

Original Article

A comparative study of the biological activity of an aqueous extract of pomegranate peel and *Lantana camara* leaves as an eco- friendly larvicidal agent against *Culex* mosquitoes

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Abstract: Mosquitoes pose a significant threat to public health, acting as vectors for deadly pathogens that infect both humans and animals. This study investigated the effectiveness of two aqueous extracts at concentrations of 0, 1, 1.5, and 2 g/L from pomegranate (*Punica granatum*) peels, as an agricultural residue, and *Lantana camara* leaves to control *Culex* mosquito larvae. The results show that both aqueous extracts are highly effective, low-cost, and eco-friendly against larvae, providing a suitable alternative to manufactured chemicals for controlling *Culex* mosquito larvae. These effects increase over time and at higher concentrations. The aqueous extract of pomegranate peel showed a greater effect after 72 hours than the *L. camara* leaf extract, compared with the control. Phytochemical screening identified alkaloids, coumarins, flavonoids, phenols, saponins, and tannins in both plants as bioactive compounds responsible for the antiparasitic activity. In addition, Quinones were found only in pomegranate peel, and Steroids were found in *L. camara* leaves. GC-MS analysis revealed 30 compounds in pomegranate peel, most notably 4 major antiparasitic compounds, including Hexadecanoic acid (0.69%) and Acridine (0.61%). In contrast, *L. camara* leaves contained 35 compounds, including 6 highly potent antiparasitic molecules, most notably 3-Allyl-6-methoxyphenol (4.09%) and 9,12-Octadecadenoic acid (2.26%). These compounds affected the larvae and caused their mortality.

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Introduction

Mosquitoes are among the most dangerous disease vectors in public health because they transmit pathogens and parasites to humans and animals (Dahmana and Mediannikov, 2020). As urban areas expand into mosquito habitats, mosquito populations become increasingly exposed to these pathogens. Environmental changes, including climate change and global warming, significantly impact the dynamics between mosquitoes and pathogens. These changes affect mosquito habitats, breeding patterns, and pathogen survival rates (Damian, 2026). *Culex*, *Anopheles*, and *Aedes* mosquitoes transmit mosquito-borne diseases such as lymphatic filariasis and malaria, as well as zoonotic pathogens like Japanese encephalitis; viral diseases include chikungunya, Zika, and dengue (Wei et al., 2026).

Mosquitoes have a holometabolous life cycle with four stages, completed in two environments: the aquatic environment for the egg, larval, and pupal

stages, and the terrestrial environment for the adult stage. The larvae undergo four instars; each instar resembles the previous one but is larger. Moreover, adult female mosquitoes are known vectors of various pathogens and are significant in public health (Liu and Yang, 2024).

Effective control of *Culex* mosquitoes depends on identifying locally productive breeding sites and understanding when, on whom, and where mosquitoes feed, especially in urban and peri-urban areas with inadequate sanitation and drainage. This ecological basis is essential for preventing or eliminating mosquito breeding sites (Hari et al., 2026).

In traditional treatment, medicinal plants were used to treat various parasitic diseases. They possessed curative and preventive effects on a wide range of parasites (AL-Snafi, 2024). Bioactive compounds from different medicinal plant species are active against microorganisms and parasitic infections. They are found in more than 400,000 plant species

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Figure 1. (A) The pond water at the botanical garden of the college, and (B) the *Lantana camara* plant.

worldwide; however, researchers have investigated only a small fraction. Researchers isolate and screen these compounds to investigate and determine their therapeutic properties (Haruna and Yahaya, 2021).

Pomegranate (*Punica granatum*: Lythraceae) is one of the oldest cultivated plants (Jebanesan et al., 2021), a fruit native to Central Asia, rich in bioactive compounds such as tannins, flavonoids, and polyphenols (Lo Muzio et al., 2025). Its peel extracts have been used successfully as pest and vector control agents and have shown significant insecticidal efficacy against mosquito larvae (Farag et al., 2021).

Lantana camara (Verbenaceae) is found in many parts of the world, and its various parts contain essential oils, phenols, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoids, phenyls, ethanoids, bisaccharides, quinones, saponins, etc. These compounds have pharmacological and biological activities and are important in treating many diseases, such as headache, fever, arthritis, rheumatism, neurological disorders, gastrointestinal disturbances, and respiratory infections (Jain et al., 2025). *Lantana camara* stem extracts were tested against parasites and showed anti-filarial activity, while the aqueous leaf extract was tested against common mosquito species (AL-Snafi, 2024).

