Tropical Biomedicine 42(4): 446-458 (2025)
https://doi.org/10.47665/tb.42.4.011
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RESEARCH ARTICLE

Larvicidal and enzyme-inhibitory effects of essential oils from *Eucalyptus globulus* and *Eucalyptus radiata* against *Culex pipiens*

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ARTICLE HISTORY

Received: 21 May 2025
Revised: 27 October 2025
Accepted: 6 November 2025
Published: 31 December 2025

ABSTRACT

Culex pipiens is among the most abundant mosquitoes in Algeria. This mosquito poses a significant public health risk as a vector of various diseases. Developing efficient and eco-friendly pesticides has also become a highly important issue to reduce the risks associated with conventional insecticides. In this context, the present study aimed to evaluate the responses of fourth-instar larvae of *Culex pipiens* (*C. pipiens*) to the effects of two essential oils (EOs) *Eucalyptus globulus* (*E. globulus*) and *Eucalyptus radiata* (*E. radiata*), both known for their bioinsecticidal properties. The oils were obtained by steam distillation and their chemical composition was determined using gas chromatography coupled to mass spectrometry (GC-MS). Larvicidal bioassays were conducted under laboratory conditions, and *in silico* analyses were performed to evaluate interactions between the major essential oils constituents and two detoxification enzymes acetylcholinesterase (AChE; PDB ID: 5X61) and glutathione S-transferase (GST; PDB ID: 18GS). *E. radiata* yielded a higher oil content (1.25 ± 0.36% w/w) than *E. globulus* (0.92 ± 0.48% w/w). GC-MS profiling revealed marked compositional differences: *E. globulus* EO was dominated by 1,8-cineole (72.05%), whereas *E. radiata* contained o-cymene (32.23%) as the main compound. Both EOs showed significant larvicidal activity ($p<0.001$) against *C. pipiens*, with *E. globulus* demonstrating greater potency (LC_{50} = 23.74 ppm) compared to *E. radiata* (LC_{50} = 53.42 ppm). *In silico* studies demonstrated that the major constituents of both essential oils exhibit strong insecticidal potential through various interactions with acetylcholinesterase and glutathione S-transferase. Compounds from *Eucalyptus globulus* showed slightly better binding affinities overall. These results support the development of safe and eco-friendly insecticides, offering a sustainable strategy for protecting both public health and the environment, especially through natural larvicides derived from essential oils targeting *Culex pipiens* mosquitoes.

Keywords : Larvicide; herbal pesticides; eucalyptol; acetylcholinesterase; *in silico*.

INTRODUCTION

Belonging to the Culicidae family, *C. pipiens* (L., 1758) (Diptera: Culicidae) is one of the most prevalent mosquito type worldwide (Bosly, 2022). In Algeria, several studies have shown that *C. pipiens* is the most common mosquito species, particularly in northern Algeria (Kharoubi *et al*., 2020; Arroussi *et al.,* 2021; Halimi *et al*., 2022).

During their blood meal, these small insects, commonly known as house mosquitoes (Al-Mekhlafi *et al*., 2018), can transmit bacteria and viruses which serves as a carrier for various vector-borne diseases (Toubal *et al*., 2022), including West Nile fever, lymphatic filariasis, and Japanese encephalitis (Zhu *et al*., 2023). Moreover, saliva analysis allowed to highlight, for the first time, the high transmission capacity of *C. pipiens* for the Rift Valley fever virus (Phlebovirus riftense, RVFV) (71.4%), compared to *Aedes albopictus* (4.3%) (Gardela *et al*., 2024). These diseases impact not only the public health (Ademar *et al*., 2025) but also the socioeconomic development of tropical and subtropical countries (Silva *et al*., 2024). Within this framework, arboviruses have become important and constant threats in these countries and the situation is further complicated by its rapid development due to climate change and intensifying globalization (Khater *et al*., 2023).

Approximately 2.5 million tons of pesticides are applied to manage the risk associated with the transmission of significant organisms, leading to a global economic impact (Isman & Machial, 2006). This is due to the high toxicity and non-biodegradable nature

of pesticides, coupled with their residues in soil, water sources, and crops, posing threats to public health (Isman & Machial, 2006 ; Ramasamy *et al*., 2024).

Hence, plant-based natural products could represent an alternative approach for sustainable control (El Haddad *et al*., 2018; Ramzi *et al*., 2022). Additionally, it has been observed that certain plants produce compounds that act as natural defenses against insect attacks. EOs are primarily composed of terpenoids and phenylpropanoids and have a range of beneficial properties, including insecticidal activities (Bursali, 2024). Moreover, they are eco-friendly, cost-effective, biodegradable (Khater, 2012) and considered useful larvicides, repellents, and deterrents (Salman *et al*., 2020; Baz *et al*., 2021). In order to control mosquito populations, treating the aquatic larval stage is considered to be the most logical and simple approach compared to adults (Mir *et al*., 2022).

