

Investigation of the miticide and repellent effect of extracts of leaves of *Eucalyptus camaldulensis* and *Myrtus communis* against adults and larvae of the mosquito *Culex quinquefasciatus*

Dr. Ghazwan Thamer Khadir Al-Rashidi

Ministry of Education, General Directorate of Nineveh Education, Mosul, Iraq

Abstract

The study revealed a wide range of secondary metabolites, volatile oils, alkaloids, phenols, terpenoids, flavonoids, glycosides, saponins, and steroids in different proportions. Alcohols, glycerol, and cholesterol were also identified in the leaf extracts of *Eucalyptus camaldulensis* and *Myrtus communis*. While FAMES were analyzed, it was found that the ethanolic and aqueous extracts of *E. camaldulensis* leaves had a high ability and significant difference in killing the larval stage of the *Culex quinquefasciatus* mosquito at rates between 96% and 89% at a concentration of 10 mg/L, respectively, of the total number of larvae (20) within three days under standard conditions. The mortality rate of the larval stage decreased when the concentration was reduced to 1 mg/L, reaching 46% and 39%, respectively, compared to the control group, in which natural mortality did not exceed 0.2%. As for the leaf extract of *E. camaldulensis*, *M. communis* witnessed a significantly lower percentage of larval mortality for the ethanolic and aqueous extracts, as it recorded percentages ranging between 91% and 82% at a concentration of 10 mg/L, respectively. When the concentration decreased to 1 mg/L, it recorded significant percentages of 46% and 39%, respectively. According to the study, the repellent ability of pregnant adults for the ethanolic and aqueous extracts of *E. camaldulensis* and *M. communis* leaves was very high, as it was shown that the ethanolic and aqueous extract of *E. camaldulensis* leaves recorded a decrease in the number of egg rafts from 29.6 rafts in the control group to 6.6 and 11.5 rafts, respectively, with the number of eggs in each raft being reduced to 30% within 7 days. As for the extract of *M. communis* leaves, it recorded a significantly higher significant difference than the control group and a statistically significant difference from the leaves of *E. camaldulensis*, as the aqueous extract of *M. communis* recorded 4.6 and 6.6, respectively, within 7 days, and the number of eggs decreased by 40-50%. This may be the maximum repellent effect of all the extracts under study. It appears that *M. communis* extracts are the best at repelling female mosquitoes from egg-laying sites.

Keywords: *Culex quinquefasciatus*, secondary metabolites, *Eucalyptus camaldulensis*, *Myrtus communis*, extracts, repellent effect

Introduction

Mosquitoes play a vital role in transmitting deadly diseases that threaten human and animal life. While synthetic insecticides may be the first line of defense due to their rapid effectiveness, their continued use can lead to environmental damage. Therefore, tremendous efforts have been made to develop and find alternatives to kill and repel mosquitoes from their breeding sites (McGregor *et al.*, 2021) [2]. Mosquito species such as *Anopheles gambiae* and *Culex quinquefasciatus* are medically important as they transmit numerous viral and bacterial pathogens worldwide, such as dengue fever, yellow fever, malaria, and others (Khater *et al.*, 2016) [13]. To develop strategies to combat these dangerous diseases, we need to adequately understand the ecology and phylogeny of disease vectors. The evolutionary history of the *Culex quinquefasciatus* mosquito, and mosquito-borne pathogens are a major cause of disease worldwide, particularly in the Middle East and Africa. (Ali *et al.*, 2021) [2] Effective vector control and prevention of diseases transmitted by them requires knowledge of mosquito species, as there are approximately 45 recorded species in Iraq, and insecticide treatments are less effective at the present time on the larval stage, especially in the rainy season (Yousuf *et al.*, 2014) [31]. Resistance to insecticides, which may not be environmentally friendly, may lead to the killing of the real enemies of mosquitoes, allowing mosquitoes of all kinds to spread more. The development of this resistance is not a new phenomenon; It has become a global issue

(Vijayakumar *et al.*, 2020) [27]. Dengue fever and other mosquito-borne diseases are a serious public health problem, and current vector control methods may not be effective in reducing mosquito populations and thus limiting the transmission of epidemic diseases. There is an urgent need for new, alternative, or complementary tools and strategies to control mosquito species. The best alternatives may be plant extracts and natural enemies of the organism (Aydin *et al.*, 2017) [4], such as other insect species, bacteria, and parasites. Mosquito control, which focuses primarily on killing the larval stage of mosquitoes or depriving and expelling them from areas where they may breed, is easy to kill mosquito larvae while they are still present in water before maturity, which is a confined environment and easy to manage (Marimuthu *et al.*, 2016) [17]. Control methods targeting the larval stage are the most effective, in addition to the strategy of expelling adults from egg-laying sites (Narayanan *et al.*, 2021) [20]. The emergence of resistance to synthetic insecticides in particular poses a major challenge to vector control techniques. Plants are rich sources of biologically active compounds and produce many secondary metabolites into highly defensive chemicals to control insect pests. Here, plants have an advantage over synthetic insecticides in particular, which increases their preference for use over chemical insecticides (Rants'o *et al.*, 2023) [22]. Mosquitoes have become resistant to many insecticides, so the discovery of new plant-based insecticides is essential and inevitable. Synthetic insecticide applications are an important tool for pest control, but they have negative