This study investigated the effectiveness of aqueous extracts from pomegranate peel and *L. camara* in eliminating the larval stage of mosquitoes. To identify the chemical compounds in both plants that have antiparasitic effects or influence

mosquito growth stages, we used Gas Chromatography-Mass Spectrometry (GC-MS). The goal is to compare the efficacy of these two natural extracts in combating, reducing, and controlling mosquito populations.

Materials and Methods

Collecting water samples containing larvae: Water samples containing mosquito larvae were collected using a 250 mL dipper from the pond water (made for research purposes with pH = 7.1) at the botanical garden of the Ibn Al-Haitham College of Education for Pure Sciences, Department of Biology, in the Al-Adhamiya district, Baghdad Governorate (Fig. 1A). We collected samples from January to May 2026 using clean plastic containers and transported them to the parasitology laboratory. The mosquito larvae were identified as members of the genus *Culex*.

Plant extract preparation: In January 2026, pomegranate fruit samples were collected from local markets and thoroughly washed to remove dirt. Peels separated from the fruits, cut into small pieces, and dried. Quantities of leaves of *L. camara* were collected from the garden of Ibn Al-Haitham College of Education for Pure Sciences, University of Baghdad (Fig. 1B). The leaves were washed with running water and dried at laboratory temperature. We then ground both pomegranate peels and *L. camara* leaves into powder using an electric grinder.

The aqueous extract was prepared according to Harborne (1984). 100 g of powdered *P. granatum* peel

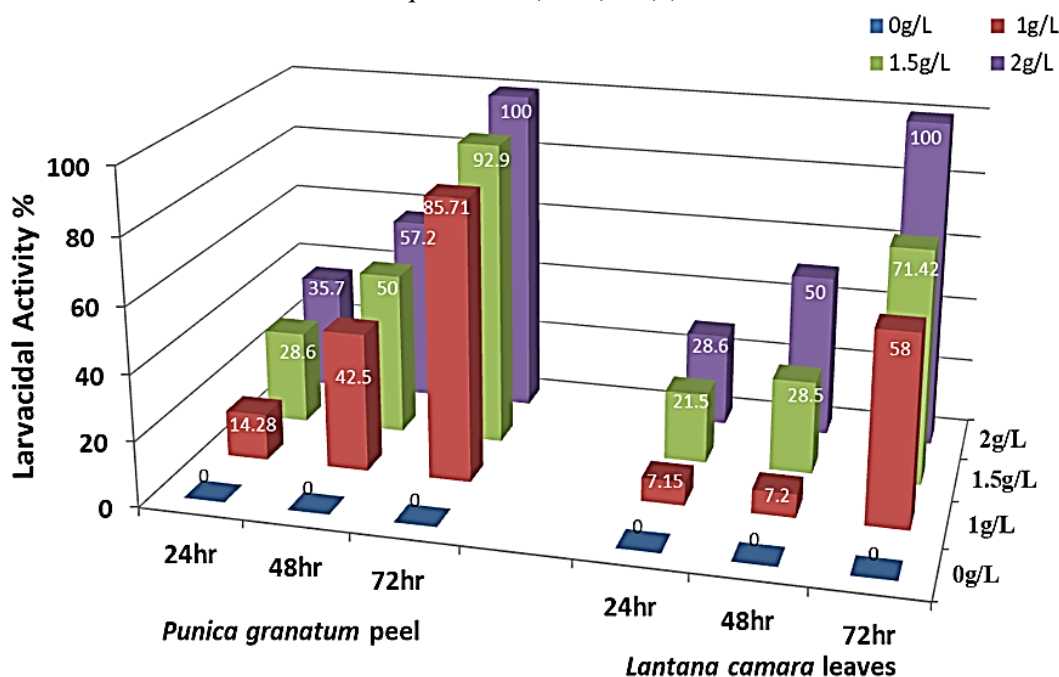


Figure 2. The larvicidal activity of *Punica granatum* peel and *Lantana camara* leaf extract against *Culex* mosquito larvae.

and *L. camara* leaves were placed in an electric mixer, 500 mL of distilled water was added, and the mixture was mixed for 5 min. The mixture was left for 30 min at laboratory temperature. Afterward, the extract was filtered through two layers of gauze to remove plant residues. Then the filtrate was centrifuged for 10-15 min at 3000 rpm. The filtrate was dried and used for further treatments.