Eucalyptus is a tree belonging to the Myrtaceae family and are usually known as eucalypt (Goldbeck *et al*., 2014; Chandel *et al*., 2019). While originally native to Australia, specific *Eucalyptus* species have successfully acclimated to diverse climates and are now found in various regions (Chandel *et al*., 2019). Currently, this plant is widely distributed in Algeria since its introduction in 1854 by Ramel (Harkat-Madouri *et al*., 2015). *Eucalyptus* EOs are recognized for their powerful insecticidal effect. These are stored in the cells of the leaves of this tree (Filomeno *et al*., 2017).

Although *E. globulus* has been widely studied, comparative data on its larvicidal activity against *C. pipiens* remain scarce. In contrast, *E. radiata*, which exhibits a distinct chemical composition, has received limited attention regarding its potential larvicidal effects on the same species. Furthermore, no previous research has simultaneously integrated GC-MS profiling, larvicidal bioassays, and molecular docking validation to provide a comprehensive comparative assessment of these two EOs.

In the present study, a comparative evaluation of *E. globulus* and *E. radiata* EOs against *C. pipiens* larvae was conducted through GC-MS chemical profiling, *in vitro* larvicidal assays, and *in silico* molecular docking of their major constituents with key detoxification enzymes. This integrated approach provides new insights into the bioactivity of *Eucalyptus* oils and their potential as eco-friendly larvicides.

MATERIALS AND METHODS

Plant collection

The leaves of *E. globulus* and *E. radiata* were harvested during May, 2024 in Afir region (36° 46’ 03” north, 3° 42’ 10” east) located 120 km east of Algiers. *E. globulus* was taxonomically identified by Dr. Bouzid Mosbah, and a voucher specimen (Eg006501) was deposited in the Herbarium of the Constantine Forestry Conservation Department. *E. radiata* was identified by a botanist from the University of Boumerdes (Dr. Rouane Asma). The choice of *Eucalyptus* species was based on their availability and known bioinsecticidal properties.

Mosquito rearing

C. pipiens larvae used in the bioassays were collected from untreated sites at Reghaïa Lake (36° 46’ 17” N, 3° 20’ 38” E), located 29 kilometers east of Algiers.

The collected larvae were transported to the laboratory for sorting according to the stages of development. Mosquito larvae were reared under controlled laboratory conditions in Pyrex glass jars containing 150 mL of lodging water. The jars were placed in screened cages (20 × 20 × 20 cm) and maintained at 27 ± 2 °C, 70–97% relative humidity, and a 12:12 h light–dark photoperiod, until the larvae reached the fourth instar stage (Mir *et al*., 2022).

Larvae identification was facilitated by using the Culicidae identification software of Mediterranean Africa developed by IRD of Montpellier (Brunhes *et al.,* 2000) and confirmed by the identification key of Himmi *et al.* (1995).

Extraction and yield of the EOs

The EOs were extracted using steam distillation method. During distillation, fresh leaves were exposed to steam for 4h. Then the EOs were collected by decantation and dried by Sodium Sulfate (Na_2SO_4) to eliminate any trace of water. The EOs yield was calculated for 100g and expressed as a percentage (w/w) based on the fresh weight of the plant material.

EO yield (%) = [weight of the extracted EO (g)/ weight of sample (g)] x 100

Gas chromatography-Mass spectrometry analysis

To determine the chemical composition of EOs, a gas chromatograph coupled with mass spectrometry (GC-MS) was used. The device used is a GC-MS series “7890/5975 MSD Agilent”, equipped with an HP5MS (5% Phenyl Methyl Silox) capillary column of 30 meters in length, 250 µm in internal diameter and 0.25 µm in film thickness. Helium (He) was used as the carrier gas at a flow rate of 1.5 mL/min. A volume of 0.2 µl was injected in 1/50 split mode. The injector temperature was maintained at 250°C. Programming the oven temperature started at 60°C for 8 minutes, followed by an increase of 4°C per minute until reaching 250°C, a temperature maintained for 25 minutes. The ionization mode used is electron impact at 70 eV, and the analysis was carried out in scan mode and the m/z mass interval was set from 40 to 550 amu. The identification of the constituents is carried out by comparing the retention indices (RI), calculated from a series of alkanes (C_6-C_{28}), as well as the spectral data (mass spectra) of the individualized compounds with the characteristics of the reference products present in the “NIST MS” spectral library.

Treatment and larvicidal activity

The larvicidal activity of both EOs (*E. radiata* and *E. globulus*) was performed against newly emerged fourth-instar larvae of *C. pipiens* according to World Health Organization (WHO, 2005) with minor modifications. Based on preliminary tests, each EO was dissolved in 1 ml of ethanol and then diluted in 100 ml lodging water in order to obtain the desired concentrations (10, 30, 50 and 70 ppm). Twenty larvae were exposed to 100 ml of each concentration. The same number of larvae were placed in control cups containing 100 ml of site water only for the negative control, while the solvent control were exposed to ethanol only. Three repetitions were performed for each concentration. Results were read at each 24-hour interval.

