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ORIGINAL RESEARCH ARTICLE

A comparative Analysis of Algerian natural extracts as Solo and Synergistically against *Culex pipiens* (Diptera: Culicidae) Larvae

Mouna Menakh[1] · Saber Boutellaa[1] · Khaoula Benabied[2] · Raouya Zaouani[2] · Mohamed Abou-Mustapha[3]

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Abstract

This study assessed the larvicidal and emergence inhibitory effects of propolis from western honey bee (*Apis mellifera* L.), Mastic Tree (*Pistacia lentiscus* L.), and Bay Laurel (*Laurus nobilis* L.) extracts on common house mosquito *Culex pipiens* L. larvae, a significant public health threat as a vector for various diseases. Our analysis encompassed individual and combined evaluations of these natural products. Gas chromatography–mass spectrometry (GC-MS) analysis revealed distinctive chemical compositions in *P. lentiscus* and *L. nobilis* essential oils, featuring noteworthy compounds such as spathulenol, *β*-caryophyllene, linalool, and 1,8-cineole. HPLC analysis showed richness of phenolics in all extracts, including benzoic acid, quercetin, and catechin hydrate. Individual larvicidal assessments demonstrated *L. nobilis* essential oil as the most potent, with an LC_{50} of 31.94 ppm and an LT_{50} of 6.14 h. Followed by *P. lentiscus* essential oil (LC_{50} of 46.59 ppm, LT_{50} of 33.77 h), *L. nobilis* ethanolic extract (LC_{50} of 73.17 ppm, LT_{50} of 11.55 h), propolis (LC_{50} of 89.22 ppm, LT_{50} of 25.40 h), and *P. lentiscus* ethanolic extract (LC_{50} of 135.60 ppm, LT_{50} of 47.69 h). Remarkably, combinations of extracts from *P. lentiscus* and *L. nobilis*, particularly their essential oils, exhibited stronger larvicidal effects than individual extracts. Notably, specific volume ratios, such as 1:4, 2:3, and 2:2, showed consistent synergistic activity, as did combinations with ethanolic extracts and propolis. Additionally, the essential oils inhibited larval emergence, with synergistic effects observed in specific combinations. These results highlight the potential of these natural extracts, both alone and in combination, as effective and eco-friendly larvicidal agents against *Cx. pipiens*.

Keywords *Pistachia lentiscus* · *Laurus nobilis* · Propolis · Natural products · Larvicidal effect · *Culex pipiens*

Introduction

Mosquitoes, particularly common house mosquito *Culex pipiens*, pose a serious threat to public health due to their ability to transmit various vector-borne diseases, such as the West Nile disease (Giatropoulos et al. 2023; Iftikhar et al. 2023). Angioedema, urticaria, and other systemic allergic reactions are brought on by the *Cx. pipiens* (Taktak et al. 2022). The control of mosquito populations is a crucial component of global health efforts (Sayah et al. 2014). Traditional chemical insecticides have played a pivotal role in mosquito control strategies; however, their widespread use has raised concerns about environmental impact and the development of insecticide resistance (Hammoud et al. 2022; Yaseen and Ali 2022). In recent years, there has been a growing interest in exploring alternative, environment friendly, and sustainable approaches to combat mosquito vector (Baz et al. 2021; Tanvir et al. 2022; Zhang et al. 2023; Jambagi et al. 2023).

Natural products derived from plants and other natural sources have interested chemical and biological activities (Menakh et al. 2020) and have gained prominence as potential alternatives to synthetic insecticides (Traboulsi et al. 2005; Elbanoby 2020; Yaseen and Ali 2022). These organic substances have a number of benefits, such as biodegradability, low toxicity to creatures other than the

✉ Saber Boutellaa
s.boutellaa@centre-univ-mila.dz

[1] Laboratory of Natural Sciences and Materials, Abdelhafid Boussouf University Center Mila, Mila 43000, Algeria

[2] Department of Biological and Agricultural Sciences, Institute of Natural and Life Sciences, Abdelhafid Boussouf University Center Mila, Mila 43000, Algeria

[3] Centre de Recherche Scientifique et Technique en Analyses Physico-chimiques CRAPC, BP 384, Bou-Ismail, Tipaza 42004, Algeria

target, and potential effectiveness against mosquito larvae (Traboulsi et al. 2002). Essential oils and botanical extracts from plants are among the promising natural sources that have demonstrated significant insecticidal efficacy against mosquito larvae (Hammoud et al. 2022; Tanvir et al. 2022).

Pistacia lentiscus (Anacardiacae) and *Laurus nobilis* (Lauraceae), commonly known as mastic and bay laurel, respectively, are native to the Mediterranean region and have been recognized for their diverse therapeutic properties (Atmani et al. 2009; Traboulsi et al. 2005). These plants have a long history of usage in traditional medicine, and current research suggests they may contain natural insecticidal chemicals (Verdian-Rizi 2009; Baz et al. 2021; Cetin et al. 2011; Ben Jemâa et al. 2012). Additionally, propolis, a resinous substance collected by bees from plant buds and exudates, has garnered attention for its antimicrobial and insecticidal properties (Yaseen and Ali 2022).

The purpose of this study was to determine the larvicidal activity and emergence inhibitory effects of propolis, *P. lentiscus* and *L. nobilis* extracts against the third instar *Cx. pipiens* larvae. In addition, this research aimed to assess the effectiveness of these natural products both individually and in combination, contributing valuable insights to the development of novel and sustainable mosquito control strategies. The dual approach of investigating the extracts individually and in synergy provides a comprehensive understanding of their potential and highlights any synergistic effects that may arise from their combination. Ultimately, this study targeted expanding our arsenal of eco-friendly solutions in the persistent battle against mosquito-borne diseases.