environmental impacts and are incompatible with organic farming (Marimuthu *et al.*, 2016) ^[17]. Biological data collected through rearing and fieldwork are compared and discussed in light of known life cycle data (Silva *et al.*, 2020; Gosik *et al.*, 2019) ^[9, 25]. Plants are among the organisms that contain the most chemical compounds, or what are called secondary metabolites, which are very effective in eliminating the larval stage of mosquitoes, and which are safe, cheap, and at the same time environmentally friendly when used scientifically and systematically to eliminate the larval stages of mosquitoes. The leaves and fruits of *Eucalyptus camaldulensis* and *Myrtus communis* may be considered. These plants are among the promising plants in the field of controlling mosquito species through their extracts from leaves, fruits, or seeds (Marc *et al.*, 2021) ^[5]. This study will be summarized to determine the extent of the killing effect of the aqueous and alcoholic extracts of the leaves of *Eucalyptus camaldulensis* and *Myrtus communis* on the larval stage and the repellent effect on adult mosquitoes of the species *Culex quinquefasciatus*.

Objective of the study

To determine the minimum and maximum lethality of aqueous and alcoholic extracts of the leaves of *Eucalyptus camaldulensis* and *Myrtus communis* against the larval stage of the mosquito *Culex quinquefasciatus*, and the minimum repellent against pregnant adults.

Materials and Methods

Mosquito Collection and Rearing: Immature stages of *Culex quinquefasciatus* mosquitoes, egg rafts, and mosquito larvae of different ages were obtained from some rainwater drainage channels at the University of Mosul. The samples were transported to the laboratories of the Research Unit of the Department of Life Sciences, College of Education, University of Mosul, in April 2025. The tested mosquito strains were classified at the Natural History Museum, University of Baghdad. The larvae were taken and placed in oval plastic containers containing water, which had been left for 48 hours. The containers were sized 35 x 20 cm at the center and 10 cm high. After hatching, the larvae were fed a mixture of ground yeast and biscuits. The water was changed every four days to prevent the formation of a yeast layer on the water surface (WHO, 2023). After emergence, the adults were placed in cages and fed a 15% honey solution for 3 days. The adult females were then fed at night with a naked breast pigeon, and the eggs were subsequently laid next to plastic trays. The light period in the insectary was 12:12 hours, the relative humidity was 65%, and the temperature was 27±3°C.

Plant Collection

Eucalyptus camaldulensis and *Myrtus communis* leaves were collected from slum areas in Mosul. They were manually cleaned of impurities and taken to the laboratory. The young, well-formed leaves were isolated, cut, and stored well for experiments and the extraction of secondary metabolites. They were placed in distilled water for 48 hours until the water turned light brown. Samples were then taken for chemical analysis (WHO, 1996).

Phytochemical Screening of the Extract

Eucalyptus camaldulensis and *Myrtus communis* leaves underwent preliminary phytochemical screening to

determine their chemical constituents. The method described by the American Aromatic Plant Association (AOAC) (1990) ^[20] was used to screen the two plants. GC-MS tests were then performed at the University of Technology in Baghdad, and IR testing was performed at the Department of Chemistry at the University of Mosul. The secondary metabolites were identified, and they were found to contain tannins, alkaloids, phenols, terpenoids, flavonoids, cardiac glycosides, saponins, steroids, and alcohols in varying proportions. The extracts were stored in special storage tubes to prepare aqueous and alcoholic extracts for subsequent sensitivity tests against the mosquito species under study (Mohamed *et al.*, 2022) ^[19].
Extract Preparation

Aqueous Extract Preparation

10 grams of crushed leaves from each plant were weighed, 100 ml of distilled water was added, and the extract was left in a vibrating device for 24 hours. The extract was then filtered to remove all impurities using special filter paper. The solution was collected in sterile tubes and stored in the refrigerator for sensitivity testing on mosquito larvae and adults.

Preparation of Ethanol Extracts

Eucalyptus camaldulensis and *Myrtus communis* leaf fragments were used. 10 g of each sample was added to 100 ml of 70% diluted ethanol to prepare the extracts and left to stand for 24 hours. The plant extract of each solvent/fraction was left in the overlying liquid, which was filtered and evaporated. The extract was then collected as a dry powder, weighed, and diluted to the required concentrations for sensitivity experiments (Yaméogo *et al.*, 2021) ^[30].