After the exposure time, larvae were scored dead when they failed to move after probing with a needle in the siphon or cervical region. The observed mortality poucentages of the different series were determined and then corrected (Abbott, 1925) and lethal concentrations with their 95 % confidence limits (95 % CL) were calculated.

Molecular docking

Proteins preparation

This study targeted two enzymes, acetylcholinesterase and glutathione S-transferase, with their crystal structures retrieved from the RCSB Protein Data Bank (PDB) using PDB IDs 5X61 for acetylcholinesterase and 1G8S for glutathione S-transferase. The 3D structures were prepared and optimized by removing water molecules, excess atoms, and other non-essential components prior to docking using ArgusLab.

Ligands preparation

This work focused on four phytochemical major compounds: (1,8-cineole; CID 2758), (α-pinene: CID 6654), (o-cymene: CID 10703), (cryptone: CID 92780 and their 3D molecular structures were downloaded in the SDF format from PubChem's chemical database (https://pubchem.ncbi.nlm.nih.gov) and converted to PDB format using Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/).

Molecular docking simulation

AutoDockTools 1.5.6 software (https://vina.scripps.edu) (Trott & Olson, 2009) was used to configure the ligands, proteins, and parameters prior to conducting the docking analysis. Only polar hydrogen atoms were added to the target's proteins. The prepared protein and ligand structures were saved in PDBQT format to generate energy grid maps. A grid box with dimensions of 126 × 126 × 126 Å and a grid spacing of 0.375 Å was employed for the calculations. Discovery Studio 4.5 software was used to visualize the results which generated 2D and 3D images to illustrate the interactions.

Statistical analysis

Data are presented as mean ± Standard Error (SE). Comparison between series was performed by one-way ANOVA, followed by Tukey's test (P<0.05). The probit-log(concentration) regression model was used to calculate LC_{50} and LC_{90} values, 95% confidence interval (CI), slopes, intercept, Chi-square values and degrees of freedom (Df). All statistical analyses were performed by using SPSS Statistics 26.0 Software.

RESULTS

Extraction and yield

In this study, the steam distillation method of *E. globulus* and *E. radiata* produced yellow to pale-yellow EOs with a liquid appearance emitting a strong odor. The extraction yield, expressed as distillated oil weight/100 g fresh leaves, is 0.92±0.48% (w/w) for *E. globulus* and 1.25±0.36% (w/w) for *E. radiata,* which was higher than that of *E. globulus*.

GC-MS analysis of EOs

The GC-MS profiling identified 33 distinct compounds, representing 97.667% of the total composition of *E. globulus* EO. The main compound is eucalyptol (1,8-cineole), representing 72.053%, followed by α-pinene (8.572%), pinocarveol (3.022%) and (+)-aromadendrene (3.014%) (Figure 1, Table 1).

The chemical profiling of *E. radiata* EO revealed a diverse chemical composition, with a marked predominance of certain compounds. The analysis identified a total of 33 components, representing 95.504% of the total oil composition. Among these, some are present in significant quantities and strongly influence the properties of the oil. The main compound is o-cymene, which constitutes 32.229% of the EO, followed by cryptone with 9.685%. Other notable compounds include spathulenol (13.109%), 4-terpineol (4.981%), and 3-carene (5.525%) (Figure 2, Table 2).

Mosquito larvicidal activity of EOs

EOs were tested at 10, 30, 50 and 70 ppm against fourth-instar larvae, with mortality recorded after 24 h and 48 h (Figure 3). No mortality was detected in the solvent and negative controls.

Mortality increased significantly with both concentration and exposure time. The lowest concentration (10 ppm), increased significantly the mortality rate from 45 ± 1.15% (P<0.001) to 68 ± 1.67% (P<0.001) (n=3) for *E. globulus* EO, while it rises the mortality rate from 30±1.35% (P<0.001) to 55±1.45% (P<0.001) (n=3) for *E. radiata* EO after 24h and 48h respectively.

The lethal concentrations LC_{50} and LC_{90} values of *E. globulus* and *E. radiata* EOs against *C. pipiens* larvae after 24 and 48 hours of treatment with their interval at 95%, slope, intercept, X^2, Df are listed in Table 3.

According to Cheng *et al*. (2003); Dias et Moraes (2014) EOs with an LC_{50} below 100 µg/mL (equivalent of 100 ppm) are considered effective larvicides, while those with an LC_{50} below 50 µg/mL (equivalent of 50 ppm) are categorized as highly active. The LC_{50} values obtained from *E. globulus* EO were 23.74 ppm and 6.69 ppm, while those for *E. radiata* were 53.42 ppm and 8.16 ppm after 24 h and 48 h, respectively. Considering the mortality rate at each concentration, the median lethal concentration (LC_{50}) obtained classifies the EOs as potential larvicidal agents.