Materials and methods

Samples collection

The leaves of *P. lentiscus* and *L. nobilis* were collected in flowering stage from Mila (North Eastern Algeria), with GPS coordinates (Latitude 36°35'46.98"N, Longitude 6°15'55.11"E, Elevation 260 m). After harvest, samples were transferred to the laboratory and were air-dried then powdered. The propolis sample was obtained from Mila farm then it was frozen at -50 ºC and was ground in the grinder to obtain powder form.

Preparation of extracts

Using a modified clevenger-type apparatus, 100 g of dried leaves were hydrodistilated for three hours to extract the essential oils. The collector solvent in this case was diethyl ether. Following solvent evaporation, the oil was kept at 04 °C in sealed vials shielded from light (Alimi et al. 2023).

Propolis, *P. lentiscus* and *L. nobilis* ethanolic extracts were prepared using a Soxhlet apparatus. Approximately 50 g of samples were extracted in 250 mL of 80% ethanol for 4 h then, the solutions were filtered, concentrated under vacuum pressure at 45 °C and were kept at 4 °C prior analysis (Basyirah et al. 2018).

Gas chromatography–mass spectrometry (GC-MS)

The analysis of essential oils using GC-MS was executed using an Agilent 6890 system coupled with a 5973 mass spectrometry detector, employing electron impact ionization at 70 eV. The chosen HP-5 MS capillary column (30 m × 0.25 mm, coated with 5% phenyl methyl silicone and 95% dimethylpolysiloxane, 0.25 µm film thickness; Hewlett-Packard, CA, USA) was integral. The temperature programming involved an increase from 60 to 250 °C over 8 min at a rate of 2 °C/min. Helium N60 served as the carrier gas at a flow rate of 0.6 mL/min, and the split ratio was maintained at 100:1. The scan duration and mass range were set at 1 s and 50–550 m/z, respectively. The identification of components included the comparison of fragmentation patterns in mass spectra and utilizing a computer to search through commercial reference libraries (WILEY and NIST05). Kovats retention indices were determined under identical conditions by comparing them to a homologous series of n-alkanes (C8–C40) (Sriti Eljazi et al. 2018).

HPLC-DAD screening of phenolics

Shimadzu reverse phase high performance liquid chromatography (Shimadzu Cooperation, Japan) system that consists of a Shimadzu model LC-20AT solvent delivery unit and a Shimadzu model SPD-M20A diode array detector and is monitored by LC-solution software was used to analyze ethyle acetate and butanolic extracts as well as 27 standard phenolics. 35 °C was chosen as the column temperature. Aqueous acetic acid 0.1% (A) and methanol served as the mobile phases for the chromatographic separation, which was carried out on an Inertsil ODS-3 guard column (4 µm, 4.0 mm x 150 mm) column (B). Elution was done in gradients ranging from 2 to 100%. Sample stock solutions were created in methanol at a concentration of 8 mg.mL-1 and filtered through an Agilent 0.45 µm filter. 20 µL of fluid was injected. A diode array detector (DAD) operating at a wavelength of 254 nm was used to find the phenolics. The results

were presented as micrograms per gram of dry weight, and their characterization was based on a comparison of the retention times (Menakh et al. 2021; Tel-Çayan et al. 2015).

Mosquito colony

The mosquito larvae employed in this investigation came from a laboratory colony of *Cx. pipiens* biotype *molestus* that was maintained at 26–27 °C, 50–60% relative humidity, and a 16:8 h photoperiod (L: D). Adult mixed-sex mosquitoes were housed in mesh-covered cages with dimensions of 33 cm in length, 33 cm in breadth, and 33 cm in height, and were given 10% sucrose solution. Because of autogeny, females did not get blood for the development of their eggs. Up until pupation, larvae were fed ad libitum with dried wheat bread in receptacles filled with tap water. The egg-laying cages were equipped with beakers containing 100 mL of water (WHO 2005).

Larvicidal bioassay test

Using the World Health Organization's recommended procedures (WHO 2005), the larvicidal impact of our extracts was evaluated. To ensure that the extract was completely soluble in water, 99 ml of distilled water with 1 mL of 0.3% Tween 80 was added as an emulsifier along with the selected extracts to create the stock solution. A variety of concentrations (25–200 ppm for essential oils and 50–400 ppm for ethanolic extracts) were added to batches of 20 early third instar *Cx. pipiens* larvae that were moved to 250 mL cups containing 100 mL of distilled water. Each concentration was experienced in five replications and a control group consisted of 1 mL of 0.3% Tween 80 and 99 mL of distilled water only (Fig. 1). Experiments were repeated three times. Mortalities in both larvae and pupae were observed at regular intervals of 1, 6, 24, 48, and 72 h during continuous exposure, all while maintaining normal feeding conditions for the larvae.