Larval toxicity test: Aqueous extracts of *P. granatum* peel and *L. camara* leaves were prepared at 0, 1, 1.5, and 2 g/L in pond water and distributed in volumes of 100 mL into clean, visible plastic cups containing 14 *Culex* mosquito larvae at various life stages. We prepared three cups for each concentration, plus a control cup without treatment. We covered the cups with white gauze to prevent insect spread. Mean larval mortality was calculated for each treatment after 24, 48, and 72 hr. The mortality% was calculated using the following equation: Mortality % = (No. of dead larvae / No. of tested larvae) X 100

Chemical compound analysis of plant extract

Phytochemical screening of plant extracts:

Phytochemical screening of alkaloids, coumarins, flavonoids, phenols, saponins, steroids, tannins, and quinones was done for pomegranate peel and *L. camara* leaves in aqueous extracts according to

Harborne (1984).

GC–MS analysis: Chemical analysis of plant extracts was performed using a GC–MS system (model QP2010Plus) with a capillary column (Optima 5HT-0.25mm for 30m) (Shimadzu, Japan) at the Ibn Al-Bitar Research Center, Corporation of Scientific Research, Baghdad. For analysis, 0.4 g of pomegranate peel and *L. camara* leaf powder were dissolved in 10 mL of methanol (Merck Co.) at 60°C. The plant suspension was filtered through a 0.45 µm filter before being examined. After running the sample, all data were presented as a PDF file, and individual compounds were identified using reference data from the system library database based on mass spectral data and retention times for each sample (Brito et al., 2017).

Statistical analysis: The Statistical Analysis System (SAS) software (2021) was used to perform a one-way analysis of variance (ANOVA) to assess differences between the control and treatment groups, with $P < 0.05$ indicating statistical significance.

Results

The larvicidal activity of *P. granatum* peel and *L. camara* leaves extracts against *Culex* mosquito larvae at concentrations of 0, 1, 1.5, and 2 g/L during

Table 1. Phytochemical group screening of *Punica granatum* peel and *Lantana camara* leaves in aqueous extracts.

Active compound group	<i>P. granatum</i> peel	<i>L. camara</i> leaves	Positive results (after treating aqueous extracts)	References
Alkaloids	+	+	1 mL of extract added to 2 mL HCl with drops of Mayer's reagent. Green color or white precipitate indicates the presence of alkaloids	Harborne (1984).
Coumarins	+	+	1 mL of the extract + 1 mL 10% NaOH. Formation of a yellow color indicates the presence of coumarins.	
Flavonoid	+	+	1mL of the extract +1 mL 2N NaOH. Formation of yellow color indicates the presence of flavonoids.	
Phenols	+	+	1 mL of the extract + 2 ml DW + few drops of 10% FeCl ₃ . Blue/green color indicates phenols.	
Saponins	+	+	1 mL of extract is shaken in a test tube for 15 min; formation of foam indicates Saponins.	
Steroids	-	+	1mLof extract + 2 mL CHCl ₃ +1 mL H ₂ SO ₄ . Reddish-brown ring at the interface indicates steroids.	
Tannins	+	+	After adding 5% ferric chloride. Formation of dark blue or greenish black indicates the presence of Tannins.	
Quinones	+	-	1 mL of extract +1 mL H ₂ SO ₄ ; formation of red color indicates quinones	