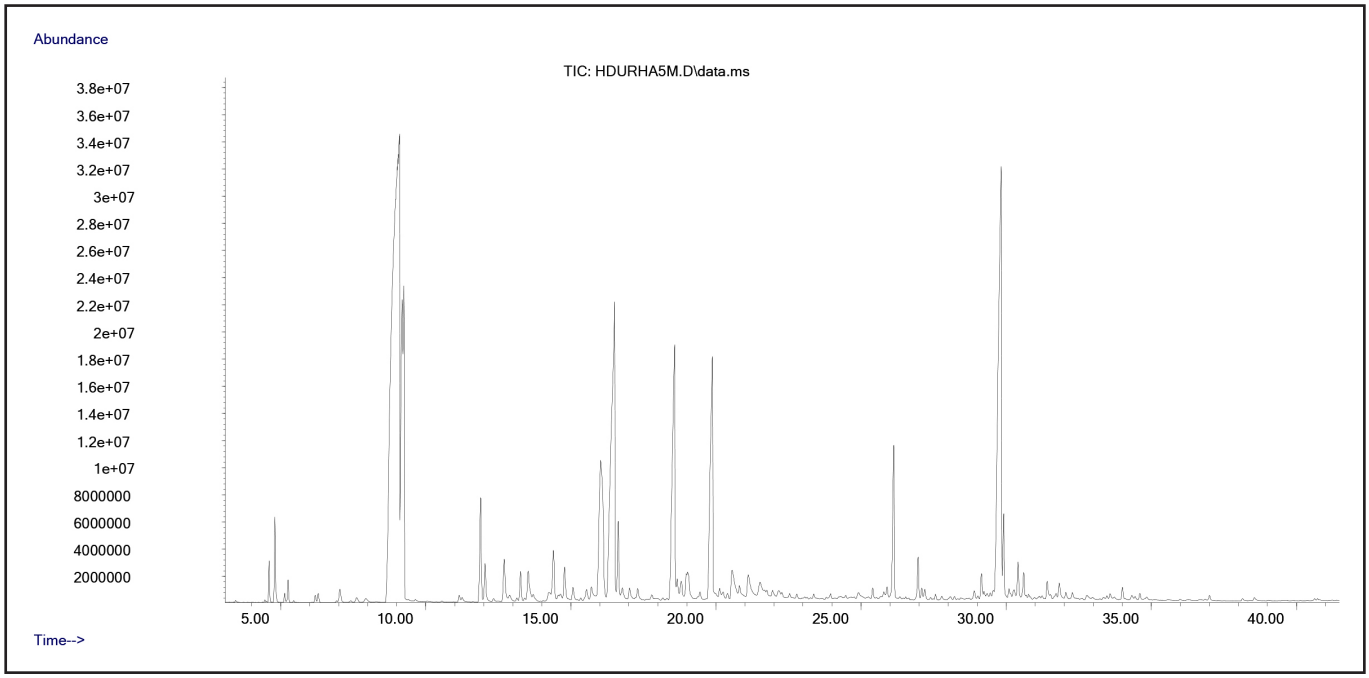


Figure 1. Chromatogramm of *E. globulus* essential oil.

Table 1. Chemical characterization of *E. globulus* EO using GC–MS, showing retention time, compound group, and relative percentage composition

