 0.05.

3. Results

3.1. The larvicidal activity of tested extracts

Data presented in Fig. 1 show the larvicidal activity of tested extracts; rosemary, lemon balm, chicory, and chard (leaves and stems) against the 3rd instar larvae of *C. pipiens*. The larval mortality results showed a concentration-dependent pattern as larval mortality increased when the concentration increased. Additionally, the obtained data showed varying levels of mortality with different tested extracts with LC₅₀ values ranging from 9.795 µg/mL for rosemary extract to 363.093 µg/mL for chard stem extract. Based on the obtained LC₅₀ values, tested extracts may be arranged in descending order as the following: rosemary extract and lemon balm extract then chard leaves extract and chicory extract, and last chard stem extract with LC₅₀ values of 9.795, 26.505, 150.867, 179.128, and 363.093 µg/mL, respectively.

Overall, the tested extracts exhibited moderate to high toxicity particularly rosemary and lemon balm extracts which showed pronounced larvicidal activity. These extracts can effectively control *C. pipiens* mosquitoes at the larval stage. The current findings indicate that the most potent extract against the mosquito larvae was rosemary extract which demonstrated absolute larvicidal action with a toxicity index of 100 % when applied at a concentration of 80 µg/mL (Fig. 1).

3.2. Impact of LC₅₀ and LC₉₀ concentrations of the tested extracts on the total carbohydrate, lipid, and protein contents of the *C. pipiens* larvae

Additionally, the data presented in Fig. 2 shows the effect of treatment with the LC₅₀ and LC₉₀ concentrations of the tested extracts on the total carbohydrate, lipid, and protein contents of the *C. pipiens* larval tissue homogenate. For total carbohydrate content, the rosemary and lemon balm extracts were the most effective, significantly reducing the total carbohydrate content. The LC₉₀ concentration caused a decrease in the total carbohydrate content to less than 4.0 mg/g in the tissues homogenate of *C. pipiens* larvae, compared to its content of 16.3 mg/g in the control group (Fig. 2A). Similarly, rosemary and lemon balm extracts also reduced the total lipid contents to reach about 0.0002 mg/g against 0.0009 mg/g for the larval tissue homogenate of the control group following the exposure to LC₉₀ concentration (Fig. 2B). The other tested extracts revealed almost the same pattern of weak effectiveness toward

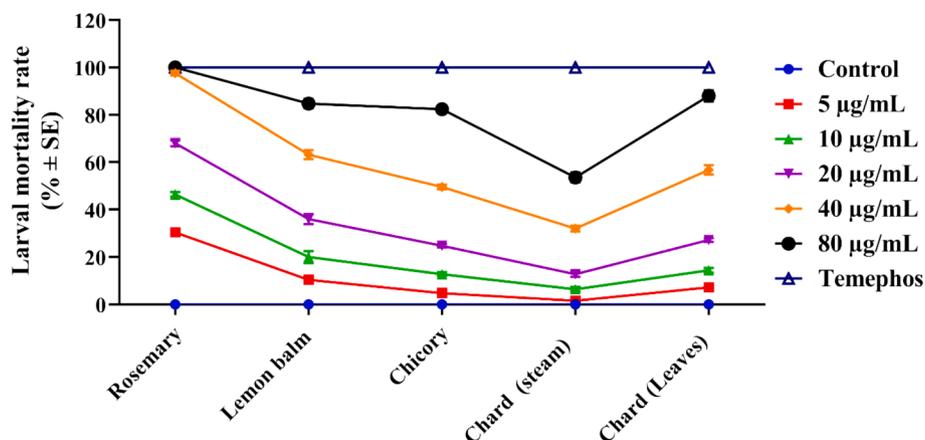


Fig. 1. Larvicidal activity of different concentrations of the rosemary, lemon balm, chicory, chard (stem), and chard (leaves) extracts against *C. pipiens*. Larval mortalities are presented as Mean \pm SE, ($n = 5$). Temephos are used as appositve control.

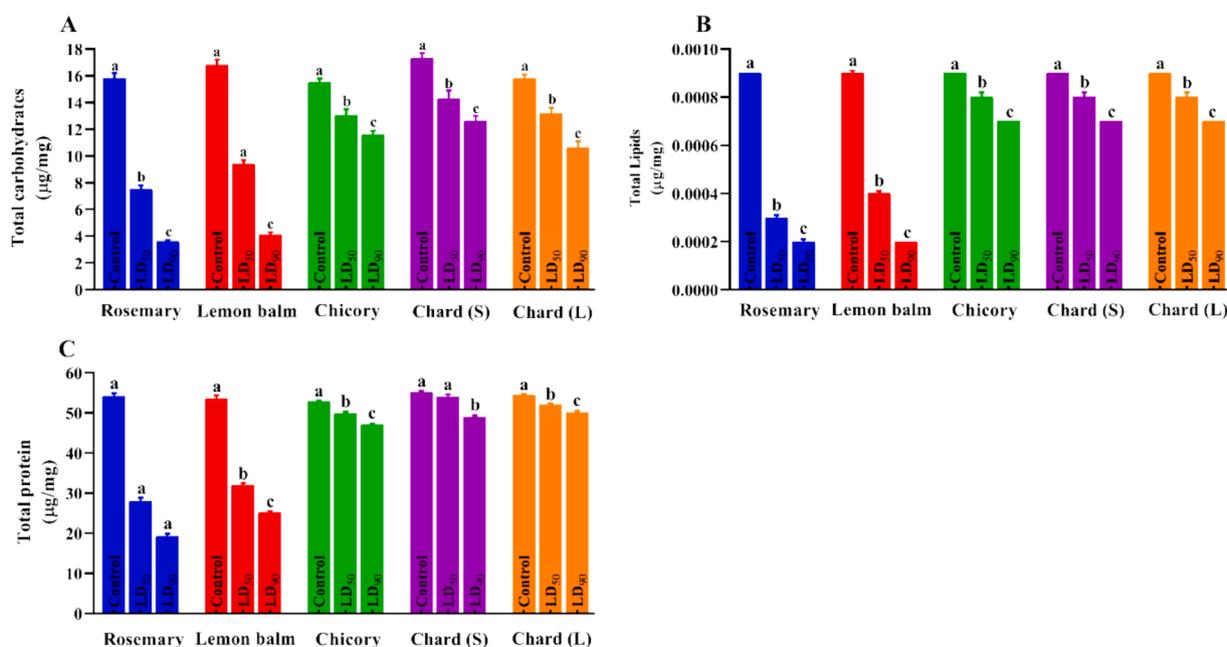


Fig. 2. Effect of LC₅₀ and LC₉₀ of the tested extracts on the total carbohydrate (A), total lipid (B), and total protein (C) contents of the *C. pipiens* larval tissue homogenate 3 days following exposure. Data illustrated as Mean \pm SE, ($n = 3$). Chard (s) = chard stem; Chard (L) = chard leaves. Different letters are significantly different between the treated and control groups ($P < 0.05$).

larval lipid profile even when the LC₉₀ concentrations of those extracts were applied.

The total protein contents were reduced when lethal concentrations of these four extracts were applied, with very slight activity recorded for other tested extracts. The total protein contents were reduced to 19.2 and 25.1 mg/g of the *C. pipiens* larval tissue homogenate treated with the LC₉₀ concentrations of rosemary and lemon balm extracts, respectively compared to 54.5 mg/g in the control group (Fig. 2C).

3.3. Impact of LC₅₀ and LC₉₀ concentrations of the tested extracts on the activity of AChE and GST and of the *C. pipiens* larvae

Data illustrated in Fig. 3 disclose the outcomes of the effects of LC₅₀ and LC₉₀ concentrations of different tested extracts on the activity of acetylcholinesterase in the *C. pipiens* larvae after exposure. The obtained findings revealed different patterns of enzymatic activity among tested extracts. The AChE activity was reduced to 2 to 3-fold after treatment with the LC₅₀ concentrations of rosemary and lemon balm, and this

reduction became much more pronounced in those larvae exposed to the LC₉₀ concentrations, while other extracts exert comparable activity to the control group.