+: Present, -: Absent,

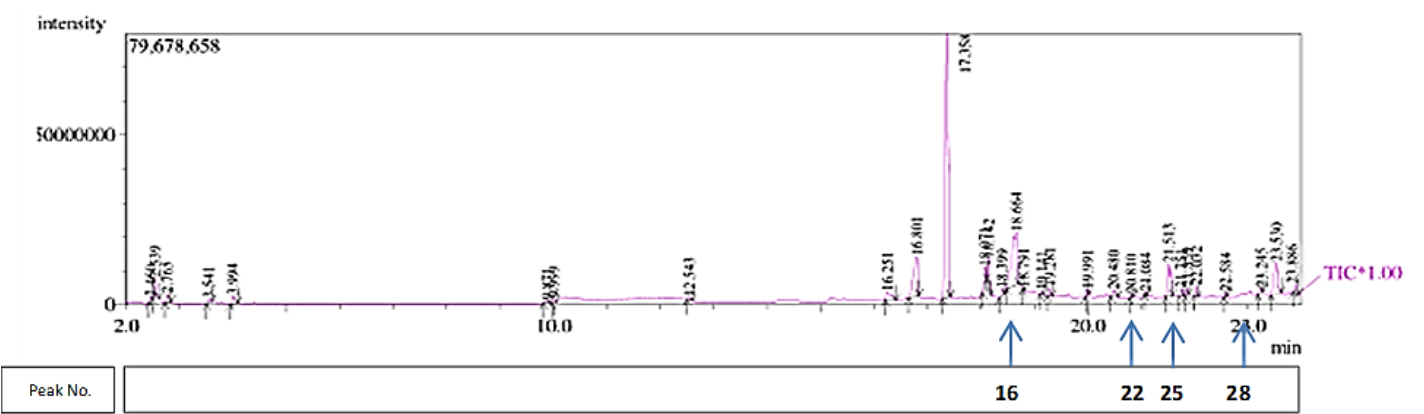


Figure 3. GC-MS analysis of *Punica granatum* peel powder, indicating the peak numbers of compounds active against parasites and/or mosquito larvae.

the time period of 24, 48, and 72 hr was illustrated in Figure 2. Table 1 shows the phytochemical screening of *P. granatum* peel and *L. camara* leaves in aqueous extracts.

All detected chemical compounds exhibit multiple biological activities. This was determined by the appearance of peaks of chemical compounds at different retention times (RT), as well as variations in their percentages. The analysis also indicated that these compounds included aromatic compounds, alkaloids, tannins, flavonoids, and others. However, this research focused specifically on identifying compounds effective against parasites and/or mosquito larvae.

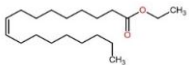
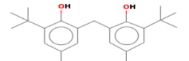
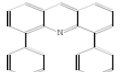
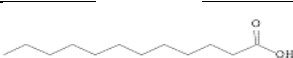
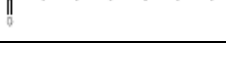
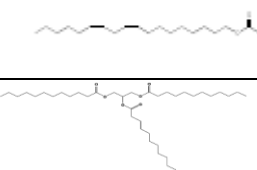
GC-MS chromatogram analysis showed that the *P. granatum* peel powder contained 30 chemical

compounds (Fig. 3). In comparison, 35 compounds were found in the aqueous extracts of *L. camara* leaves (Fig. 4). Of the 30 compounds in *P. granatum* peel powder, only 4 were found to have antiparasitic activity. In comparison, 6 separate compounds from *L. camara* leaves have the same activity. Table 2 summarizes the most significant compounds from the aqueous extracts of both plants.

Discussions

Although mosquito larvae are food for freshwater fish such as *Coptodon zillii* (Tang et al., 2025; Obaid et al., 2026), their numbers continue to increase, especially in ponds and rivers. Therefore, much research focuses on controlling their spread. One such method is using medicinal plants in biotherapeutic treatments. The

Table 2. GC-MS analysis data of anti-parasitic compounds with their chemical structures from *Punica granatum* peel and *Lantana camara* leaves.

Plant extracts	GC-MS Details				Chemical strictures
	Peak no.	R. Time (min)	Peak report TIC area (%)	Name and Formula	
<i>P. granatum</i> peel powder	16	18.791	0.17	Ethyl Oleate (C ₂₀ H ₃₈ O ₂)	
	22	21.084	0.14	Phenol, 2,2'-methylenebis 6 (C ₂₃ H ₃₂ O ₂)	
	26	21.859	0.69	Hexadecanoic acid (C ₁₉ H ₃₈ O ₄)	
	28	23.245	0.61	Acridine (C ₂₅ H ₁₇ N)	
<i>L. camara</i> leaves powder	12	5.872	0.95	Spiro[3.5]nona-5,7-dien-1-one (C ₁₂ H ₁₆ O)	
	13	9.415	4.09	3-Allyl-6-methoxyphenol (C ₁₀ H ₁₂ O ₂)	
	15	12.025	0.91	Dodecanoic acid (C ₁₂ H ₂₄ O ₂)	
	25	18.102	2.26	9,12-Octadecadienoic acid (C ₁₉ H ₃₆ O ₂)	
	32	21.508	0.77	Linalyl acetate (C ₂₀ H ₃₆ O ₂)	
	33	23.092	2.15	Dodecanoic acid,1,2,3-propanetriyl ester (C ₃₉ H ₇₄ O ₆)	