N°	Compound [a]	Rt (min)	Molecular formulae	Compound groups	Calculated IK [b]	Theoretical IK [c]	% [d]
1	α-Thujene	4.374	$C_{10}H_{16}$	Monoterpene hydrocarbon	917	918	tr
2	α-Pinene	4.872	$C_{10}H_{16}$	Monoterpene hydrocarbon	924	925	8.572
3	Camphene	5.260	$C_{10}H_{16}$	Monoterpene hydrocarbon	934	937	0.137
4	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	5.452	$C_{10}H_{14}$	Monoterpene hydrocarbon (unsaturated)	939	954	0.076
5	β-Pinene	6.298	$C_{10}H_{16}$	Monoterpene hydrocarbon	961	964	0.438
6	β-Myrcene	7.062	$C_{10}H_{16}$	Monoterpene hydrocarbon	981	983	Tr
7	α-Phellandrene	7.665	$C_{10}H_{16}$	Monoterpene hydrocarbon	996	998	0.273
8	Eucalyptol	9.528	$C_{10}H_{18}O$	Monoterpene ether	1033	1033	72.053
9	τ-Terpinene	10.587	$C_{10}H_{16}$	Monoterpene hydrocarbon	1056	1056	0.208
10	Dehydro-p-cymene	12.035	$C_{10}H_{14}$	Monoterpene hydrocarbon (aromatic)	1086	1088	0.289
11	Solusterol (iso-Amyl isovalerate)	12.994	$C_{10}H_{20}O_2$	Ester (monoterpene derivative)	1106	1106	0.348
12	α-Campholenal	13.109	$C_{10}H_{16}O$	Monoterpene aldehyde	1120	—	0.083
13	Pinocarveol	14.276	$C_{10}H_{16}O$	Monoterpene alcohol	1133	1134	3.022
14	Pinocarvone	15.274	$C_{10}H_{14}O$	Monoterpene ketone	1154	—	1.392
15	Borneol	15.402	$C_{10}H_{18}O$	Monoterpene alcohol	1157	1159	0.136
16	4-Terpineol	15.931	$C_{10}H_{18}O$	Monoterpene alcohol	1168	1174	0.263
17	5-Isopropenyl-2-methylenecyclohexanol	16.435	$C_{10}H_{16}O$	Monoterpene alcohol	1178	1186	0.591
18	α-Terpineol	16.548	$C_{10}H_{18}O$	Monoterpene alcohol	1181	1185	0.757
19	Myrtenol	16.749	$C_{10}H_{16}O$	Monoterpene alcohol	1185	1189	0.111
20	D-Carvone	18.603	$C_{10}H_{14}O$	Monoterpene ketone	1233	1234	0.12
21	Caryophyllene	24.743	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1410	1416	0.08
22	Calarene	25.145	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1423	1423	0.147
23	(+)-Aromadendrene	25.425	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1431	1439	3.014
24	τ-Gurjunene	25.535	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1435	1444	0.111
25	α-Caryophyllene	25.842	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1444	1445	tr
26	Alloaromadendrene	26.075	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1452	1454	0.575
27	β-Eudesmene	26.871	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1477	1474	0.041
28	Varidiflorene	27.176	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1486	1487	0.212
29	Epiglobulol	29.101	$C_{15}H_{26}O$	Sesquiterpene alcohol	1551	—	0.756
30	Ent-Spathulenol	29.630	$C_{15}H_{24}O$	Sesquiterpene alcohol	1569	1578	0.081
31	Globulol	29.857	$C_{15}H_{26}O$	Sesquiterpene alcohol	1577	1579	2.963
32	Viridiflorol	30.042	$C_{15}H_{26}O$	Sesquiterpene alcohol	1583	1585	0.586
33	β-Eudesmol	30.349	$C_{15}H_{26}O$	Sesquiterpene alcohol	1615	1609	0.232
						Total	**97.667**

[a] Identified compounds are listed in the order of elution using a nonpolar HP-5MS capillary column.
[b] Calculated retention indices obtained on a nonpolar HP-5MS capillary column.
[c] Literature retention indices reported for columns of similar polarity (HP-5MS or DB-5).
[d] Relative percentages of identified compounds, calculated from GC–MS peak areas using an HP-5MS column.
tr: trace amounts (<0.05%).
% percentage.