Otherwise, the GST enzyme activity was moderately increased under exposure to rosemary and lemon balm extracts compared to the control larvae. However, chicory and stem and leaves chard extracts did not have significance compared to the untreated larvae when applied for either AChE or GST enzyme activity evaluation (Fig. 3 and Fig. 4). Importantly, based on the larvicidal potency, the methanolic extracts of rosemary and lemon balm had the lowest LC₅₀ values of 9.795 and 26.505 µg/mL, respectively, indicating high larvicidal influence. According to the obtained results, it became important to identify the kinds of natural constituents present in the extracts that showed the most effectiveness, "rosemary and lemon balm". Recently, the analytical tools specifically the liquid chromatography (LC) connected with masses detectors (MS) gave an obvious image of the precursor's masses that were characterized for specific natural compounds.

In this context, we performed UPLC-MS/MS with the aid of

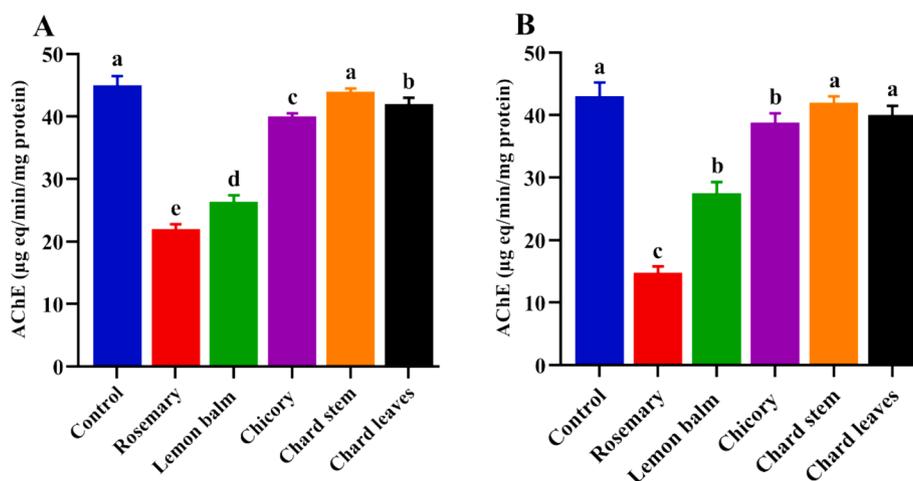


Fig. 3. Effect of lethal concentrations, LC₅₀ (A), and LC₉₀ (B) of different tested extracts on the enzymatic activity of Acetylcholinesterase (AChE) in the third instar larvae of the *C. pipiens* evaluated 3 days post-exposure. Data presented as Mean ± SE, (n = 3). Different letters are significantly different between the treated and control groups ($P < 0.05$).

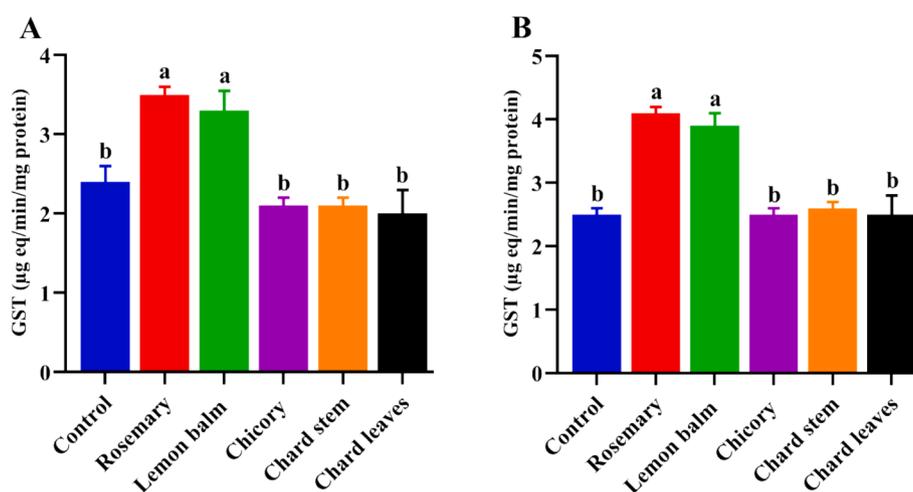


Fig. 4. Effect of tested lethal concentrations, LC₅₀ (A), and LC₉₀ (B) of different tested extracts on the enzymatic activity of Glutathione S-transferase (GST) in the third instar larvae of the mosquito, *C. pipiens* evaluated 3 days post-treatment. Data presented as Mean ± SE, (n = 3). Different letters are significantly different between the treated and control groups ($P < 0.05$).

molecular networking through the GNPS platform to explain the biological activity response depending on (Fig. 5 and Fig. 6; Table 2 and Table 3).

3.4. Metabolomic profiling of the most active extracts using UPLCMS/MS analysis

The phytochemical analysis of *R. officinalis* L. leaves extract (Fig. 5 and Table 2) exploited 23 metabolites that represented different classes of compounds including, benzoic acid derivatives, lipids of triterpenes derivatives, and methylated flavonoids that represented the most three classes, as well as fatty acids derivatives (Fig. 7). On the other hand, the phytoconstituents of *M. officinalis* L. leaves extract (Fig. 6 and Table 3) introduced 41 compounds arranged in different classes such as steroidal derivatives, flavonoids and their glycosides derivatives, phenolic acid derivatives, and fatty acid derivatives (Fig. 8).

Globally, molecular networking (MN) was used to explore visually the variance in the recorded metabolome of the investigated species and to make metabolite identification and categorization easier. The developed MNs were then analyzed using a MolNetEnhancer methodology, which improves the data annotation by merging results from various

computational tools.

Rosemary MN was constructed of 101 nodes arranged as 12 clusters, each with a minimum of two connected nodes, and 61 self-nodes that are represented as a pie chart (Fig. 7). While, the 289 nodes in the created lemon balm MN were divided into 36 clusters, each with a minimum of two connected nodes, and 138 single nodes that are represented as a pie chart (Fig. 8).

4. Discussion

Mosquitoes are an age-old nuisance pest and mosquito-borne diseases signify an increasing global health challenge. *C. pipiens* (Diptera: Culicidae) is a commonly spread mosquito in Egypt, transmitting dreadful diseases leading to high morbidity and sometimes mortality in humans and animals (Abdel-Shafi et al., 2016). Mosquito combating is necessary to prevent the spread of many pathogens that can be transmitted through these vectors, which is beneficial to humans and the environment as it reduces the spread of diseases and pollution of the environment and food chain. Additionally, using natural products to control the spread of mosquitoes will be useful in preventing the transmission of food pathogens carried by the mosquitoes and also in