Emergence inhibition effect

To prevent the adults that have successfully emerged from escaping into the environment, it is necessary to encase the entire test and control cups in netting while determining the extract concentrations for 50 and 90% inhibition of adult emergence (IE_{50} and IE_{90}). Mortality and survival are recorded every two or three days until all adults have appeared. The experiment maintains a temperature range of 25–28 °C with a preferred photoperiod of 12 L: 12D. The impact was expressed as IE% based on larvae that failed to develop into viable adults. This calculation includes moribund and dead larvae/pupae and adult mosquitoes not fully separated from pupal cases. The experiment concludes

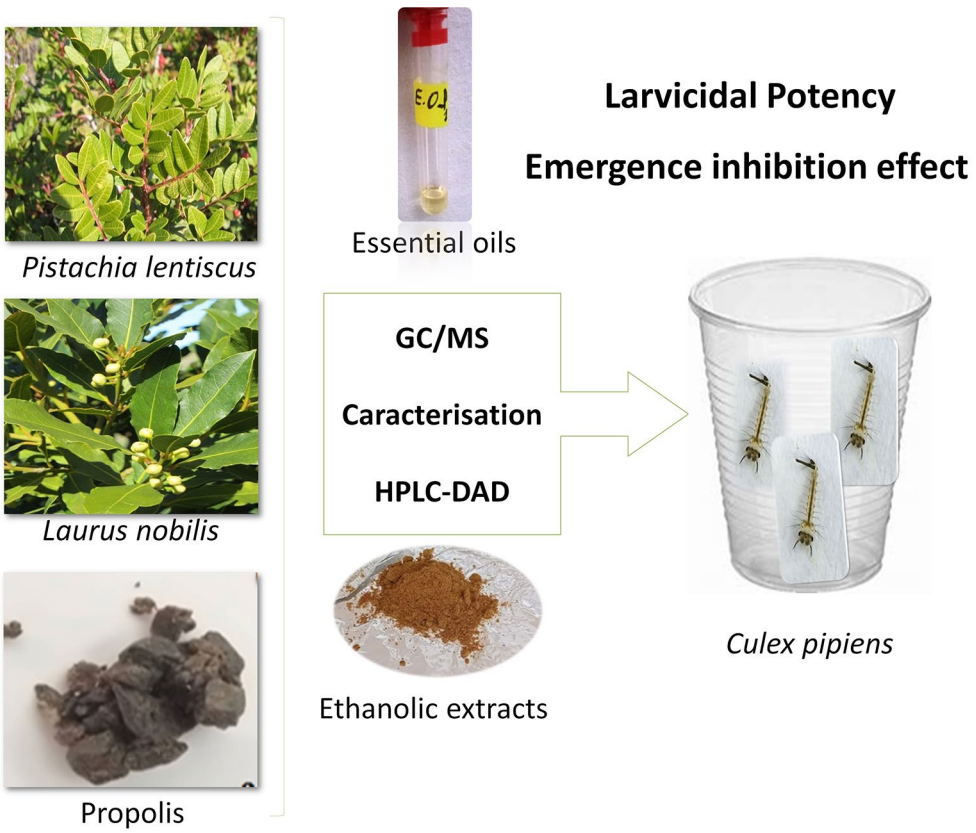

Fig. 1 Methodological design for evaluating the larvicidal activity of propolis, *P. lentiscus*, and *L. nobilis* extracts on *Cx. pipiens* larvae

Table 1 Chemical composition of the essential oil from *P.lentiscus*

N°	Compound	RT	RI	%
1	α-pinene	4.74	939	2.40
2	Camphene	5.18	942	0.24
3	β -Pinene	6.87	954	2.01
4	α-Thujene	7.41	957	0.57
5	α-Terpinene	8.06	1062	0.01
6	*p*-Cymene	8.52	1065	3.23
7	β-Phelladrene	8.76	1066	4.82
8	γ -Terpinene	10.56	1179	3.46
9	α-Terpinolene	13.28	1197	2.38
10	4-Terpineol	18.13	1229	2.10
11	α-Terpineol	19.09	1236	0.63
12	Pulegone	22.31	1258	4.00
13	Phellandral	24.56	1273	2.64
14	Bornyl acetate	25.52	1442	2.44
15	Carvacrol	26.98	1447	7.12
16	2-Bornene	29.73	1457	1.32
17	Copaene	31.03	1512	0.27
18	β-Caryophyllene	33.69	1525	11.60
19	α-Caryophyllene	35.68	1535	1.85
20	Aromadendrene	36.11	1537	1.37
21	γ-Cadinene	37.12	1542	5.29
22	Germacrene-D	37.47	1543	8.03
23	α-Muurolene	38.78	1550	1.30
24	α-Amorphene	39.47	1553	0.89
25	α-Farnesene	39.72	1554	0.33
26	δ-Cadinene	40.18	1557	3.81
28	Spathulenol	43.14	1571	16.73
29	β-Cadinene	45.90	1585	1.18
31	α-Cadinol	47.39	1592	6.73
Grouped components				
Monoterpene hydrocarbons		**20.43%**		
Sesquiterpene hydrocarbons		**35.91%**		
Oxygenated monoterpenes		**16.50%**		
Oxygenated sesquiterpenes		**23.46%**		
Other		**2.44%**		
Total identified		**98.75%**		

RT retention time. RI retention index

when control larvae/pupae have all died or emerged as adults (WHO 2005).

Mixing extracts with propolis

We utilized the ten-point approach to explore the potential enhancement of specific extracts' effects by combining them in different ratios. According to this idea, the half-lethal concentrations of substances A and B are influenced by the potency of a and b. As a result, we evaluated the mixes with the co-toxic factor approach. In particular, the concentration gradient order of the following five ratios was taken into consideration: 1:4, 2:3, 2:2, 3:1, and 4:1 (Liang et al. 2020).