Biocalibration Tests

Laboratory experiments were conducted at a relative humidity of 75-85%, a temperature of 27 ± 2°C, and a photoperiod of 12 hours/day. The larvicidal activities of *E. camaldulensis* and *M. communis* leaf extracts were determined according to the World Health Organization (WHO) method (1996) ^[28]. Twenty third- and early fourth-instar *C. quinquefasciatus* larvae were transferred via a suction tube to special 2-liter test beakers filled with 1 liter of tap water (which had been left for 48 hours). All test beakers were standardized and provided with adequate food to prevent larval death from starvation or competition. Extract concentrations (1 ml/100 ml, 5 ml/100 ml, and 10 ml/100 ml) of both the aqueous and ethanolic extracts were then applied. Three replicates were applied to each extract, with the control group or the control group without any extract. After 72 hours, the overall results were recorded, along with the larval mortality rate. Repellent tests for pregnant female *Culex quinquefasciatus* mosquitoes:

One hundred adult mosquitoes about to emerge were placed in a special cage for rearing first-generation mosquitoes to produce adult males and females. After hatching, a 15% honey solution was fed to the males and females for seven days to allow mating opportunities. A pigeon with its breast feathers removed was then placed inside the cage to give the pregnant females an opportunity to lay eggs. Five trays were then placed inside the cage. The first contained larval rearing water, which served as a positive control (Pavela *et al.*, 2019) ^[21]. The second and third trays contained *Myrtus communis* extract (10% aqueous and alcoholic extract of

each tray). The fourth and fifth trays contained water containing a 10% aqueous and alcoholic extract of *E. camaldulensis* leaves. The number of egg boats in each of the four trays was counted daily until day seven, and the results were recorded.

Statistical Analysis

The variable Y (average larval mortality rate after 24 hours) was plotted against the variable X (corresponding concentrations) for regression analysis using Microsoft Excel 2016. Regression lines were constructed to determine the 50% and 95% lethal concentrations (LC50 and LC95) for *Culex quinquefasciatus* larvae (Bader *et al.*, 2021 [5]).

Results and Discussion

Measurement of leaf extract components using GC-MS

Secondary metabolites in the leaves of *E. camaldulensis* and *M. communis* were measured and identified using the GCMS Analysis Results technique at the Environment and Water Department at the Environmental Research Center, University of Technology. They were found to contain tannins, alkaloids, phenols, terpenoids, flavonoids, cardiac glycosides, saponins, and steroids in varying proportions, according to Table (1). Alcohols, glycerol, and cholesterol were identified as derivatives of trimethylsilyl (TMS), while FAMES (fatty acids) were analyzed as parent compounds (Siciliano *et al.*, 2005) [24]. Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Hewlett-Packard 6890 gas chromatograph equipped with a Rtx-5 capillary column (J&W Scientific, 30 m × 0.25 mm inner diameter × 0.15 μm film thickness), and an SSQ 710 equipped with an HP 6890 gas chromatograph. These results indicate that the leaves and secondary metabolites in the tissues of these plants contain an abundance of phenolic, volatile, and aromatic compounds. Insecticides, insect growth regulators, and microbial agents are commonly used to target mosquito larvae. However, these are harmful to humans and the environment and may kill natural enemies of mosquitoes (Gosik *et al.*, 2019) [9] and may lead to the opposite result after these larvae acquire resistance to insecticides (Elimam *et al.*, 2009) [7]. Therefore, finding “environmentally friendly”

alternatives to pesticides is of paramount importance. GC-MS/MS technology was used to examine the metabolic contents of various leaf extracts from *E. camaldulensis* and *M. communis*. Cardenolides, such as 15-hydroxy-calactin, 16-hydroxy-calactin, 12-hydroxy-calactin, calactin, and oscaridin, were found abundantly in the leaves of *E. camaldulensis* and *M. communis* (Jiraungkoorskul, 2016) [11]. In addition to what was mentioned above, the secondary metabolites produced by *E. camaldulensis* and *M. communis* plants have a wide range of physiological and biological activities, including deterrent and anti-feeding activity for larval feeding, according to a study. Alves *et al.* (2018) [3] examined the phytochemical screening and biological activity of other genera close to the plants under study, and presented the results of the chemical screening. The phytochemical properties of these plants were obtained. The results obtained from the current study were similar to previous results.

Table 1: The percentages of chemical compounds produced from plant leaf extract using (GC-MS) technology:

Compound	Compound Nature	Leaves ratio in	
		<i>M. communis</i>	<i>E. camaldulensis</i>
Octadecadienal	Alcohols	++	+
Tannins	Terpenes	+++	+
Flavonoids		++	+
n-Hexadecanoic acid	Acids	+	+++
Glycosides	Glucosides	+	++
Alkaloids	Alcohols	+	++
Phenols	Phenols	++++	+++
Terpenoids	Terpenes	++	++
Ethyllic acid	Acids	+	+
Dipirartril-tropico	Sulfates	++++	++
Oleic Acid	Fatty Acids	+	++
1-Nitro-2-propanone	Propane	++	+
Oxacycloheptadec-8-en-2-one	Volatile Oils	++++	+++
Musk ambrette	Volatile Oils	++++	+++

Where (+) is a specific standard ratio and is a comparative indication of the density of compounds in the leaf extract of the plants under study.