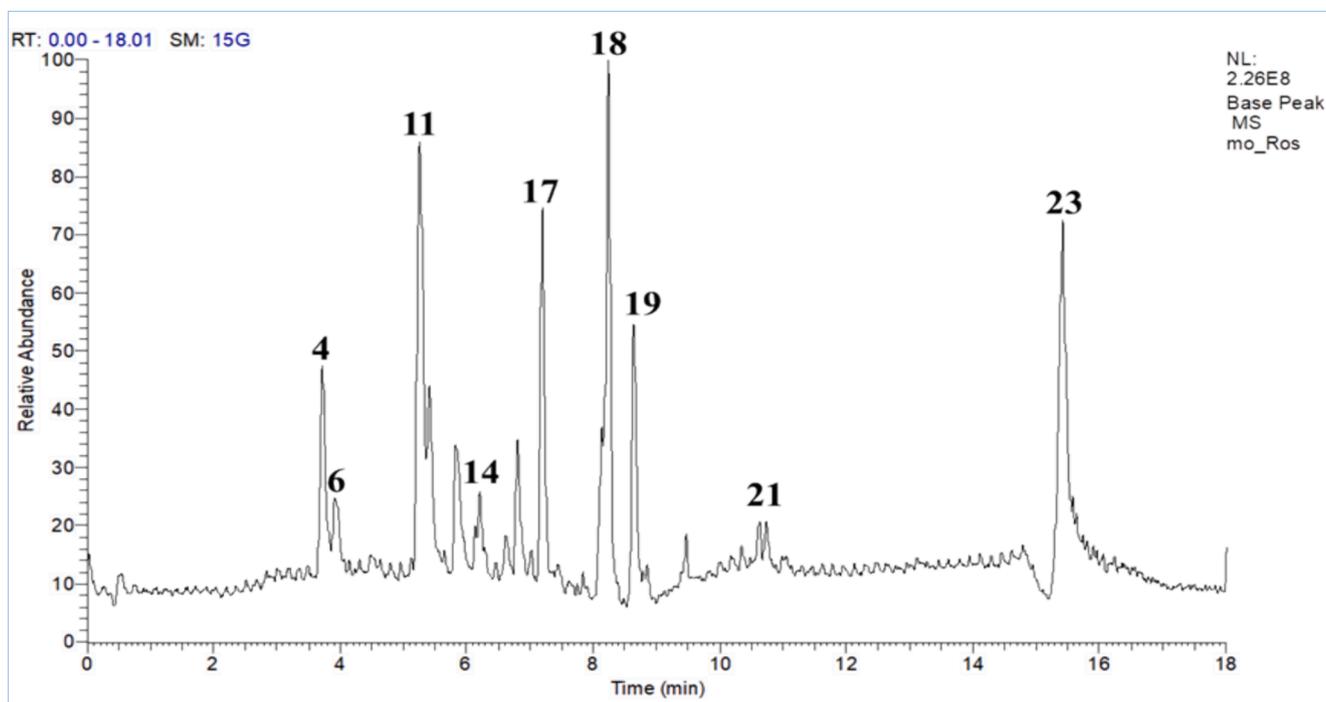


Fig. 5. Base peak chromatogram of *R. officinalis* L. leaves extract.

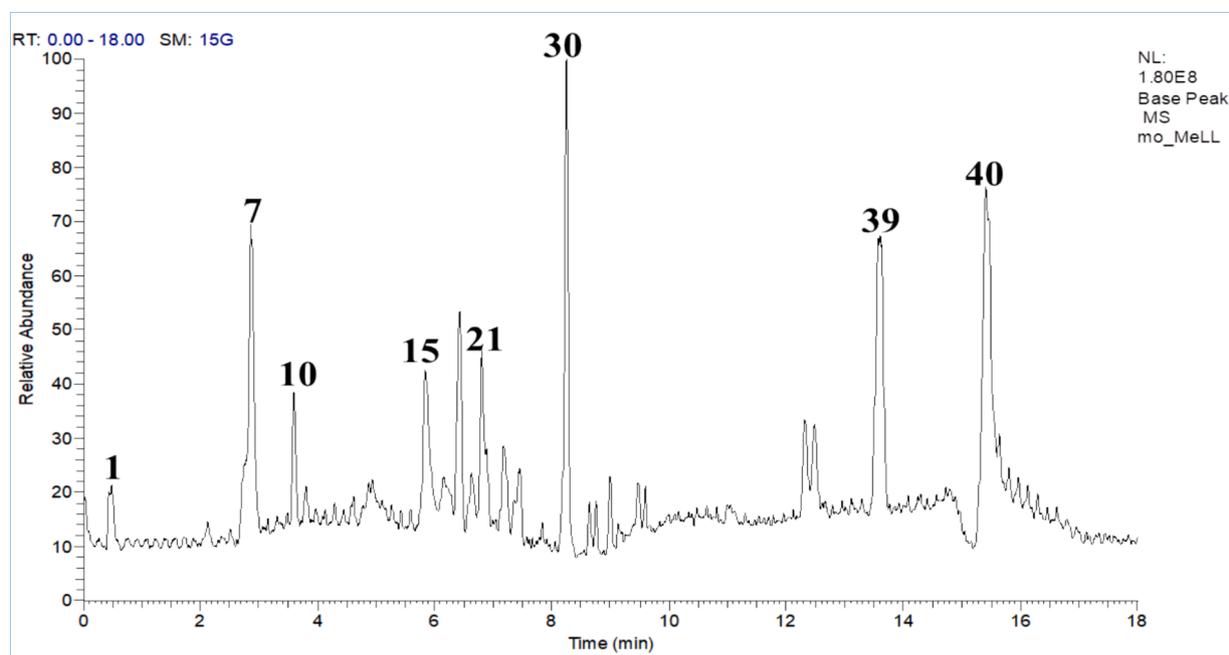


Fig. 6. Base peak chromatogram of *M. officinalis* L. leaves extract.

decreasing the negative side effects of synthetic chemical insecticides as environmental and food contaminants (Onen et al., 2023). Furthermore, employing environmentally safe natural substances for disease and vector control ensures safety for the environment and helps combat mosquito resistance to chemical insecticides (Niroumand et al., 2016; Tantawy et al., 2020; Demarque and Espindola, 2021). Natural products such as medicinal plant extracts and essential oils exert high larvicidal activities against many mosquitoes including *C. pipiens* larvae (Chellappandian et al., 2018; Sultan, 2020; El-Akhal et al., 2021; Hillary et al., 2024). In this study, four Egyptian plants, rosemary, lemon balm, chicory, and chard have been chosen based on their traditional uses and

applied as natural larvicidal agents against the larvae of *C. pipiens*.

Current findings indicate that the rosemary and lemon balm extracts had the highest effectiveness in inducing mortality in larvae of *C. pipiens* compared to the other tested extracts (chicory and chard), wherein they recorded the lowest LC_{50} and LC_{90} . When compared together rosemary extract exhibited a higher effect than the lemon balm extract. In the same context, several studies performed to increase the effectiveness of plant extracts or their essential oils as biocontrol agents for controlling the spread of mosquitoes including *C. pipiens* as reported in the published reports (Shalaby and Khater, 2005; Koliopoulos et al., 2010; Chafik and Afaf, 2020; Zeghib et al., 2020; Pavela et al., 2021; Ramzi

Table 2
Phytochemical analysis of *R. officinalis* L.

NO.	Rt (min)	Precursor mass (– or +)	Fragments	Compound Name	Chemical Class	Ref.
1	0.19	191	147, 127, 103, 93, 87	Quinic acid	Cyclohexanecarboxylic acid	(Borrás-Linares et al., 2014)
2	2.83	477	315, 299, 285	Cirsimarín	Flavone glycoside	(Borrás-Linares et al., 2014)
3	3.07	461	283, 297	Homoplantaginín	Flavonoid glycoside	
4	3.72	345	301, 283	Rosmanol	Diterpene lactone	(Borrás-Linares et al., 2014)
5	3.84	329	314, 285, 268	Tri-hydroxy-di-methoxyflavonol	Flavonoid	(Borrás-Linares et al., 2014)
6	3.91	345	301, 283	Rosmanol isomer	Diterpene lactone	
7	4.29	331	313, 283, 285, 287, 269	Carnosic acid	Benzenediol abietane diterpene	(Borrás-Linares et al., 2014)
8	4.47	313	283, 298	Cirsimaritin	Flavonoid	(Borrás-Linares et al., 2014)
9	5.26	359	283, 300, 329	Rosmanol methyl ether	Diterpene lactone	(Borrás-Linares et al., 2014)
10	5.41	299	284, 227, 137	Diosmetin	Methylated flavone	(Velamuri et al., 2020)
11	5.56	329	314, 285, 268	Tri-hydroxy-di-methoxyflavonol	Diterpenoid	Asmaey, MA. 2023
12	5.83	343	315, 299, 269, 243	Rosmadiol	Phenolic diterpene lactone	(Borrás-Linares et al., 2014)
13	6.07	283	268	Genkwanin	Methylated flavone	(Borrás-Linares et al., 2014)
14	6.21	315	285	Rosmaridiphenol	Diphenolic diterpene	(Borrás-Linares et al., 2014)
15	6.73	359	283, 329, 300	Epirosmanol methyl ether	Diterpene lactone	(Borrás-Linares et al., 2014)
16	6.82	487	–	Asiatic acid	Pentacyclic triterpenoid	(Borrás-Linares et al., 2014)
17	7.21	345	301, 287	12-Methoxy-carnosic acid	Benzenediol abietane diterpene	(Borrás-Linares et al., 2014)
18	8.50	453	407	Micromeric acid	Triterpenoid	(Borrás-Linares et al., 2014)
19	8.62	391	207, 181, 149, 113	Bis(2-ethylhexyl) phthalate	Phthalate ester	Nasr et al., 2018
20	8.69	455	–	Betulinic acid	Pentacyclic triterpenoid	(Borrás-Linares et al., 2014)
21	10.73	311(+)	161, 135	Avobenzone	Dibenzoyl methane derivative	GNPS
22	11.18–12.69	282.27 (+)	265, 247, 149, 135	9-Octadecenamíde	Fatty amides	GNPS
23	15.42	305	248, 174.9, 159,	3-(2-Carboxyethylpentasulfanyl) propanoic acid	Fatty acid derivatives	GNPS

m/z: Precursor mass, (–): negative ion, (+): positive ion

et al., 2022; Priya et al., 2023).