Table 2 Chemical composition of the essential oil of leaves of *L. nobilis*

N°	Compound	RT	RI	%
1	α-pinene	4.873	936	0.68
2	Camphene	5.325	939	15.01
3	Sabinene	6.314	946	2.84
4	β-Pinene	7.08	952	0.19
5	1.8-Cineole	9.549	1022	23.29
6	γ-Terpinene	10.869	1031	0.24
7	4-Terpineol	11.355	1084	2.21
8	α-Terpinolene	12.658	1093	0.1
9	Linalool	14.795	1107	25.68
10	trans-Pinocarveol	16.041	1115	0.37
11	Borneol	17.825	1177	0.28
12	Nerol	18.088	1179	0.49
13	Phellandral	22.397	1258	0.44
14	Carvacrol	24.397	1272	0.25
15	Elemol	25.003	1276	0.23
16	Linalyl acetate	25.952	1282	0.74
17	l-Bornyl acetate	27.347	1291	0.51
18	Eugenol	31.09	1317	5.02
19	β-Elemene	32.708	1327	0.31
20	Methyleugenol	34.52	1340	15.48
21	germacrene A	39.218	1479	0.33
22	α-Amorphene	39.966	1485	0.16
23	δ-Cadinene	40.544	1539	0.47
24	Spathulenol	43.601	1562	2.96
25	Alloaromadendrene	46.351	1583	0.48
26	β-Eudesmol	47.414	1590	0.99
Grouped components				
Monoterpene hydrocarbons		**19.05%**		
Sesquiterpene hydrocarbons		**1.75%**		
Oxygenated monoterpenes		**53.01%**		
Oxygenated sesquiterpenes		**4.18%**		
Other		**21.75%**		
Total identified		**99.74%**		

Statistical analysis

The statistical analysis of mean percentage of larval deaths was conducted using the SPSS software. Probit analysis was employed to calculate key parameters such as LC_{50}, LC_{90}, IE_{50}, IE_{90}, LT_{50}, LT_{90}, upper confidence limit (UCL), lower confidence limit (LCL), and Chi-square (Finney 1971). Results were considered statistically significant at a significance level of $P \leq 0.05$.

Results

GC-MS analysis

The essential oils from *P. lentiscus* and *L. nobilis* exhibited distinct chemical compositions, as detailed in Tables 1 and 2, respectively, through GC-MS analysis. In the *P.*

lentiscus essential oil, sesquiterpene hydrocarbons constituted the highest fraction at 35.91%, succeeded by oxygenated sesquiterpenes (23.46%), monoterpene hydrocarbons (20.43%), and oxygenated monoterpenes (16.50%). The prominent compounds identified were spathulenol (16.73%), β-caryophyllene (11.60%), germacrene-D (8.03%), and carvacrol (7.12%). In contrast, the *L. nobilis* essential oil showcased oxygenated monoterpenes as the predominant fraction at 53.01%, followed by monoterpene hydrocarbons (19.05%), oxygenated sesquiterpenes (4.18%), and sesquiterpene hydrocarbons (1.75%). Major compounds in this oil were linalool (25.68%), 1,8-cineole (23.29%), methyleugenol (15.48%), camphene (15.01%) and eugenol (5.02%).

HPLC analysis

The HPLC analysis results of *P. lentiscus*, *L. nobilis*, and propolis ethanolic extracts, presented in Table 3, showcased the presence of distinct compounds. *P. lentiscus* and propolis extracts revealed 5 compounds each, while the *L. nobilis* extract contained 6 compounds. In the *P. lentiscus* extract, the identified compounds were benzoic acid (17.35%), catechin hydrate (9.45%), galic acid (2.58%), ascorbic acid (2.11%), and quercetine (0.75%). The propolis extract contained caffeine (2.17%), quercetine (2.00%), ascorbic acid (0.22%), linoleic acid (1.61%), and myrecitine (1.40%). Additionally, the *L. nobilis* extract revealed quercetine (7.89%), Benzoic acid (3.39%), myrecitine (2.94%), 3-hydroxyflavone (1.75%), coumaric acid (1.43%), and catechin hydrate (1.09%) as its identified compounds (Fig. 2).

Individual larvicidal potency

The individual larvicidal effects of *P. lentiscus*, *L. nobilis*, and propolis extracts against *Cx. pipiens* larvae were detailed in Table 4. The findings highlighted *L. nobilis* essential oil as the most potent extract, exhibiting an LC_{50} of 31.94 ppm and an LT_{50} of 6.14 h. Following this, *P. lentiscus* essential oil showed moderate effectiveness with an LC_{50} of 46.59 ppm and an LT_{50} of 33.77 h. Subsequently, *L. nobilis* ethanolic extract displayed an LC_{50} of 73.17 ppm with an LT_{50} of 11.55 h, while propolis exhibited an LC_{50} of 89.22 ppm and an LT_{50} of 25.40 h. Lastly, P. lentiscus ethanolic extract showed comparatively lower effectiveness, with an LC_{50} of 135.60 ppm and an LT_{50} of 47.69 h.

Table 3 Bioactive phenolics obtained from ethanolic extracts of *P. lentiscus, L. nobilis* and propolis

N°	Compounds (%)	RT (min)	Propolis	*P. lentiscus*	*L. nobilis*
1	Ascorbic acid	2.89	0.22	2.11	-
2	Gallic acid	6.98	-	2.58	-
3	Catechin hydrate	14.23	-	9.45	1.09
4	Caffeine	19.21	2.17	-	-
5	Coumaric acid	22.99	-	-	1.43
6	Benzoic acid	27.15	-	17.35	3.39
7	Myrecitine	31.06	1.40	-	2.94
8	Quercetine	35.11	2.00	0.75	7.89
9	3-Hydroxyflavone	46.70	-	-	1.75
10	Linoleic acid	52.68	1.61	-	-

(-) not found

Synergistic larvicidal potency

The outcomes of the synergistic larvicidal effect of *P. lentiscus*, *L. nobilis*, and propolis extracts against *Cx. pipiens* larvae were depicted in Table 5. Our findings indicated that combining these extracts in various ratios enhanced their effectiveness against mosquito larvae. Specifically, when *P. lentiscus* and *L. nobilis* essential oils were blended in volume ratios of 1:4, 2:3, and 2:2, the resulting CTC values were 106.25, 101.91, and 104.20, respectively. These effective combinations displayed CTCs exceeding 100, implying a synergistic effect. Furthermore, combinations of *P. lentiscus* and *L. nobilis* ethanolic extracts with propolis at all ratios exhibited synergistic effects (CTC > 100). However, when *P. lentiscus* and *L. nobilis* ethanolic extracts were mixed at all ratios, the CTCs were less than 100, indicating an antagonistic effect.