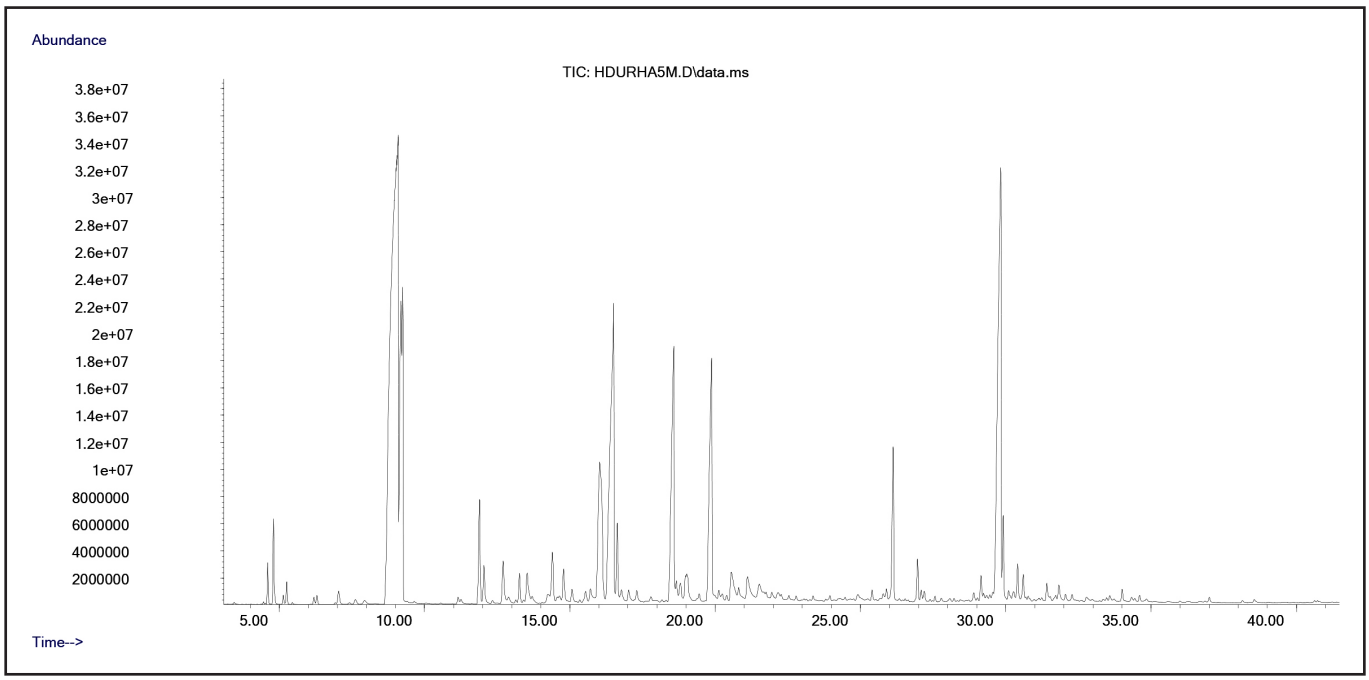


Figure 2. Chromatogramm of *E. radiata* essential oil.

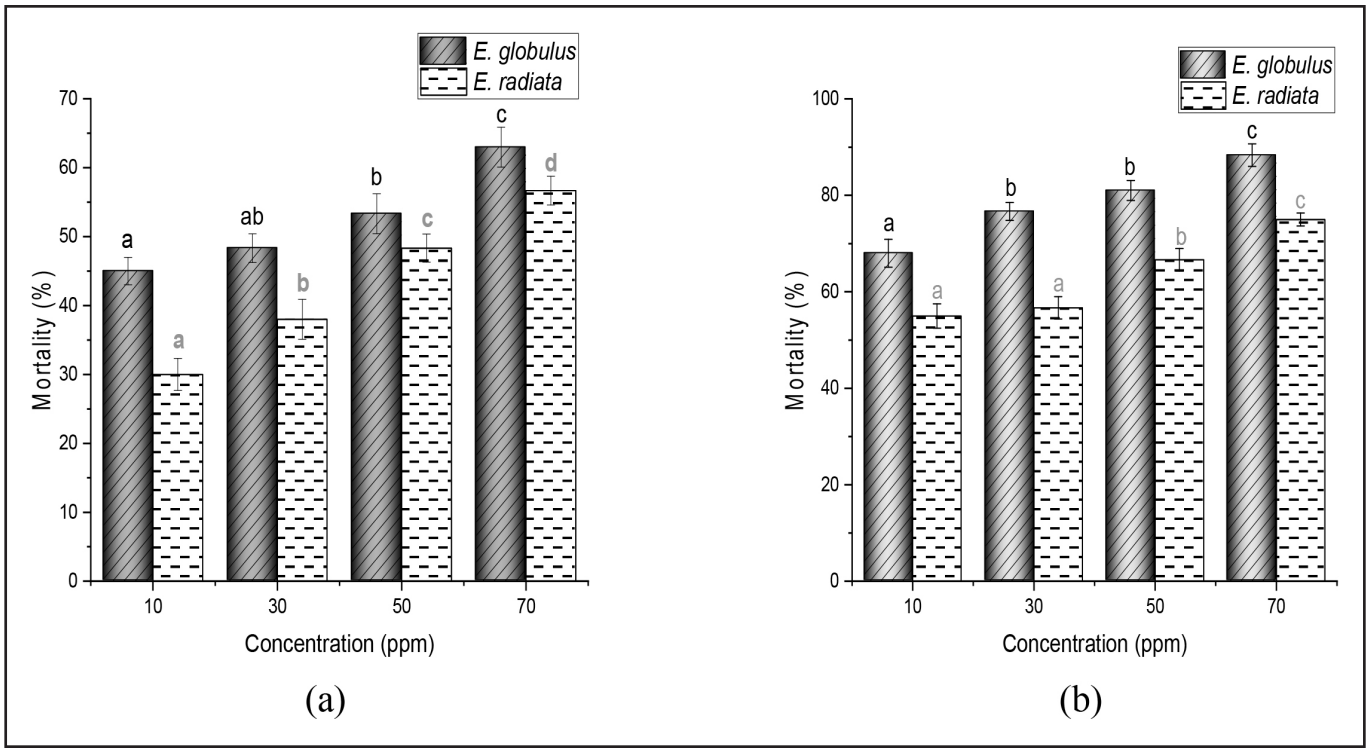


Figure 3. Larvicidal activity of *E. globulus* and *E. radiata* EOs against the early fourth instar larvae of *Culex pipiens* after 24h (a) and 48h (b) exposure times (mean ± SE, n = 3 replicates each containing 20 larvae). Values with the same letter for each species do not show statistically significant differences at $P < 0.05$ by Tukey-Kramer and Sheffé.