The current results are promising and applicable; herein the rosemary and lemon balm herbals are available and can thrive in various geographical areas. Likewise, the exposure of the rosemary and lemon balm extracts also declined the carbohydrates, lipid, and total protein contents in the *C. pipiens* under exposure to LC₅₀ and LC₉₀. These results are consistent with previous studies (Uritu et al., 2018; Ramzi et al., 2022). Carbohydrates constitute the main source of glucose essential for variable biological processes in the insect body (Hamama et al., 2022). The depletion in the carbohydrate content may be attributed to utilizing the larval tissues' reserved carbohydrate sources as a result of plant extract stress (Shehata, 2018). Exposure to rosemary and lemon balm extracts led to the reduction of lipid and protein content in *C. pipiens*, possibly indicating a shift in energy metabolism towards lipid catabolism due to the stress induced by the active components in the extracts.

Sharma and colleagues suggested that the reduction in total protein content may be attributed to hormones that regulate protein synthesis interacting with the active components present in the tested plant extracts (Sharma et al., 2009; Sharma et al., 2011). Furthermore, Senthilkumar and coauthors (Senthilkumar et al., 2009) reported that the protein level in *Anopheles Stephensi* larvae treated with some botanical extract was reduced and ascribed that to the disruption action of the plant extracts on the protein synthesis mechanism. The obtained results are harmonious with those stated before (Chafik and Afaf, 2020; Alghamdi 2021). Chafik and Afaf found that exposure to the essential oils of *R. officinalis* causes a decrease in numerous morphometric parameters such as the width of the thorax, weight, and body volume of the

larval stage 4 individuals of *C. pipiens* compared to the control (Chafik and Afaf, 2020). Meanwhile, Alghamdi found that the extracts of natural products from *Juniperus procera* and *Dodonaea viscosa* plants cause some morphological deformations in the *C. pipiens* larvae during the larval development stage into a pupa and in the emergence stage from a pupa into a complete insect (Alghamdi, 2021).

In the current study, the biochemical response following the exposure to LC₅₀ and LC₉₀ of the different tested extracts was studied by detecting the AChE and GST activities. Tested extracts decreased significantly ($P < 0.05$) the AChE activity compared to its activity in the control group. AChE is a pivotal enzyme to regulate the terminal nerve impulse, it is responsible for the hydrolysis of the neurotransmitter acetylcholine in insects. AChE is a well-known biomarker of neurotoxicity (Rajagopalan et al., 2023). The inhibitory effect of tested plant extracts on AChE activity is detailed in several published reports using various insecticides against different insect species including *C. pipiens* (Zhou et al., 2016; Al-Solami, 2021; Ali et al., 2021; Riaz, 2023).

Furthermore, GSTs play an essential role in the biotransformation and detoxification of exogenous substances, and in protecting organisms against oxidative stress. The increased level of GST activity indicated that the targeted larvae detoxification system was stimulated by the tested extracts. Here, the meaningful rise of GST activity under the LC₅₀ and LC₉₀ of rosemary and lemon balm extracts on the GST activity is in parallel with those reported (Zeghib et al., 2020) using rosemary extract and its essential oil (Chaudhari et al., 2021); using median lethal concentrations (50 and 90) of *M. officinalis* against *C. pipiens* and other insects.

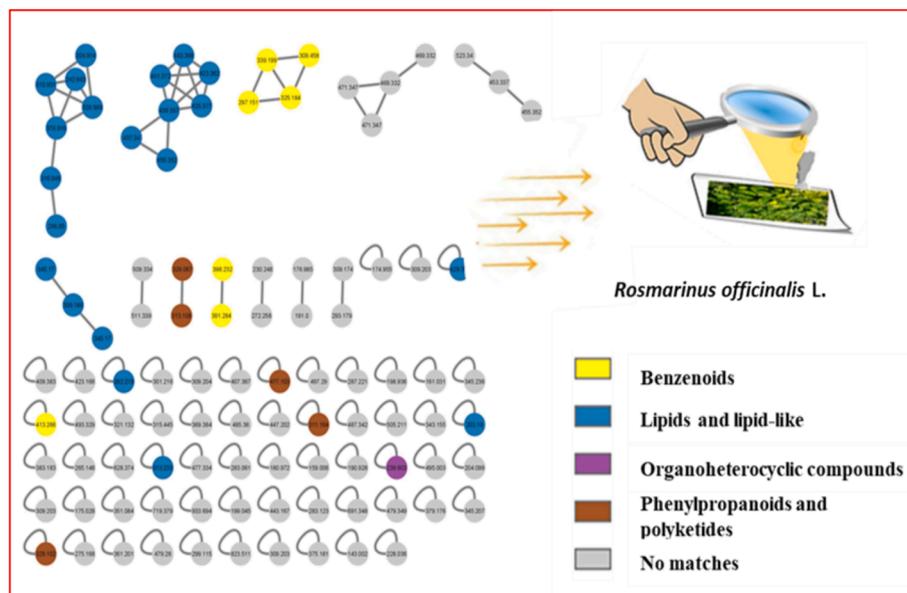
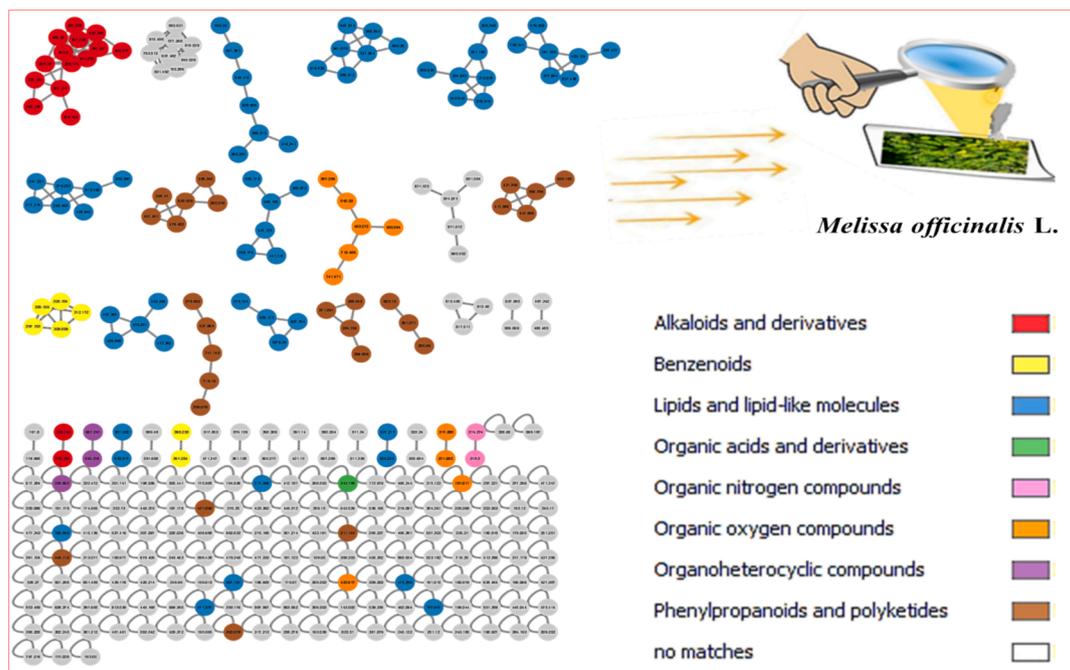
Table 3
Phytochemical analysis of *M. officinalis* L. leaves extract.