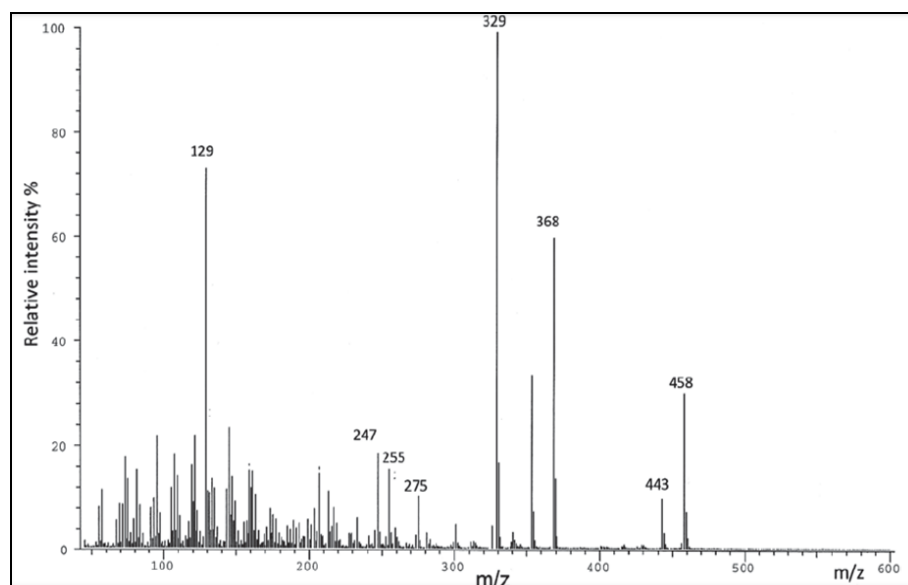


Fig 1: shows secondary metabolites according to the GC-MS technique test and at different proportions for the plants under study

The lethal effect of the aqueous extract of *E. camaldulensis* and *M. communis* leaves against *Culex quinquefasciatus* larvae.

Sensitivity test

Sensitivity test of the aqueous solution

The sensitivity of *Culex quinquefasciatus* larvae was tested by preparing the aqueous extract for 24 hours. The standard error of the logarithmic dose (SE(Y)) was 1.47, while the SE(X) was 0.65. The aqueous extract of *E. camaldulensis* leaves showed a high level of toxicity against third-instar *Culex quinquefasciatus* larvae. The mortality rate of third and fourth instars at concentrations of 1/100 mg/L, 5/100 mg/L, and 10/100 mg/L was 39%, 67%, and 89%, respectively, of the total number of larvae (20) over a three-day period. Larval mortality began after 8 to 16 hours. The *E. camaldulensis* leaf extract outperformed the *M. communis* leaf extract by a very small percentage, which may be due to the difference in the standard concentrations

of the chemical compounds in both extracts (Singh *et al.*, 2015) [26] as shown in Table (1). Compared to the control group, there was a huge and significant difference in the mortality rate, as the mortality rate or natural death rate of the larvae in the control group reached 0.02% of the total larvae rate over 7 days. It is worth noting that the highest mortality rate was on the third day of the sensitivity test for third-instar larvae to the aqueous extract of plants under investigation. Study: This study has shown the possibility of gradually eliminating harmful chemical pesticides by the aqueous extract of *E. camaldulensis* and *M. communis* leaves, due to its natural product and the large amount of environmentally friendly compounds that are lethal to mosquito larvae. This study may be consistent with the study (Mahmoudi *et al.*, 2021) [15] in which it was discovered that the fresh leaf extract of *E. camaldulensis* has larvicidal properties against the *Diptera* order specifically, especially mosquito larvae.

Table 2: The killing effect of the aqueous extract of *E. camaldulensis* leaves and *M. communis* leaves against third-instar larvae of *Culex quinquefasciatus* at a density of 20 larvae/IJS

Average Total Loss	Average Larval Mortality	Time/Hour										Concentration of The Extract	Type of Plant				
		72	64	56	48	36	30	24	16	8	0						
0 ed												0 ed	E. camaldulensis				
a											1.3	0.5 c		0.7 b	1.1 ab	1.2 a	a
A																	
0 de																	
a																	
	% 89 A	2.5 a	1.3 d	1.8 c	2.0 b	1.9 b	2.3 a	2.8 a	2.3 a	1.0 de							Control Group

Vertically similar lowercase letters mean there is no significant difference between them.