Table 2. Chemical characterization of *E. radiata* EO using GC–MS, showing retention time, compound group, and relative percentage composition

N°	Compound [a]	Rt (min)	Molecular formulae	Compound groups	Calculated IK [b]	Theoretical IK [c]	% [d]
1	Tricyclene	4.462	$C_{10}H_{16}$	Monoterpene hydrocarbon	914	918	tr
2	Cumene	4.526	C_9H_{12}	Aromatic hydrocarbon	915	919	tr
3	α-Thujene	4.608	$C_{10}H_{16}$	Monoterpene hydrocarbon	917	918	0.367
4	α-Pinene	4.807	$C_{10}H_{16}$	Monoterpene hydrocarbon	922	925	0.856
5	Camphene	5.261	$C_{10}H_{16}$	Monoterpene hydrocarbon	934	937	0.233
6	β-Pinene	6.299	$C_{10}H_{16}$	Monoterpene hydrocarbon	961	964	tr
7	β-Myrcene	7.046	$C_{10}H_{16}$	Monoterpene hydrocarbon	980	983	0.227
8	o-Cymene	9.045	$C_{10}H_{14}$	Aromatic monoterpene	1026	1026	32.229
9	3-Carene	9.191	$C_{10}H_{16}$	Monoterpene hydrocarbon	1028	1023	5.525
10	Eucalyptol	9.252	$C_{10}H_{18}O$	Oxygenated monoterpene	1029	1030	3.27
11	Fenchone	11.898	$C_{10}H_{16}O$	Oxygenated monoterpene	1083	1086	1.526
12	Dehydro-p-cymene	12.046	$C_{10}H_{12}$	Aromatic monoterpene	1086	1088	0.6
13	Linalol	12.707	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1099	1100	0.686
14	Thujone	13.269	$C_{10}H_{16}O$	Oxygenated monoterpene (ketone)	1111	1110	0.389
15	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	13.534	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1117	1120	0.51
16	Camphor	14.401	$C_{10}H_{16}O$	Oxygenated monoterpene (ketone)	1135	1139	0.87
17	Borneol	15.554	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1160	1159	0.212
18	4-Terpineol	16.031	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1170	1174	4.981
19	Crypton	16.507	$C_9H_{14}O$	Aromatic oxygenated compound	1180	1183	9.685
20	α-Terpineol	16.638	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1183	1185	0.92
21	cis-Piperitol	16.777	$C_{10}H_{18}O$	Oxygenated monoterpene (alcohol)	1197	1194	0.211
22	Cuminal	18.578	$C_{10}H_{12}O$	Aromatic oxygenated compound (aldehyde)	1232	1234	6.287
23	Piperitone	19.025	$C_{10}H_{16}O$	Oxygenated monoterpene (ketone)	1245	1250	0.919
24	Phellandral	19.877	$C_{10}H_{16}O$	Oxygenated monoterpene (aldehyde)	1270	1271	5.802
25	Cuminol	20.559	$C_{10}H_{14}O$	Aromatic oxygenated compound (alcohol)	1289	1287	0.923
26	Carvacrol	21.113	$C_{10}H_{14}O$	Aromatic oxygenated compound (phenol)	1305	1304	0.624
27	Copaene	23.382	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1370	1371	0.092
28	Alloaromadendrene	26.128	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1453	1454	2.374
29	Varidiflorene	27.199	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	1487	1487	0.164
30	Spathulenol	29.829	$C_{15}H_{24}O$	Oxygenated sesquiterpene (alcohol)	1576	1576	13.109
31	Ent-Spathulenol	29.915	$C_{15}H_{24}O$	Oxygenated sesquiterpene (alcohol)	1579	1578	1.35
32	Epiglobulol	30.099	$C_{15}H_{26}O$	Oxygenated sesquiterpene (alcohol)	1585	—	0.085
33	Ledol	30.411	$C_{15}H_{26}O$	Oxygenated sesquiterpene (alcohol)	1596	1590	0.478
						Total	**95.504**

[a] Identified compounds are listed in the order of elution using a nonpolar HP-5MS capillary column.
[b] Calculated retention indices obtained on a nonpolar HP-5MS capillary column.
[c] Literature retention indices reported for columns of similar polarity (HP-5MS or DB-5).
[d] Relative percentages of identified compounds, calculated from GC–MS peak areas using an HP-5MS column.
tr: trace amounts (<0.05%).
% percentage.