No.	Rt (min)	Precursor mass (- or +)	Fragments	Compound Name	Chemical Class	Ref.
1	0.48	387	341, 179	Hydroxyjasmonic acid glucoside	hydroxyjasmonic acid glucoside	(Ben et al., 2020)
2	1.82	137	93	4-Hydroxybenzoic acid	monohydroxybenzoic acid	(Miron et al., 2013)
3	1.83	153	123, 109	Protocatechuic acid	dihydroxybenzoic acid	(Miron et al., 2013, Wang et al., 2015)
4	2.26	179	161, 135, 121	Caffeic acid	Phenolic acid	(Wang et al., 2015)
5	2.38	151	136, 109	Vanillin	organic compound	(Tsiaka et al., 2022)
6	2.74	521	359, 197, 179, 161	Rosmarinic acid hexoside	hydroxycinnamic acid ester glycoside	(Silva et al., 2023)
7	2.87	359	197, 179, 161	Rosmarinic acid	hydroxycinnamic acid ester	(Ben et al., 2020)
8	2.88	719	359, 161	Sagerinic acid	cyclobutane lignans	(Ben et al., 2020)
9	2.94	193	178, 149, 134	Ferulic acid	hydroxycinnamic acid	(Wang et al., 2015)
10	3.60	205	179, 162, 161, 133	Caffeic acid vinyl ester	hydroxycinnamic acid derivative	
11	3.87	353	191	Chlorogenic acid	Phenolic acid	(Tsiaka et al., 2022)
12	4.33	395	197	Dimer (3,4-dihydroxyphenyl) lactic acid	hydroxy monocarboxylic acid	(Miron et al., 2013)
13	4.87	345	330, 315, 300, 287	Rosmanol	Diterpene lactone	(Gürbüz et al., 2019)
14	4.93	593	285	Kaempferol-3-O-rutinoside	flavonol glycoside	
15	5.90	487	469, 443, 305	3,6,19-trihydroxy-Urs-12-en-28-oic acid	Pentacyclic triterpenoid	GNPS
16	6.16	301	301, 283, 185, 229, 257	Sternbin	Flavanone	GNPS
17	6.18	377	329, 309, 265	1-decoxy-1,4-dioxo-4-[(E)-prop-1-enoxy]butane-2-sulfonic acid		GNPS
18	6.21	455 (+)	409, 391, 265, 191	Micromeric acid	Pentacyclic triterpenoid	GNPS
19	6.43	293	276, 275, 257, 197	13-Hydroxy-9,11,15-octadecatrienoic acid	unsaturated hydroxy fatty acid	GNPS
20	6.63	325	197, 191, 183, 147	10-(4-Sulfophenyl)decanoic acid	Organic Fatty acid derivative	GNPS
21	6.84	507	279, 153	16-Acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-9,11,15,16-tetrahydro-3H-cyclopenta[a]phenanthren-17-ylidene]-6-methylhept-5-enoic acid		GNPS
22	6.90	555	493, 359, 225	Salvianolic acid K isomer	Lignans	(Silva et al., 2023)
23	7.18	345	330, 315, 299, 286	Rosmanol	Diterpene lactone	(Gürbüz et al., 2019)
24	7.21	399	353, 191, 179, 135	CQA ester	Phenolic acid ester	(Liu et al., 2022)
25	7.37	473 (+)	455, 427, 409, 249	Maslinic acid (2-hydroxyoleanolic acid)	pentacyclic triterpenoid	GNPS
26	7.44	455 (+)	409, 391, 265, 191	Micromeric acid isomer	Pentacyclic triterpenoid	GNPS
27	7.59	561	515, 399, 353, 191, 179, 161, 133	diCQA ethyl ester	Phenolic acid ester	(Liu et al., 2022)
28	7.67	677	515	CQA hexosyl hexoside	Phenolic acid glycoside	(Liu et al., 2022)
29	7.85	325	257, 216, 197, 183, 163	10-(4-Sulfophenyl)decanoic acid	Organic Fatty acid derivative	GNPS
30	8.28	457(+)	439, 411, 407	Ursolic acid	pentacyclic triterpenoid	GNPS
31	8.52	609	577, 551, 489	Penta methoxy dihydro biapigenin	Biflavonoid	(Emam et al., 2021)
32	8.65	391	331, 313, 279, 167, 149	6-Hydroxylithocholic acid	Steroid	GNPS
33	8.74	477	431, 345, 264	3,4-dihydroxy-2-methyloctadecoxy]-6-(hydroxymethyl)oxane-3,4,5-triol		GNPS
34	8.99	593 (+)	533, 431	ND		
35	9.60	535	507, 447, 405,300	longirostreroneA	organic heterotricyclic compound	GNPS
36	12.34	309	267, 255, 215, 209, 146, 157, 97	1-[2-methyl-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]ethanone		GNPS
37	12.49–14.84	339	275, 265, 183	[5-Acetyloxy-3-(hydroxymethyl)-2-oxo-6-propan-2-ylcyclohex-3-en-1-yl] 3-methylpentanoate		GNPS
38	12.60	325	265, 239, 216, 197, 189, 183	10-(4-Sulfophenyl) decanoic acid	Organic Fatty acid derivative	GNPS
39	13.60	282	265, 247	Oleic acid	Fatty acid	GNPS

(continued on next page)

Table 3 (continued)

No.	Rt (min)	Precursor mass (- or +)	Fragments	Compound Name	Chemical Class	Ref.
40	15.40	305	299, 191, 179, 162, 147	3-(2-Carboxyethylpentasulfanyl)propanoic acid		GNPS
41	16.14	327	283, 269,	(10E,15E)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid	Fatty acid	GNPS

Fig. 7. Molecular networking (MNW) was prepared using MS/MS data from *R. officinalis* L. (rosemary) leaf extract. Nodes are labelled with a parent mass.Fig. 8. Molecular networking (MNW) was prepared using MS/MS data from *M. officinalis* L. (lemon balm) leaf extract. Nodes are labelled with a parent mass.

The potent effect of the rosemary and lemon balm extracts is attributed to their active components that can be toxic to the mosquito vector, *C. pipiens*. The increased activity of *R. officinalis* L. compared to *M. officinalis* L. leaves extract could be explained by its high content of polyphenols and terpenoids, especially those belonging to rosmarinic acid derivatives. These types of metabolites have been identified that

might enhance the toxicity toward *C. pipiens* larvae and agree with those results published before (David et al., 2000; Kannathasan et al., 2011; Hadidy et al., 2022). To establish a scientific structural-activity relationship, we should consider the shared compounds between the two active extracts and the compounds exclusive to rosemary, which is the most active one.

The shared compounds between the active extracts could contribute significantly to their combined larvicidal activity. For instance, terpenoids, such as rosmanol, and micromeric acid are found in both extracts and belong to a class of compounds recognized for their bioactivity, including insecticidal potential. However, their potential role in larvicidal activity warrants investigation. Rosmanol is a diterpene, while micromeric acid is a triterpenoid. Both are a large and diverse class of naturally occurring organic metabolites derived from isoprene.

On the other hand, there are compounds exclusively identified in rosemary such as rosmanol methyl ether, rosmadial, rosmaridiphenol, *epi*-rosmanol methyl ether, and others. We suggest that the presence of metabolites corresponding to terpenoids with rosmarinic acid derivatives may increase larvicidal activity. It is possible that the larvicidal activity of these extracts is due to the presence of these and other bioactive compounds. However, further research would be needed to determine the exact mechanism of action and to perform a detailed structure–activity relationship analysis.