Vertically similar uppercase letters mean there is no significant difference between them.

Methanol solution sensitivity test

The methanol extract proved to be more effective as a larvicide than the aqueous extract. Table (3) shows that there are significant differences in the mortality rate at all concentrations under study, with the mortality rate of third-instar larvae reaching 96% at a concentration of 10 mg/L, which is the absolute maximum larval mortality rate. The other concentrations also outperformed the aqueous extract in comparison. With the stability of the superiority of the methanolic extract of *E. camaldulensis* leaves over the extract of *M. communis* leaves by a very small percentage, as the best percentage of mortality was recorded at a concentration of 10 mg/L, and this may be due to the difference in the standard concentrations of the chemical compounds in both extracts that were previously mentioned, and with the stability of the natural death rate in the control group (Ghanem *et al.*, 2022) [8] and according to Figure (2), it may be clear from this that the extract of these plants may

lead to the development of new methods for insect monitoring and control of the mosquito *Culex quinquefasciatus* [30]. These plant extracts may be new alternatives to synthetic insecticides in future mosquito control plans. These results are similar to a study by Baranitharan and Dhanasekaran (2014) [6], in which *C. procera* leaves were extracted from methanol and tested for their larvicidal activity against dengue vectors. Larvicidal bioassays using the alcoholic extract of *C. procera* leaves showed efficacy with LC50 and LC90 values of 78.39% and 100%, respectively. After prolonged exposure of the larvae to the extracts, the potential toxicity of the extract increased, with the LC50 values decreasing by 2.3%. From this it is clear that the aqueous and alcoholic extract is highly efficient in killing and destroying the larval stage, which may reach 65% to 73% on average for the *E. camaldulensis* leaf extract and 59% to 70% on average for the *M. communis* leaf extract (Kraemer *et al.*, 2015).

Table 3: The killing effect of the Ethanolic extract of *E. camaldulensis* leaves and *M. communis* leaves against third-instar larvae of *Culex quinquefasciatus* at a density of 20 larvae/IJS

Average Total Loss	Average Larval Mortality	Time/Hour										Concentration of The Extract	Type of Plant
		72	64	56	48	36	30	24	16	8	0		
% 73	% 46 DE	1.5 a	1.4 a	1.2 b	1.3 ab	0.8 d	1.2 a	1.1 b	1.3 a	0.3 ce		1 mg/L	E. camaldulensis A
	% 75 B	2.8 a	1.2 d	1.8 c	2.2 ab	1.7 c	1.3 d	1.7 c	2.0 b	0.6 ce		5 mg/L	
% 70	% 96 A	3.5 a	1.3 d	1.8 c	1.2 ab	1.9 bc	2.3 b	2.8 a	2.3 b	2.0 bc		10 mg/L	M. communis AB
	% 48 DE	1.5 a	1.2 b	1.2 b	1.3 b	1.6 a	1.5 a	1.2 b	1.1 c	0.3 eh		1 mg/L	
% 70 CE	2.2 a	1.2 c	1.5 b	1.2 a	1.3 c	1.2 c	1.5 b	2.1 a	0.5 eh			5 mg/L	

Vertically similar lowercase letters mean there is no significant difference between them.

Vertically similar uppercase letters mean there is no significant difference between them.

The repellent effect of the ethanolic extracts of the leaves of *E. camaldulensis* and *M. communis* against adult *Culex quinquefasciatus* mosquitoes:

It is known that pregnant female *C. quinquefasciatus* mosquitoes lay their eggs in the water in boat-like clusters, each containing 200-300 fertilized eggs in normal conditions (Jones *et al.*, 2021) [12]. Many studies, including this current study, have revealed that the mother mosquito carries maternal characteristics, choosing the most suitable environment for laying eggs. This phenomenon was exploited to repel mosquitoes, which we call indirect control of mosquito spread. 100 mosquito pupae, about to emerge, were placed in a special cage for rearing first-generation mosquitoes to obtain adult males and females at a fixed concentration of 10 mg/L, which was mentioned in detail previously and is shown in Table (4). Regardless of the number of adults, under laboratory conditions, it was found that There were significant differences between the *E. camaldulensis* plant extract and the control group, reaching more than 40% for the aqueous extract, where a decrease was recorded in the number of egg rafts from 29.6 rafts carrying 7250 eggs in the control group to 11.5 rafts carrying 2300 eggs in the *E. camaldulensis* aqueous extract group. The ethanolic extract of the same plant also witnessed a significant difference, reaching 6.6 rafts, which is equivalent to a decrease of up to 75% in egg laying, with an average of 1260 eggs per week. The extract of *M. communis* leaves showed a significantly higher moral difference than the control group and a statistically