Emergence inhibition efficacy

As depicted in Table 6, L. *nobilis* E.O and *P. lentiscus* E.O displayed intriguing inhibitory effects on the emergence of *Cx. pipiens* larvae, with LC_{50} values of 27.64 ppm and 42.33 ppm, respectively. However, *P. lentiscus*, *L. nobilis*, and propolis E.E exhibited a moderate impact, showcasing LC_{50} values of 121.50 ppm, 70.23 ppm, and 83.44 ppm, respectively. Regarding their synergistic effects, the combination of *P. lentiscus* and *L. nobilis* essential oils demonstrated synergistic activity when mixed in volume ratios of 1:4 and 2:2, resulting in CTC values of 102.79 and 126.33, respectively. Additionally, the synergistic effects were observed consistently across all ratios when combining *P. lentiscus* and *L. nobilis* ethanolic extracts with propolis, reliably resulting in CTC values exceeding 100.

Discussion

Mosquito-borne diseases pose significant health risks globally, making effective mosquito control strategies imperative (Aziz et al. 2016). Traditional chemical insecticides, while effective, raise concerns about environmental and human health (Abutaha 2022). Consequently, there's a growing interest in exploring natural products as eco-friendly alternatives for mosquito control (Engdahl et al.

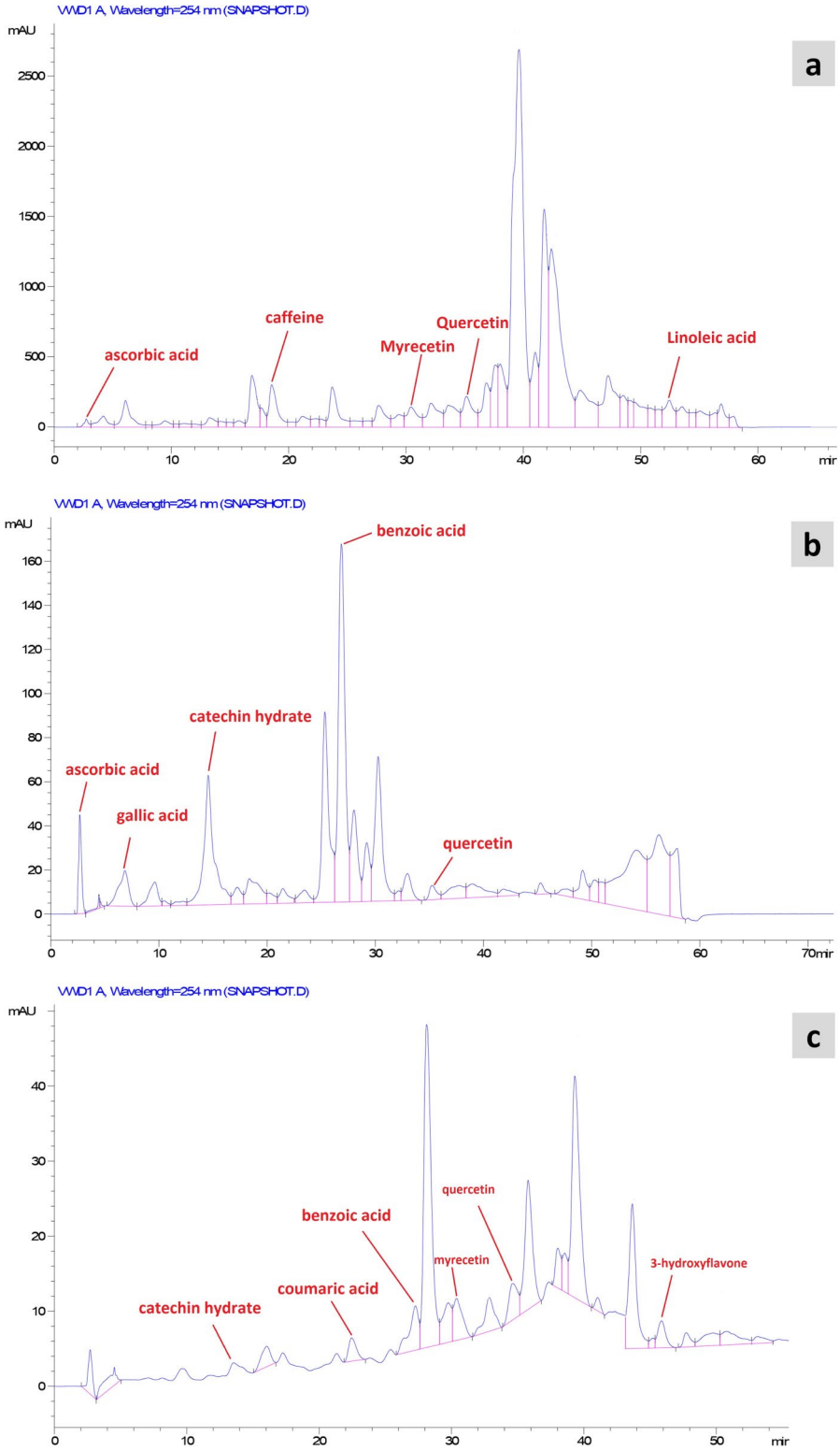

Fig. 2 HPLC Chromatogram of propolis (**a**), *P. lentiscus* (**b**) and *L. nobilis* (**c**) and ethanolic extracts

2022; Giatropoulos et al. 2023; Hamama et al. 2022). In this context, our study aimed to investigate the larvicidal and emergence inhibitory effects of natural extracts derived from Propolis, *Pistacia lentiscus*, and *Laurus nobilis* against *Culex pipiens* larvae. Our investigation encompassed an evaluation of the individual efficacy of these extracts and an exploration of their potential synergistic effects when combined.