The potential mechanisms that may explain the toxicity of extracts from *R. officinalis* and *M. officinalis* on *C. pipiens* larvae could be due to several factors. The presence of phenolic and terpenoid compounds with antioxidant properties may damage the gut epithelial membrane in mosquito larvae (War et al., 2012, Raguvaran et al., 2023). Additionally, the extracts may reduce feeding activity (Marques et al., 2016), hinder insect digestive enzymes and processes (Czerniewicz et al., 2017; Yadav and Kant-Upadhyay 2022), and inhibit detoxification enzymes, including glutathione S-transferase (Wang et al., 2014). Additionally, the synergistic interaction between fatty acids and polyphenolic compounds is known to play a crucial role in insecticidal activity (Tak et al., 2017). In summary, although we can propose hypotheses regarding the larvicidal activity of these compounds based on their known properties, it is crucial to undertake a comprehensive scientific approach. This approach should involve the isolation of individual compounds, conducting structure–activity studies, and performing mechanistic investigations. Only through such a rigorous scientific process can we establish a robust structural–activity relationship and fully elucidate the potential of these natural products for effective mosquito control.

Limitations and future perspectives

Further studies could focus on investigating the potential synergistic effects of combining the most effective extracts in comparison with other natural or common synthetic larvicides to broaden their applicability in mosquito control programs. It will also be important to isolate and elucidate the structure of the major compounds that are accountable for the observed activities. Additionally, large-scale field trials would be necessary to assess the environmental impact of this larvicide and larvae resistance to it compared to traditional larvicides. If successful, this research could lead to the development of a safe, sustainable, and effective tool for controlling mosquito populations.

5. Conclusion

In this study, four Egyptian herbal extracts were evaluated as natural larvicides against the third instar larvae of the mosquito *C. pipiens*. Based on the obtained outcomes, the extracts of *R. officinalis* L. (rosemary) and *M. officinalis* L. (lemon balm) were more effective against the larvae of *C. pipiens*. Their exposure caused a high mortality rate and recorded LC₅₀ values of 9.795 and 26.505 µg/mL, respectively. It disrupted the biochemical and physiological parameters (total carbohydrates, total lipids, total protein, AChE, and GST) when compared with *C. intybus* (Chicory) and *B. vulgaris* (Chard) leaves and steam, which recorded LC₅₀ values of 150.867, 179.128, and 363.093 µg/mL, respectively. Moreover, the results of the UPLC/MS revealed that rosemary and lemon balm extracts involved numerous bioactive compounds, such as flavonoids, terpenoids, phenolic acids, and alkaloids. In general, rosemary and lemon balm extracts can be considered promising natural control agents against *C. pipiens* mosquitos, and these actions are attributed to their contents of bioactive constituents. Further studies are required for

the development and evaluation of a safe larvicidal formula based on these plant- extracts.

CRediT authorship contribution statement

Mostafa A. Asmaey: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hanan Y. Aati:** Writing – review & editing, Project administration. **Mahmoud Emam:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Nikolas Tsafantakis:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization. **Nikolas Fokialakis:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization. **Mohamed seif:** Writing – review & editing, Writing – original draft, Visualization, Software, Data curation. **Ahmed I. Hasaballah:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

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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References

- Abbasi, E., Vahedi, M., Bagheri, M., et al., 2022. Monitoring of synthetic insecticides resistance and mechanisms among malaria vector mosquitoes in Iran: a systematic review. *Heliyon*, e08830.
- Abdel-Haleem, D.R., Marrez, D.A., El Raey, M.A., et al., 2024. Assessment of the chemical profile and potential biocontrol of *Amphora coffeaeformis* against foodborne pathogens and *Culex pipiens* L. to ensure food safety. *J. Food Saf.* 44, e13124.
- Abdel-Shafi, I.R., Shoeib, E.Y., Attia, S.S., et al., 2016. Mosquito identification and molecular xenomonitoring of lymphatic filariasis in selected endemic areas in Giza and Qualioubiya Governorates, Egypt. *J. Egypt. Soc. Parasitol.* 46, 93–100.
- Adly, E., Hegazy, A.A., Kamal, M., et al., 2022. Midguts of *Culex pipiens* L. (Diptera: Culicidae) as a potential source of raw milk contamination with pathogens. *Sci. Rep.* 12, 13183.
- Alghamdi, A.G., 2021. Toxicological and biological effects of some plant extracts on the house mosquito *Culex pipiens*, the dominant species in Jeddah governorate. *Entomol. Res.* 51, 403–412.
- Ali, K., Sagheer, M., Rashid, A., et al., 2021. Bioactivity of medicinal plant extracts as toxicants and enzyme inhibitors against insect pests of stored commodities. *J. Crop Prot.* 10, 95–109.
- Al-Solami, H.M., 2021. Larvicidal activity of plant extracts by inhibition of detoxification enzymes in *Culex pipiens*. *J. King Saud Univ.-Sci.* 33, 101371.
- Al-Tohamy, R., Ali, S.S., Saad-Allah, K., et al., 2018. Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from Egyptian flora. *J. Appl. Biomed.* 16, 289–300.
- Asmaey, M.A., Salem, M.M., Emam, M., et al., 2023. Chemical constituents from *Colchicum palaestinum* (Baker) C. Archer with the assessment of its antioxidant, wound scratch, and tyrosinase repressive potential. *S. Afr. J. Bot.* 157, 209–218.
- Asmaey, M.A., 2023. Unravelling the Secrets of α -Pyrones from *Aspergillus Fungi*: A Comprehensive Review of Their Natural Sources, Biosynthesis, and Biological Activities. *Chem. & Biodiv.* 20 (11), e202301185.
- Asmaey, M.A., Hamed, A., Shaaban, M., 2024. Natural peroxides from plants: historical discovery, biosynthesis, and biological activities. *Chem. Biodiv.*, e202400644
- Assemie, A., Gameda, T., 2023. Larvicidal activities of *Allium sativum* L. and *Zingiber officinale* Rosc. extracts against filariasis vectors in Hadiya Zone, Ethiopia. *Biomed. Res. Int.* 2023, 6636837 <https://doi.org/10.1155/2023/6636837>.
- Belhumeur, P.N., Chen, D., Feiner, S., et al., 2008. Searching the world's herbaria: a system for visual identification of plant species. In: *Computer Vision—ECCV 2008: 10th European Conference on Computer Vision, Marseille, France, October 12–18, 2008, Proceedings, Part IV* 10. Springer.
- Ben, A.B., Rouabhi, R., Gasmi, S., et al., 2020. LC-MS analysis and antioxidant activity of the hydro-alcoholic extract of *Melissa officinalis* L. from Algeria. *Chem. J. Moldova* 15, 78–87.