significant moral difference from the leaves of *E. camaldulensis*, as the aqueous extract of *M. communis* recorded 6.6 boats with a number of eggs reaching 1150 eggs, while the ethanolic extract of the same plant recorded a repellent rate of only 4.6 boats within 7 days with a number of eggs estimated at 506 eggs only, i.e. an estimated 90%, and this is considered the maximum repellent effect of all the extracts under study. According to Figure (3), it is clear that the extracts of *M. communis* are the best ever in repelling female mosquitoes from egg-laying sites, as well as the appearance of what is called the phenomenon of egg confinement in females (Seghier *et al.*, 2020) [23]. We note that the later the females lay eggs, the fewer eggs in the egg boats due to the mother mosquitoes consuming the protein present in the eggs to remain for a longer period for egg-laying, according to this study and other studies (Hussin *et al.* 2020) [10], and from the above, indirect control by exploiting volatile oils and phenolic compounds with pungent and aromatic odors resulting from these plant extracts may be one of the most effective methods for controlling the *C. quinquefasciatus* mosquito. According to this study and the data and figures mentioned above, it has been shown that female mosquitoes are very sensitive to the presence of these extracts in the water where these females may lay eggs and spread widely. The presence of these compounds in the water, which are environmentally friendly and at the same time repellent to mosquitoes, may be one of the safest methods for biological control of mosquitoes (Alahmed *et al.*, 2019) [1].

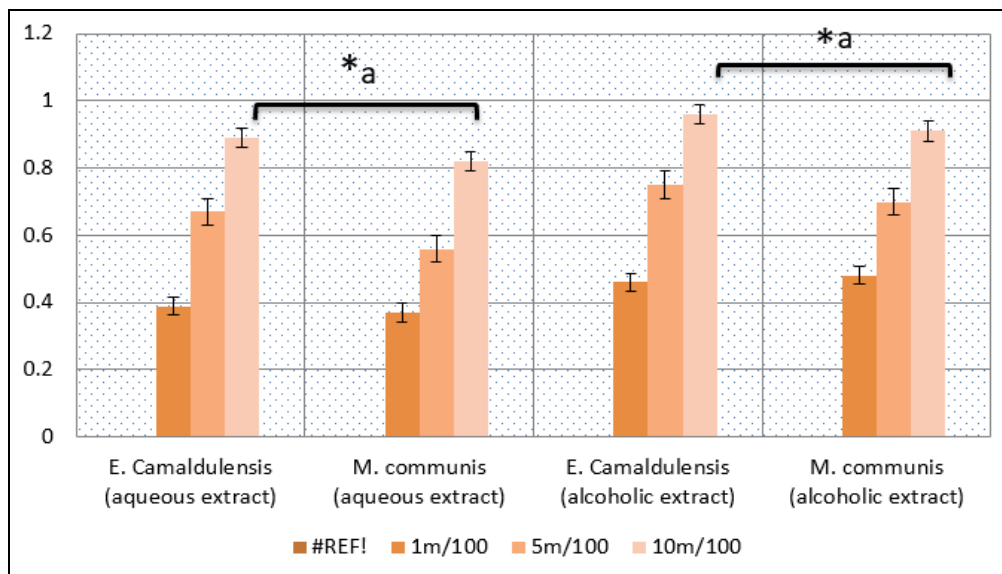


Fig 2: Comparison of the killing effect of the ethanolic extract of *E. camaldulensis* leaves and *M. communis* leaves against third-instar larvae of *Culex quinquefasciatus* with the control group

Table 4: The repellent effect of pregnant *C. quinquefasciatus* mosquitoes against extracts of *E. camaldulensis* leaves and *M. communis* leaves

Number of eggs	Number of egg boats	Time/Days Number of days it takes to lay eggs							Natural aquatic environment 10% concentration	Type of plant
		seventh	sixth	fifth	fourth	third	second	First		
2300±12.1	11.5±1.8	2.4 ±0.2	3.3 ±0.2	2.6 ±0.4	2.2 ±0.2	1.2 ±0.2	0.0 ±0.0	0.0 ±0.0	aqueous extract	E. camaldulensis
1260±11.2	6.6±1.4	1.6 ±0.2	2.0 ±1.0	1.2 ±0.4	1.0 ±0.2	1.0 ±0.1	0.0 ±0.0	0.0 ±0.0	Ethanolic extract	
1150±8.2	6.6±1.4	2.0 ±0.4	1.6 ±0.2	1.6 ±0.2	1.4 ±0.2	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	aqueous extract	M. communis
506±4.4	4.6±1.1	2.4 ±0.2	2.2 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	Ethanolic extract	
7250±20.2	29.6±3.2	3.8 ±0.6	4.2 ±0.6	5.6 ±0.8	4.5 ±1.2	3.8 ±0.4	4.2 ±0.4	3.6 ±0.4	Control Group larval rearing water	