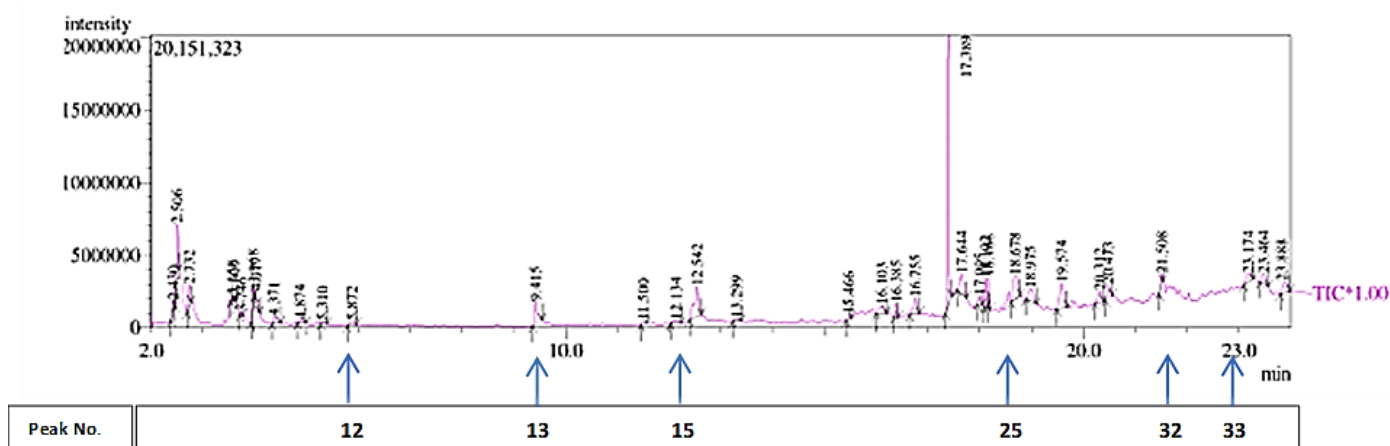


Figure 4. GC-MS analysis of *Lantana camara* leaves powder, indicating the peak numbers of compounds active against parasites and/or mosquito larvae.

present study results showed that the larvicidal activity of *P. granatum* peel and *L. camara* leaf aqueous extract is time- and concentration-dependent, with mortality increasing from 24 hr to 72 hr. Low

mortality (%) was observed at 24 hr, while moderate mortality was detected at 48 hr, confirming that the observed effects are due to the plant extract. The highest effect was observed at 72 hr across all

concentrations (0, 1, 1.5, and 2 g/L) ($P \leq 0.05$). The increase in larval mortality over time is similar to results from other studies on plant extracts such as *L. camara* extracts (Mondal et al., 2023; Sharma and Ajazuddin, 2024).

Punica granatum peel aqueous extract had higher efficacy, reaching 100% mortality at 72 hr. It had much higher efficacy than *L. camara* over the earlier 48 hr at concentrations of 1 and 1.5 g/L. Both plant aqueous extracts reached 100% mortality after 72 hr and even showed considerable activity at earlier exposure times. This mortality pattern and the larvicidal effect in both extracts likely resulted from the presence of powerful bioactive compounds, such as tannins and phenolics, in both extracts. These compounds disrupt larval metabolism and membrane integrity. The findings align with recent research showing that plant-based larvicides increase mortality with prolonged exposure, and that their efficacy varies by plant species and phytochemical composition (Lo Muzio et al., 2025).

The bioactive compounds in both aqueous extracts included alkaloids, coumarins, flavonoids, phenols, saponins, and tannins. Quinones were found only in *P. granatum* peel, and steroids only in *L. camara* leaves. Quinones affect cellular stress, destroy internal tissue structures, and accelerate mortality (Wang et al., 2006), while sterols, a subgroup of steroids, have been shown to delay insect development and increase mortality (Dahlin and Ruthes, 2024). Recent studies have reported many biological activities of *L. camara* extracts because of their rich content of phytochemical bioactives such as terpenoids, alkaloids, and flavonoids, which can act as larvicides at different parasite life stages (Ramírez et al., 2025). For example, Rajan and Varghese (2017) found that 80 mg per 100 ml of *L. camara* leaf extract resulted in 100% mortality after six hours of treatment, consistent with this study's findings.