- BK, S.K., Moural, T., Zhu, F., 2022. Functional and structural diversity of insect glutathione S-transferases in xenobiotic adaptation. *Int. J. Biol. Sci.* 18, 5713.
- Borrás-Linares, I., Stojanovic, Z., Quirantes-Piné, R., et al., 2014. *Rosmarinus officinalis* leaves as a natural source of bioactive compounds. *Int. J. Mol. Sci.* 15, 20585–20606.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Chafik, M., Afaf, B.J., 2020. The evaluation of the larvicidal activity of the essential oil extracted from *Officinalis rosmarinus* on morphometric parameters *Culex pipiens*. 10.
- Chaudhari, A.K., Singh, V.K., Kedia, A., et al., 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. *Environ. Sci. Pollut. Res.* 28, 18918–18940.
- Chellappandian, M., Thanigaivel, A., Vasantha-Srinivasan, P., et al., 2018. Toxicological effects of *Sphaeranthus indicus* Linn. (Asteraceae) leaf essential oil against human disease vectors, *Culex quinquefasciatus* Say and *Aedes aegypti* Linn., and impacts on a beneficial mosquito predator. *Environ. Sci. Pollut. Res. Int.* 25, 10294–10306.
- Conley, A.K., Fuller, D.O., Haddad, N., et al., 2014. Modeling the distribution of the West Nile and Rift Valley Fever vector *Culex pipiens* in arid and semi-arid regions of the Middle East and North Africa. *Parasites Vectors* 7, 289. <https://doi.org/10.1186/1756-3305-7-289>.
- Conti, B., Canale, A., Bertoli, A., et al., 2010. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol. Res.* 107, 1455–1461. <https://doi.org/10.1007/s00436-010-2018-4>.
- Crompton, M., Birt, L., 1967. Changes in the amounts of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. *J. Insect Physiol.* 13, 1575–1592.
- Czerniewicz, P., Sytykiewicz, H., Durak, R., et al., 2017. Role of phenolic compounds during antioxidative responses of winter triticale to aphid and beetle attack. *Plant Physiol. Biochem.* 118, 529–540. <https://doi.org/10.1016/j.plaphy.2017.07.024>.
- David, J.-P., Rey, D., Pautou, M.-P., et al., 2000. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. *J. Invertebr. Pathol.* 75, 9–18.
- Demarque, D.P., Espindola, L.S., 2021. Challenges, advances and opportunities in exploring natural products to control arboviral disease vectors. *Front. Chem.* 9, 779049.
- Djombou Feunang, Y., Eisner, R., Knox, C., et al., 2016. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. *J. Cheminf.* 8, 1–20.
- DuBois, M., Gilles, K.A., Hamilton, J.K., et al., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Ebadollahi, A., Ziaee, M., Palla, F., 2020. Essential oils extracted from different species of the Lamiaceae plant family as prospective bioagents against several detrimental pests. *Molecules* 25, 1556.
- El-Akhal, F., Ramzi, A., Farah, A., et al., 2021. Chemical composition and larvicidal activity of *Lavandula angustifolia* subsp. *angustifolia* and *Lavandula dentata* spp. *dentata* essential oils against *Culex pipiens* larvae, vector of West Nile virus. *Psyche: J. Entomol.* 2021, 1–7.
- El-Seedi, H.R., Yosri, N., Khalifa, S.A.M., et al., 2021. Exploring natural products-based cancer therapeutics derived from Egyptian flora. *J. Ethnopharmacol.* 269, 113626 <https://doi.org/10.1016/j.jep.2020.113626>.
- Elsoghair, A.A., El-Gaby, M.S., Shabaan, M.A., et al., 2024. Eco-friendly dyeing of cotton fabrics using a blend of *Olea europaea* L. and *Ziziphus spina-christi* L. Leaf extracts: chemical profiling and color fastness properties. *Egypt. J. Chem.* 67, 207–213.
- Emam, M., Abdel-Haleem, D.R., Salem, M.M., et al., 2021. Phytochemical profiling of *Lavandula coronopifolia* poir. Aerial parts extract and its larvicidal, antibacterial, and antibiofilm activity against *Pseudomonas aeruginosa*. *Molecules* 26, 1710.
- Enayati, A.A., Ranson, H., Hemingway, J., 2005. Insect glutathione transferases and insecticide resistance. *Insect Mol. Biol.* 14, 3–8. <https://doi.org/10.1111/j.1365-2583.2004.00529.x>.
- Ernst, M., Kang, K.B., Caraballo-Rodríguez, A.M., et al., 2019. MolNetEnhancer: enhanced molecular networks by integrating metabolome mining and annotation tools. *Metabolites* 9, 144.
- Farag, S.M., Moustafa, M.A., Fónagy, A., et al., 2024. Chemical composition of four essential oils and their adulticidal, repellence, and field oviposition deterrence activities against *Culex pipiens* L. (Diptera: Culicidae). *Parasitol. Res.* 123, 110.
- Gaber, M., Ahmad, A.A., El-Kady, A.M., et al., 2022. Dengue fever as a reemerging disease in upper Egypt: diagnosis, vector surveillance and genetic diversity using RT-LAMP assay. *PLoS One* 17, e0265760.
- Gürbüz, P., Martínez, A., Pérez, C., et al., 2019. Potential anti-Alzheimer effects of selected Lamiaceae plants through polypharmacology on glycogen synthase kinase-3 β , β -secretase, and casein kinase 1 δ . *Ind. Crop Prod.* 138, 111431.
- Haba, Y., McBride, L., 2022. Origin and status of *Culex pipiens* mosquito ecotypes. *Curr. Biol.* 32, R237–R246. <https://doi.org/10.1016/j.cub.2022.01.062>.
- Hadidy, D.E., El Sayed, A.M., Tantawy, M.E., et al., 2022. Larvicidal and repellent potential of *Ageratum houstonianum* against *Culex pipiens*. *Sci. Rep.* 12, 21410. <https://doi.org/10.1038/s41598-022-25939-z>.
- Hamama, H.M., Zyaan, O.H., Ali, O.A.A., et al., 2022. Virulence of entomopathogenic fungi against *Culex pipiens*: impact on biomolecules availability and life table parameters. *Saudi J. Biol. Sci.* 29, 385–393.
- Hillary, V.E., Ceasar, S.A., Ignacimuthu, S.J., 2024. Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomol. Exp. Appl.* 172, 195–214.
- Kannathasan, K., Senthilkumar, A., Venkatesalu, V., 2011. Mosquito larvicidal activity of methyl-p-hydroxybenzoate isolated from the leaves of *Vitex trifolia* Linn. *Acta Trop.* 120, 115–118.
- Kao, C.-H., Hung, C.-F., Sun, C.-N., 1989. Parathion and methyl parathion resistance in diamondback moth (Lepidoptera: Plutellidae) larvae. *J. Econ. Entomol.* 82, 1299–1304.
- Karumaratne, S., Surendran, S., 2022. Mosquito control: a review on the past, present and future strategies.
- Khater, H., Soliman, D.E., Slim, A., et al., 2023. Larvicidal efficacy of fifteen plant essential oils against *Culex pipiens* L. mosquitoes in Egypt. *Egypt. J. Vet. Sci.* 54, 183–192.
- Knight, J.A., Anderson, S., Rawle, J.M., 1972. Chemical basis of the sulfo-phosphovanillin reaction for estimating total serum lipids. *Clin. Chem.* 18, 199–202.
- Koliopoulos, G., Pitarokili, D., Kioulos, E., et al., 2010. Chemical composition and larvicidal evaluation of *Mentha*, *Salvia*, and *Melissa* essential oils against the West Nile virus mosquito *Culex pipiens*. *Parasitol. Res.* 107, 327–335.
- Liu, C., Wahefu, A., Lu, X., et al., 2022. Chemical profiling of Kaliziri injection and quantification of six caffeoyl quinic acids in beagle plasma by LC-MS/MS. *Pharmaceuticals* 15, 663.
- Marques, T.R., Caetano, A.A., Alves, D.S., et al., 2016. *Malpighia emarginata* DC. bagasse acetone extract: phenolic compounds and their effect on *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae). *Chil. J. Agric. Res.* 76, 55–61.
- Miron, T., Herrero, M., Ibáñez, E., 2013. Enrichment of antioxidant compounds from lemon balm (*Melissa officinalis*) by pressurized liquid extraction and enzyme-assisted extraction. *J. Chromatogr. A* 1288, 1–9.
- Nasr, H.M., Hawas, U.W., Mousa, S.A., et al., 2018. Isolation and identification of *Emericella nidulans* secondary metabolites. *Chem. Res. J* 3, 114–119.
- Nazeh, M., Asmaey, M.A., Eladly, A., et al., 2024. Metabolomic profiling of streptomycetes griseorubens with the evaluation of their antioxidant and anticancer potentialities. *Egypt. J. Chem.*
- Niroumand, M.C., Farzaei, M.H., Razkenari, E.K., et al., 2016. An evidence-based review on medicinal plants used as insecticide and insect repellent in traditional Iranian medicine. *Iran. Red Crescent Med. J.* 18.
- Onen, H., Luzala, M.M., Kigozi, S., et al., 2023. Mosquito-borne diseases and their control strategies: an overview focused on green synthesized plant-based metallic nanoparticles. *Insects* 14, 221.
- Oni, M.O., Ogungbete, O.C., Oguntuase, S.O., et al., 2019. Inhibitory effects of oil extract of green *Acalypha* (*Acalypha wilkesiana*) on antioxidant and neurotransmitter enzymes in *Callosobruchus maculatus*. *J. Basic Appl. Zool.* 80, 1–13.
- Pavela, R., Pavoni, L., Bonacucina, G., et al., 2021. Encapsulation of *Carlina acaulis* essential oil and carlina oxide to develop long-lasting mosquito larvicides: nanoemulsions versus nanoemulsions. *J. Pest. Sci.* 94, 899–915.
- Priya, S.S., Vasantha-Srinivasan, P., Altemimi, A.B., et al., 2023. Bioactive molecules derived from plants in managing dengue vector *Aedes aegypti* (Linn.). *Molecules* 28, 2386.
- Raguvaran, K., Kalpana, M., Manimegalai, T., et al., 2023. Bioefficacy of isolated compound l-isooleucine, N-allyloxy-carbonyl-, and dodecyl ester from entomopathogenic actinobacteria *Actinokineospora fastidiosa* against agricultural insect pests, human vector mosquitoes, and antioxidant activities. *Environ. Sci. Pollut. Res. Int.* 30, 42608–42628. <https://doi.org/10.1007/s11356-022-23565-w>.
- Rajagopalan, V., Venkataraman, S., Rajendran, D.S., et al., 2023. Acetylcholinesterase biosensors for electrochemical detection of neurotoxic pesticides and acetylcholine neurotransmitter: a literature review. *Environ. Res.* 115724.
- Ramzi, A., El Ouali Lalami, A., Ez zoubi, Y., et al., 2022. Insecticidal effect of wild-grown *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their main monoterpenes against *Culex pipiens* (Diptera: Culicidae). *Plants* 11, 1193.
- Rani, L., Thapa, K., Kanojia, N., et al., 2021. An extensive review on the consequences of chemical pesticides on human health and environment. *J. Clean. Prod.* 283, 124657.
- Riaz, N.J.P.J., 2023. Effect of different plants extract on acetylcholinesterase activity of *Aedes aegypti* and *Musca domestica* adults. *Pak. J. Sci.* 74, 12–19.
- Robert, L., 2001. Mosquitoes (Culicidae). Guide to Entomological Surveillance during Contingency Operations. Walter-Reed Army Institute of Research, Washington, DC.
- Senthilkumar, N., Varma, P., Gurusubramanian, G.J.P., 2009. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles Stephensii* (Liston) 104, 237–244.
- Shahat, M.A., El-Sheikh, T.M., Hammad, K.M., et al., 2020. Activity of *Otostegia fruticosa* (Lamiaceae) leaves extracts against lymphatic filariasis vector, *Culex pipiens* L. (Diptera: Culicidae). *Egypt. Acad. J. Biol. Sci. A, Entomol.* 13, 175–186.
- Shalaby, A., Khater, H.J.J., 2005. Toxicity of certain solvent extracts of *Rosmarinus officinalis* against *Culex pipiens* larvae. *Environ. Sci.* 69, 80.
- Sharma, P., Mohan, L., Srivastava, C.N.J.P.R., 2009. Anti-juvenile activity of *Azadirachta indica* extract on the development and morphology of filaria vector, *Culex quinquefasciatus* (Diptera: Culicidae) Say. *Parasitol. Res.* 105, 1193–1203.
- Sharma, P., Mohan, L., Dua, K.K., et al., 2011. Status of carbohydrate, protein and lipid profile in the mosquito larvae treated with certain phytoextracts. *Asian Pac. J. Trop. Med.* 4, 301–304.
- Shehata, A.Z.J., 2018. Repellent activity of *Deverra triradiata* (Apiaceae) extracts against *Anopheles sergentii* Theobald, *Culex pipiens* Liston and *Culex antennatus* Becker mosquitoes. *J. Egypt. Soc. Parasitol.* 48, 599–604.
- Shehata, A.Z., 2019. Biological activity of *Prunus domestica* (Rosaceae) and *Rhamnus cathartica* (Rhamnaceae) leaves extracts against the mosquito vector, *Culex pipiens* L. (Diptera: Culicidae). *Egypt. Acad. J. Biol. Sci. F. Toxicol. Pest Control* 11, 65–73.
- Silva, B.N., Cadavez, V., Caleja, C., et al., 2023. Phytochemical composition and bioactive potential of *Melissa officinalis* L., *Salvia officinalis* L. and *Mentha spicata* L. extracts. *Foods* 12, 947.
- Simpson, D., Bull, D., Lindquist, D., 1964. A semimicrotechnique for the estimation of cholinesterase activity in boll weevils. *Ann. Entomol. Soc. Am.* 57, 367–371.
- Sultan, K.M., 2020. Larvicidal efficacy of some natural products against *Culex pipiens* larvae. *Egypt. Vet. Med. Soc. Parasitol. J.* 16, 102–113.