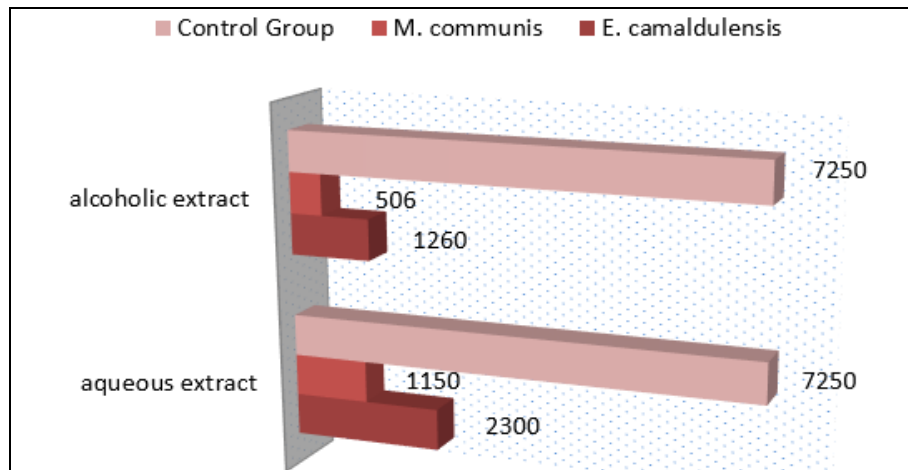


Fig 3: Comparison of the repellent effect of the ethanolic extract of *E. camaldulensis* leaves and *M. communis* leaves on *Culex quinquefasciatus* adults with the control group

Conclusion

This study demonstrated that the presence of a wide range of secondary metabolites in the leaf extracts of *Eucalyptus camaldulensis* and *Myrtus communis* has a high ability to kill the larval stage of the mosquito *Culex quinquefasciatus* under standard conditions. It was also found that the ability of the *M. communis* extract to repel female mosquitoes from egg-laying sites is significantly greater than that of the *E. camaldulensis* leaf extract.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Alahmed AM, Munawar K, Khalil S, Harbach RE. Assessment and an updated list of the mosquitoes of Saudi Arabia. *Parasit Vectors.*,2019;12(1):1-9.
- Ali EOM, Babalghith AO, Bahathig AOS, Toulah FHS, Bafaraj TG, Al-Mahmoudi SMY, *et al.* Prevalence of Larval Breeding Sites and Seasonal Variations of *Aedes aegypti* Mosquitoes (*Diptera: Culicidae*) in Makkah Al-Mokarramah, Saudi Arabia. *Int J Environ Res Public Health.*,2021;18(14):7368. doi:10.3390/ijerph18147368
- Alves AC, da Silva TI, de Azevedo FR, Virgulino RR, Costa CE, Feitosa JV, *et al.* Attractive activity of plant extracts for the oviposition of *Aedes aegypti* L. (*Diptera: Culicidae*). *Idesia.*,2018;36(2):225-31.
- Aydin T, Bayrak N, Baran E, Cakir A. Insecticidal effects of extracts of *Humulus lupulus* (hops) L. cones and its principal component, xanthohumol. *Bull Entomol Res.*,2017, 1-7.
- Bader A, Omran Z, Al-Asmari AI, Santoro V, De Tommasi N, D'Ambola M, *et al.* Systematic Phytochemical Screening of Different Organs of *Calotropis procera* and the Ovicidal Effect of Their Extracts to the Foodstuff Pest *Cadra cautella*. *Molecules.*,2021;26(4):905. doi:10.3390/molecules26040905
- Baranitharan M, Dhanasekaran S. larvicidal properties of *Commiphora caudate* (*Bursaceae*) against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston), *Culex quinquefasciatus*. *Int J Curr Microbiol App Sci.*,2014, 6:262-268.
- Elimam AM, Elmalik KH, Ali FS. Efficacy of leaves extract of *Calotropis procera* Ait. (*Asclepiadaceae*) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *Saudi J Biol Sci.*,2009;16(2):95-100. doi:10.1016/j.sjbs.2009.10.007
- Ghanem GA, Gebily DA, Ragab MM, Ali AM, Soliman NE, El-Moity TH. Efficacy of antifungal substances of three *Streptomyces* spp. against different plant pathogenic fungi. *Egypt J Biol Pest Control.*,2022;32(1):1-3. doi:10.1186/s41938-022-00612-9
- Gosik R, Sprick P, Morris MG. Descriptions of immature stages of four species of the genera *Graptus*, *Peritelus*, *Philopedon*, and *Tanymecus* and larval instar determination in *Tanymecus* (*Coleoptera, Curculionidae, Entiminae*). *ZooKeys.*,2019;813:111:50. doi:10.3897/zookeys.813.30336
- Hussin NHB, Ismail SB, Ke-Xin Y. Larvicidal activity of ethanol extract of *Carica papaya* seed against *Aedes albopictus* (Skuse). *Int. J. Medical Toxicol. Leg. Med.*,2020, 23:125-128.
- Jiraungkoorskul W. Larvicidal and Histopathological Effects of *Andrographis paniculata* Leaf Extract against *Culex quinquefasciatus* Larva. *Agric Technol Biol Sci.*,2016;13(2):133-40.
- Jones RT, Ant TH, Cameron MM, Logan JG. Novel control strategies for mosquito-borne diseases. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 2021, 376.
- Khater EI, Baig F, Saleh A, Kamal H. *Aedes aegypti* mosquito vector of dengue in the Arabian Peninsula: Ecology, phylogenetics and control. *Int J Infect Dis.*,2016;53:155-6. doi:10.1016/j.ijid.2016.11.380
- Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, *et al.* Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat. Microbiol.*,2019;4:854-863.
- Mahmoudi K, Cheriti A, Boulenouar N, Bourmita Y, El Hadj MD. Efficacy of Anti-termite Extracts from Four Saharan Plants against the Harvester Termite, *Anacanthotermes ochraceus*. *Pertanika J Trop Agric Sci.*,2021;44(4):865-77.
- Marc M, Moïse BF, Joël TN, Lebel TJ. Evaluation of the insecticidal activity of the methanol extracts of