The distinct chemical compositions revealed by GC-MS analysis of the essential oils, compounds such as

Table 4 Individual larvicidal effect of *P. lentiscus*, *L. nobilis* and propolis extracts against *Cx. pipiens* larvae

Extracts	LC value (ppm)			LT value (h)		
	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2	LT_{50} (LCL-UCL)	LT_{90} (LCL-UCL)	X^2
P. lentiscus E.O	46.59 (34.66–59.77)	117.56 (86.19-214.32)	1.48	33.77 (23.57–52.75)	179.75 (93.76-965.68)	5.36
P. lentiscus E.E	135.60 (104.09-176.31)	353.45 (253.00-666.01)	0.41	47.69 (33.77–87.35)	238.27 (115.70-1751.07)	2.42
L. nobilis E.O	31.94 (22,72-40.08)	66.87 (51.68–114.10)	1.26	6.14 (4.96–9.56)	10.36 (8.66–13.76)	0.03
L. nobilis E.E	73.17 (49.29–95.46)	196.74 (143.14-379.69)	0.71	11.55 (8.96–13.37)	28.37 (22.82–37.24)	5.09
Propolis E.E	89.22 (66.02-114.58)	225.21 (164.96-414.36)	3.65	25.40 (20.86–31.27)	49.23 (37.92–89.53)	0.56

LC_{50}- LC_{90} Lethal concentration and LT_{50}- LT_{90} Lethal time kills 50% and 90% of the exposed larvae at LC_{50} respectively, UCL Upper confidence limit, LCL Lower confidence limit, X^2 Chi-square E.O essential oil, E.E ethanolic extract

sesquiterpene hydrocarbons, oxygenated sesquiterpenes, monoterpene hydrocarbons, and oxygenated monoterpenes in *P. lentiscus* oil contribute to its larvicidal potential. Notably, compounds like spathulenol, *β*-caryophyllene, germacrene-D, and carvacrol might play significant roles due to their known biological activities (Al-Ghanim et al. 2023; Benelli et al. 2020; Sun et al. 2020).

Conversely, *L. nobilis* oil, rich in oxygenated monoterpenes like linalool, 1,8-cineole, methyleugenol, camphene, and eugenol, exhibits potent larvicidal effects. These compounds possess inherent properties known for their insecticidal actions, potentially contributing to the observed efficacy (Ayllón-Gutiérrez et al. 2023; Beier et al. 2014; Benelli et al. 2018). Our findings were consistent with previous studies that have reported on the chemical compositions of these essential oils (Bachrouch et al. 2010; Bendjersi et al. 2016; Cetin et al. 2011).

Among the listed compounds, carvacrol, *β*-caryophyllene, and 1,8-cineole have demonstrated effective larvicidal activity against mosquito larvae in various scientific studies (Nararak et al. 2019; Traboulsi et al. 2002; Youssefi et al. 2019). These compounds have demonstrated substantial capacity in inhibiting larval growth and demonstrating toxicity, indicating their effectiveness in managing mosquito populations (Govindarajan et al. 2016; Nararak et al. 2019).

HPLC analysis of the ethanolic extracts from *P. lentiscus*, *L. nobilis*, and propolis revealed distinct compounds present in each. Notably, *P. lentiscus* and propolis extracts showcased 5 compounds each, while *L. nobilis* extract contained 6 compounds, including various acids, flavonoids, and other compounds known for their diverse biological activities. Through specific research studies, catechin hydrate, benzoic acid and quercetin, have revealed considerable promise as a larvicides against mosquito larvae (Elumalai et al. 2016; Selin-Rani et al. 2016; Raguvaran et al. 2022; Hekal et al. 2023). These components have indeed shown significant promise by disrupting larval growth and displaying toxic effects, indicating their potential as natural candidates for effectively controlling mosquito populations (Elumalai et al. 2016; Pessoa et al. 2018).

Different plant parts contain a diverse array of chemicals exhibiting distinct biological activities, often attributed to toxins and secondary metabolites. These substances can function as attractants or deterrents (Traboulsi et al. 2005). The present study investigated the larvicidal effects of essential oils and ethanolic extracts derived from *Laurus nobilis*, *Pistacia lentiscus*, and propolis. Our findings demonstrate a range of potency among the tested substances, with *L. nobilis* essential oil emerging as the most effective larvicidal agent. This is consistent with the work of Aissaoui et al. (2023) and Tine-Djebbar et al. (2021), who reported significant larvicidal effects of *L. nobilis* essential oil against *Cx. pipiens*.

P. lentiscus essential oil exhibited moderate efficacy, supporting previous studies by Cetin et al. (2011) and Traboulsi et al. (2002). The variability in effectiveness observed in our study and the reported LC_{50} values highlight the importance of considering geographical and environmental factors that may influence the composition of essential oils. Interestingly, the ethanolic extracts of *L. nobilis* and propolis demonstrated intermediary larvicidal effects. While limited antecedents exist regarding the insecticidal effects of propolis, our study aligns with the growing body of research on its diverse bioactive properties (Damiani et al. 2010; González-Martín et al. 2017). The multifaceted nature of propolis, encompassing insecticides, fungicides, and herbicides, underscores its potential as a valuable resource for pest control (Silva-Beltrán et al. 2021).