- Tak, J.-H., Jovel, E., Isman, M.B., 2017. Effects of rosemary, thyme and lemongrass oils and their major constituents on detoxifying enzyme activity and insecticidal activity in *Trichoplusia ni*. *Pesticide Biochem. Physiol.* 140, 9–16.
- Tantawy, A.H., Farag, S.M., Hegazy, L., et al., 2020. The larvicidal activity of natural inspired piperine-based dienehydrazides against *Culex pipiens*. *Bioorg. Chem.* 94, 103464.
- Tantawy, A.H., Farag, S.M., Abdel-Haleem, D.R., et al., 2022. Facile synthesis, larvicidal activity, biological effects, and molecular docking of sulfonamide-incorporating quaternary ammonium iodides as acetylcholinesterase inhibitors against *Culex pipiens* L. *Bioorg. Chem.* 128, 106098.
- Tiwari, M., Dubey, V., Lahiri, A., 2020. Zika virus: an explosion in world. *Int. J. Pharm. Life Sci.* 11.
- Tsiaka, T., Kritsi, E., Lantzouraki, D.Z., et al., 2022. Assessing the phytochemical profile and potential of traditional herbal infusions against aldose reductase through in silico studies and LC-MS/MS analysis. *Appl. Sci.* 12, 8361.
- Uritu, C.M., Mihai, C.T., Stanciu, G.-D., et al., 2018. Medicinal plants of the family Lamiaceae in pain therapy: a review. *Pain Res. Manag.* 2018.
- Velamuri, R., Sharma, Y., Fagan, J., et al., 2020. Application of UHPLC-ESI-QTOF-MS in phytochemical profiling of sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*). *Planta Med. Int. Open* 7, e133–e144.
- Wang, X., Li, W., Ma, X., et al., 2015. Simultaneous determination of caffeic acid and its major pharmacologically active metabolites in rat plasma by LC-MS/MS and its application in pharmacokinetic study. *Biomed. Chromatogr.* 29, 552–559.
- Wang, Z., Zhao, Z., Abou-Zaid, M.M., et al., 2014. Inhibition of insect glutathione S-transferase (GST) by conifer extracts. *Arch. Insect Biochem. Physiol.* 87, 234–249. <https://doi.org/10.1002/arch.21192>.
- War, A.R., Paulraj, M.G., Ahmad, T., et al., 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.* 7, 1306–1320. <https://doi.org/10.4161/psb.21663>.
- WHO, 2005. Guidelines for Laboratory Field Testing of Mosquito Larvicides. WHO, Geneva.
- Yadav, N., Kant-Upadhyay, R., 2022. Therapeutic and insecticidal potential of plant terpenes: a review. *Int. J. Green Pharm.* 16, 14.
- Zeghib, F., Tine-Djebbar, F., Zeghib, A., et al., 2020. Chemical composition and larvicidal activity of *Rosmarinus officinalis* essential oil against west Nile vector mosquito *Culex pipiens* (L.). *J. Essent. Oil Bear. Plants* 23, 1463–1474.
- Zhou, B.-G., Wang, S., Dou, T.-T., et al., 2016. Aphicidal activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferases activities in *Myzus persicae* (Hemiptera: Aphididae). *J. Insect Sci.* 16, 11.
- Zibae, A., Bandani, A.R., Tork, M., 2009. Effect of the entomopathogenic fungus, *Beauveria bassiana*, and its secondary metabolite on detoxifying enzyme activities and acetylcholinesterase (AChE) of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae). *Biocont. Sci. Technol.* 19, 485–498.