- Calotropis procera* (Asclepiadaceae) and *Albizia lebbek* (Mimosaceae) on larvae of *Culex quinquefasciatus* Say, 1823. J Basic Appl Zool.,2021;82(1):1-8. doi:10.1186/s41936-021-00262-7
17. Marimuthu G, Mohan R, Hotib SL, Giovanni B. Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). Research in Veterinary Science.,2016;104:77-82.
 18. McGregor BL, Connelly CR. A Review of the Control of *Aedes aegypti* (Diptera: Culicidae) in the Continental United States. J Med Entomol.,2021;58(1):10-25. doi:10.1093/jme/tjaa157
 19. Mohamed Abdoul-Latif F, Elmi A, Merito A, Nour M, Risler A, Ainane A, et al. Essential oils of *Ocimum basilicum* L. and *Ocimum americanum* L. from Djibouti: Chemical composition, antimicrobial and cytotoxicity evaluations. Processes.,2022;10:1785.
 20. Narayanan M, Vijay A, Kandasamy S, Nasif O, Alharbi SA, Srinivasan R, et al. Phytochemical profile and larvicidal activity of aqueous extract of *Ocimum americanum* against mosquito vectors. Appl. Nanosci.,2021;11:3369-3381.
 21. Pavela R, Maggi F, Iannarelli R, Benelli G. Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. Acta Trop.,2019;193:236-71. doi: 10.1016/j.actatropica.2019.01.019
 22. Rants'o TA, Koekemoer LL, van Zyl RL. *In vitro* and *in silico* analysis of the *Anopheles* anticholinesterase activity of terpenoids. Parasitol. Int.,2023;93:102713.
 23. Seghier H, Tine-Djebbar F, Loucif-Ayad W, Soltani N. Insecticidal activity of *Petroselinum crispum* essential oil on mosquitoes. J. Entomol. Res.,2020;44:613-620.
 24. Siciliano T, De Leo M, Bader A, De Tommasi N, Vrieling K, Braca A, et al. Pyrrolizidine alkaloids from *Anchusa strigosa* and their antifeedant activity. Phytochemistry.,2005;66(13):1593-600.
 25. Silva NM, Santos NC, Martins IC. Dengue and Zika viruses: Epidemiological history, potential therapies, and promising vaccines. Trop. Med. Infect. Dis.,2020;5:150.
 26. Singh M, Kumari S, Attri R, Kumar S. Impact of *Calotropis procera* leaf extracts on the survival, morphology and behaviour of dengue vector, *Aedes aegypti* L. DU. J Under Res Innov.,2015;1(3):96-107.
 27. Vijayakumar S, Vidhya E, Anand GC, Nilavukkarasi M, Punitha VN, Sakthivel B. Eco friendly synthesis of TiO₂ nanoparticles using aqueous *Ocimum americanum* L. leaf extracts and their antimicrobial, anti-proliferative and photocatalytic activities. Vegetos.,2020;33:805-810.
 28. World Health Organization. Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva: WHO; 1996, 69.
 29. World Health Organization. Vector-Borne Diseases., 2020.
 30. Yaméogo F, Wangrawa DW, Sombié A, Sanon A, Badolo A. Insecticidal activity of essential oils from six aromatic plants against *Aedes aegypti*, dengue vector from two localities of Ouagadougou, Burkina Faso. Arthropod-Plant Interact.,2021;15:627-634.
 31. Yousuf MJ, Anjum SI, Faiz R. Toxicological attributes of plant chemicals and their biochemical impacts on cholinesterase and protein levels in relation with conventional insecticides against mosquito larvae of Karachi city. Toxicol. Envir Chem.,2014;96(7):1088-1095.