Comparatively, *P. lentiscus* ethanolic extract exhibited lower larvicidal effectiveness. The disparity in efficacy between essential oils and ethanolic extracts suggests that the mode of extraction plays a crucial role in determining the bioactivity of these plant-derived compounds (Hammoud et al. 2022).

Furthermore, our investigation into emergence inhibition revealed noteworthy findings. *L. nobilis* and *P. lentiscus* essential oils exhibited notable inhibitory effects against the emergence of *Cx. pipiens* larvae. However, the ethanolic extracts

Table 5 Synergistic larvicidal effect of *P. lentiscus*, *L. nobilis* and propolis extracts against *Cx. pipiens* larvae

Mixtures	LC_{50} (ppm) (LCL-UCL)	X^2	CTC	LT_{50} (h) (LCL-UCL)	X^2	CTC
P. lentiscus E.O/ *L. nobilis* E.O						
1 :4	30.06 (19.06–44.06)	0.45	106.25^{S}	7.06 (5.94–21.66)	5.25	86.96^{an}
2 :3	31.34 (19.34–49.34)	1.53	101.91 ^{S}	8.34 (7.66–22.24)	3.63	73.62^{an}
2 :2	30.65 (22.65–48.65)	2.55	104.20 ^{S}	10.65 (7.35–24.85)	2.87	57.65^{an}
3 :2	33.56 (21.56–47.56)	0.54	95.17^{an}	7.56 (6.44–14.02)	1.54	81.21^{an}
4 :1	36.03 (28.03–50.03)	3.06	88.64^{an}	11.03 (8.44–24.72)	3.86	55.66^{an}
P. lentiscus E.E/ *L. nobilis* E.E						
1 :4	74.64 (62.64–88.64)	2.86	98.03^{an}	14.64 (11.14–28.68)	0.74	78.89^{an}
2 :3	73.11 (63.11–89.11)	3.42	100.08^{ad}	11.10 (9.89–25.11)	1.95	104.05^{S}
2 :2	73.64 (61.64–87.64)	0.87	99.36^{an}	13.64 (11.56–27.23)	1.63	84.67^{an}
3 :2	76.66 (66.76–91.57)	1.75	95.44^{an}	12.66 (10.63–36.45)	3.81	91.23^{an}
4 :1	80.34 (67.34–93.34)	1.05	91.07^{an}	10.34 (8.66–24.34)	2.86	111.70^{S}
P. lentiscus E.E/Propolis E.E						
1 :4	71.48 (58.68–85.88)	1.33	102.36^{S}	11.48 (10.52–28.48)	0.64	100.60^{S}
2 :3	70.88 (56.23–84.76)	1.96	103.23^{S}	10.88 (8.33–33.17)	1.42	106.15^{S}
2 :2	71.94 (57.83–85.42)	2.85	101.70^{S}	11.94 (9.14–19.45)	0.72	96.73^{an}
3 :2	68.88 (55.33–82.06)	3.05	106.22^{S}	12.88 (8.55–36.76)	1.82	89.67^{an}
4 :1	74.87 (65.44–89.82)	0.44	103.44^{S}	11.87 (9.05–28.44)	1.94	97.30^{an}
L. nobilis E.E/ Propolis E.E						
1 :4	75.55 (61.83–86.22)	0.67	118.09^{S}	24.55 (12.34–38.97)	2.84	103.46^{S}
2 :3	78.34 (69.37–91.64)	0.98	113.88^{S}	18.34 (6.32–32.56)	2.05	138.49^{S}
2 :2	69.76 (50.23–83.62)	1.32	127.89^{S}	24.76 (12.46–33.16)	1.92	102.58^{S}
3 :2	76.45 (61.92–94.45)	1.42	116.70^{S}	23.45 (11.65–43.06)	1.74	108.31^{S}
4 :1	77.94 (72.53–99.43)	0.53	114.47^{S}	22.94 (13.26–53.12)	0.85	114.28^{S}

LC_{50} Lethal concentration and LT_{50} Lethal time kills 50% of the exposed larvae respectively, UCL Upper confidence limit, LCL Lower confidence limit, X^2 Chi-square, Ps Propolis ethanolic extract, E.O essential oil, E.E ethanolic extract. CTC Co-toxicity Index, CTC = 100 indicated an additive effect, CTC > 100 indicated a synergistic effect, and CTC < 100 indicated an antagonistic effect

from *P. lentiscus*, *L. nobilis*, and propolis exhibited more moderate impacts on the emergence inhibition. The differential impact between essential oils and ethanolic extracts on emergence inhibition aligns with previous research on the multifaceted properties of plant-derived substances (Aziz et al. 2016; Elbanoby 2020). The distinct chemical compositions and concentrations obtained through various extraction methods contribute to the nuanced effects observed in our study (Song et al. 2017). These findings prompt a deeper exploration into the specific bioactive compounds responsible for emergence inhibition, facilitating the development of targeted interventions. Moreover, the environmental conditions and geographical variations can influence the composition of essential oils and extracts, impacting their efficacy (Hammoud et al. 2022). Future studies should consider these factors for a

Table 6 Emergence inhibition efficacy of *P. lentiscus*, *L. nobilis* and propolis extracts against *Cx. pipiens* larvae