In pomegranate peel aqueous extract, GC-MS analysis revealed four compounds as antiparasitic agents. Previous research indicates that Ethyl oleate (Peak 16) disrupts cellular and membrane structures in target cells as esters, such as exhibiting modest to

notable antiparasitic activity against fish parasitic diseases (Jawaji et al., 2024), and as an active component in natural insect and plant extracts against protozoan and ectoparasitic models (Panda and Luyten, 2018). Phenol, 2,2'-methylenebis (Peak 22), known as dichlorophen, is an antiparasitic agent used for tapeworm (cestode) treatments and in veterinary animals (Degerli and Tepe, 2015). Hexadecanoic acid (palmitic acid, Peak 26) shows notable antiparasitic properties, including multi-stage antiplasmodial activity against malaria parasites and moderate effects against other vector-borne or helminthic pathogens. It works by disrupting parasite cell membranes and targeting vital life-cycle proteins (Afolayan et al., 2024). The fourth compound (Peak 28), Acridine, shows strong antiparasitic activity, such as antimalarial, antiprotozoal, and anti-flatworm activity. Key historical and active compounds include quinacrine and pyronaridine (Rupar et al., 2018; Magesh et al., 2026).

Chemical analysis of *L. camara* leaf aqueous extract showed 35 compounds; six of these chemicals have antiparasitic activity. Spiro[3.5]nona-5,7-dien-1-one (Peak 12) and its derivatives show general antiparasitic interest, particularly targeting enzymes like trypanothione reductase in parasites such as *Trypanosoma brucei* (Turcano et al., 2020). The chemical 3-Allyl-6-methoxyphenol (Peak 13) in *L. camara* leaves is a natural phenylpropanoid compound found in essential oils. This chemical shows potential activity in folk medicine against larval vectors, though it is mostly studied in lab settings rather than as an approved human medicine (Kiraithe et al., 2016). Dodecanoic acid (Peak 15), commonly known as lauric acid, shows anti-parasitic activity primarily against protozoa like *Giardia duodenalis* by accumulating in the cytoplasm and rupturing the cell membrane. It is a medium-chain saturated fatty acid found naturally in coconut oil and breast milk (Rayan et al., 2005).

9,12-Octadecadienoic acid (Peak 25) (Linoleic acid) shows potential activity against certain pathogens and parasites, but it is not a standard standalone medication for human parasitic infections.

It is frequently identified as a bioactive constituent in plant and mushroom extracts that display anti-infective, anti-nematodal, or antiparasmodial properties in laboratory studies (Ahmed et al., 2021; Broschwitz et al., 2021; Hryckowian et al., 2024). Linalyl acetate (Peak 32) (often referred to in queries as linoleyl acetate) shows modest, context-dependent antiparasitic properties primarily as a component of plant essential oils from lavender and bergamot. It exhibits activity against specific nematode and protozoan targets, often acting synergistically with companion compounds like linalool (Oader et al., 2017; Hikal et al., 2021). Dodecanoic acid, 1,2,3-propanetriyl ester (Peak 33), commonly known as trilaurin or glyceryl trilaurate, is a glycerol ester of lauric acid identified in various plant and marine extracts that exhibit baseline antimicrobial or larvicidal screening properties (Sivakumar, 2011).

Conclusions

The current study confirms that aqueous extracts of pomegranate peel and *L. camara* leaves are highly effective, low-cost, and environmentally safe larvicides against *Culex* mosquito larvae. Their larvicidal activity increases with time and concentration; significant differences appear from 24–72 hours, with 100% mortality at higher concentrations. The pomegranate peel aqueous extract showed a greater effect after 72 hours than the *L. camara* leaf extract. Phytochemical screening identified the bioactive compounds responsible for the lethal effects in both aqueous extracts. The results highlight the potential of aqueous extracts of *P. granatum* peel and *L. camara* leaves as larvicides and effective biological agents against *Culex* mosquitoes, especially when the GC-MS-identified compounds are used as pure compounds.

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