Extracts	LC_{50}	*LCL-UCL*	X^2	CTC
P. lentiscus E.O	42.33	32.23–51.65	2.45	-
P. lentiscus E.E	121.50	100.15-153.22	1.31	-
L. nobilis E.O	27.64	20.55–38.21	1.76	-
L. nobilis E.E	70.23	42.67–91.66	1.61	-
Propolis E.E	83.44	62.77-105.22	3.25	-
P. lentiscus E.O / *L. nobilis* E.O (1:4)	26.89	17.23–40.66	2.75	102.79[S]
P. lentiscus E.O / *L. nobilis* E.O (2:3)	29.59	18.37–45.77	1.43	93.41[an]
P. lentiscus E.O / *L. nobilis* E.O (2:2)	21.88	15.73–39.25	1.05	126.33[S]
P. lentiscus E.O / *L. nobilis* E.O (3:2)	30.44	18.51–37.33	0.77	90.80[an]
P. lentiscus E.O / *L. nobilis* E.O (4:1)	32.66	23.65–46.88	0.89	84.63[an]
P. lentiscus E.E / *L. nobilis* E.E (1:4)	70.34	58.24–80.44	2.42	99.84[an]
P. lentiscus E.E / *L. nobilis* E.E (2:3)	69.22	60.51–81.33	3.83	98.95[an]
P. lentiscus E.E / *L. nobilis* E.E (2:2)	70.45	59.34–80.23	1.56	99.69[an]
P. lentiscus E.E / *L. nobilis* E.E (3:2)	72.05	61.43–85.53	1.36	97.47[an]
P. lentiscus E.E / *L. nobilis* E.E (4:1)	75.33	61.11–90.05	2.62	93.23[an]
P. lentiscus E.E / *Propolis* E.E (1:4)	67.31	53.18–70.21	1.44	123.96[S]
P. lentiscus E.E / *Propolis* E.E (2:3)	68.54	51.34–76.32	0.91	121.74[S]
P. lentiscus E.E / *Propolis* E.E (2:2)	69.10	57.63–80.12	0.55	120.75[S]
P. lentiscus E.E / *Propolis* E.E (3:2)	65.88	55.04–78.25	1.82	126.65[S]
P. lentiscus E.E / *Propolis* E.E (4:1)	72.43	63.13–85.66	2.83	115.20[S]
L. nobilis E.E / *Propolis* E.E (1:4)	70.15	58.27–80.19	3.71	100.11[S]
L. nobilis E.E / *Propolis* E.E (2:3)	73.22	67.41–89.54	4.05	103.37[S]
L. nobilis E.E / *Propolis* E.E (2:2)	66.15	48.22–80.03	1.39	106.17[S]
L. nobilis E.E / *Propolis* E.E (3:2)	67.19	59.32–90.65	2.91	108.65[S]
L. nobilis E.E / *Propolis* E.E (4:1)	70.04	58.33–81.27	2.05	100.27[S]

LC_{50} Lethal concentration and LT_{50} Lethal time kills 50% of the exposed larvae respectively, UCL Upper confidence limit, LCL Lower confidence limit, X^2 Chi-square, Ps Propolis ethanolic extract, E.O essential oil, E.E ethanolic extract. CTC Co-toxicity Index, CTC = 100 indicated an additive effect, CTC > 100 indicated a synergistic effect, and CTC < 100 indicated an antagonistic effect

comprehensive assessment of the practical applicability of these natural compounds.

The combination of *P. lentiscus* and *L. nobilis* extracts exhibited remarkable synergistic effects, particularly pronounced in the essential oils, leading to heightened larvicidal activity at specific volume ratios. Notably, when these extracts were combined with propolis, consistent synergistic effects were observed, especially in augmenting larvicidal activity across various ratios. The diverse array of bioactive compounds in these extracts, coupled with the multifaceted modes of action, suggests a complementary and interactive effect on the larvae. Chemical interactions among these compounds, possibly influencing enzymatic activities and disrupting physiological processes, contribute to the observed synergy (Togbé et al. 2014). The observed larvicidal effects can be attributed to the individual or combined actions of these compounds. For example, monoterpenoids and sesquiterpenoid components, known as fast-acting neurotoxins in insects, contribute to the overall efficacy (Liang et al. 2020). Furthermore, the presence of larvicidal properties in compounds such as benzoic acid and quercetin has been well-documented in various studies (Hekal et al. 2023).

Additionally, the synergistic effects witnessed in the combined formulations may arise from the cumulative or enhanced actions of these compounds. This cumulative effect results in a more potent larvicidal outcome compared to the effects of individual extracts alone (Hertzberg and MacDonell 2002). This underscores the importance of considering not only the individual components but also the collective impact when exploring the larvicidal potential of botanical extracts, paving the way for a more nuanced understanding of their synergistic actions.

Conclusion

In conclusion, the findings from our study underscore the potential of these natural extracts, especially essential oils from *P. lentiscus* and *L. nobilis*, in exerting larvicidal and emergence inhibitory effects against *Cx. pipiens* larvae. Furthermore, the observed synergistic effects among these extracts indicate the promise of combination approaches in enhancing their efficacy as eco-friendly alternatives in mosquito control strategies. Further studies delving into the mechanisms of action and field applications of these natural extracts are needed to validate their potential for mosquito control programs while ensuring environmental safety.

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Author contributions All authors conceived the study. Saber BOUTELLAA collected the plants and propolis and prepared extracts, Mohamed Abou-Mustapha conducted GC/MS and HPLC analyses, and Mouna MENAKH, Khaoula BENABIED, and Raouya ZAOUANI conducted all other laboratory work, and Mouna MENAKH wrote the manuscript with major input from all other authors.

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Declarations

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