Table 3. Lethal concentrations LC_{50} and LC_{90} of *E. globulus* and *E. radiata* EOs against fourth-instar larvae of *C. pipiens* after 24h and 48h of exposure

Exposure time	EOs	LC_{50} (ppm)	CI 95% (LCL-UCL)	LC_{90} (ppm)	CI 95% (LCL-UCL)	Slope	Intercept	Chi-square (χ^2)	df	P
24h	EG	23.748	3.544 – 46.934	12490.732	816.307 – 9.586E+15	0.471	-0.648	0.029	2	0.985
	ER	53.424	37.032 – 106.426	2190.808	523.404 – 153422.846	0.79	-1.36	0.034	2	0.983
48h	EG	6.699	2.606 – 10.667	94.385	62.277 – 214.428	1.13	-0.95	0.031	2	0.984
	ER	8.164	0.461 – 15.945	1279.465	272.881 – 2794818.47	0.6	-0.55	0.027	2	0.986

EOs: Essential oils, EG *E. globulus*, ER *E. radiata*, LC_{50} lethal concentration that kills 50% of larvae, LC_{90} lethal concentration that kills 90% of larvae, 95% CI confidence interval of 95%, LCL lower confidence limit, UCL upper confidence limit (95% fiducial limit), df degrees of freedom.

Table 4. *In silico* insecticidal activity of *E. globulus* and *E. radiata* major compounds against acetylcholinesterase and S-glutathione transferase

Compound name	18GS Binding energy (Kcal/mol)	Bonds and amino acid residues	5X61 Binding energy (Kcal/mol)	Bonds and amino acid residues
1,8 cineole	-5.2	Arg282 (hydrophobic alkyl bond), Tyr287 (hydrophobic Pi-alkyl bond), Arg74 (conventional hydrogen bond).	-6.4	Tyr1031 (conventional hydrogen bond)
α-pinene	-5.3	Tyr287 (two hydrophobic Pi- alkyl bonds), Arg282 (two hydrophobic alkyl bonds), Tyr79 (hydrophobic Pi- alkyl bond)	-6.2	Tyr494, Trp441 (hydrophobic Pi-alkyl bond), Leu444 (hydrophobic alkyl bond)
o-cymene	-5.3	Arg282, Arg74 (hydrophobic alkyl bond), Ala86, Tyr79 (hydrophobic Pi- alkyl bonds)	-5.8	Trp782 (Two hydrophobic Pi-alkyl bonds), Ser820 (conventional hydrogen bond), Tyr819 (unfavorable)
Cryptone	-5.3	Arg74 (hydrophobic Pi- alkyl bonds)	-5.8	Tyr282, Tyr493 (hydrophobic Pi-alkyl bond)
pyriproxyfen	-6.4	Arg74 (hydrophobic Pi-alkyl bond)	-8.1	Tyr282, Tyr493 (hydrophobic Pi-alkyl bond).

For *E. globulus*, the Chi-square goodness-of-fit test confirmed that the probit model adequately fitted the observed data after 24 h (χ^2 = 0.029, df = 2, P = 0.985). After 48 h, an excellent fit was obtained (χ^2 = 0.034, df = 2, P = 0.983).

Regarding *E. radiata*, the probit analysis at 24 h showed a good fit to the data (χ^2 = 0.031, df = 2, P = 0.984). After 48 h, the Chi-square goodness-of-fit test demonstrated an excellent agreement between the probit model and the observed data (χ^2 = 0.027, df = 2, P = 0.986).

The results lead also to conclude that both EOs are effective in terms of combating *C. pipiens* larvae; however, the EO of *E. globulus* seems to be more effective than *E. radiata* EO.

Molecular docking results

The molecular docking results, summarized in Tables 4, 5, and 6, demonstrate varying binding affinities of the tested compounds toward the targets S-glutathione transferase (PDB ID: 18GS) and acetylcholinesterase (PDB ID: 5X61).

However, for the 5X61 target, 1,8-cineole really stood out with the strongest binding, and a-pinene was a close second. These two compounds clearly have a much higher affinity for 5X61 compared to the other molecules we tested.

When interacting with the 18GS target, α-pinene, o-cymene, cryptone, and 1,8-cineole exhibit very similar binding affinities. While α-pinene, o-cymene, and cryptone might have a slight edge, all four are considered to have a moderate affinity for 18GS.

DISCUSSION

EOs are lipophilic secondary metabolites obtained from aromatic plants; dominated mainly by terpenoids (Jankowska *et al*., 2017). *Eucalyptus* is one of the notable plants recognized for its fragrant aroma, which comes primarily from an EO found in the leaves (Vecchio *et al*., 2016). The genus *Eucalyptus* has leaves that contain aromatic oils with a characteristic odour whose recovery by steam distillation produces EOs (Denny, 2002). However, young leaves contain more oil than older leaves, whereas leaves from mature trees produced a slightly higher yield (Zhang *et al*., 2010).

According to Mahumane (2016) *E. radiata* is one of the highest EO yielding among *Eucalyptus* species. EO yields ranged from 0.14% to 4.31% (w/w).

Regarding *E. globulus*, studies in Algeria have shown variation in extraction yield depending on the plant part and collection site. For exmple the yield in Tebessa was estimated to 2.89 ± 0.64 % (Yahia *et al*., 2023), while similar yields were reported, ranging from 0.93% in Constantine (Atmani-Merabet *et al*., 2018) to 0.96% in Blida (Boukhatem *et al*., 2014). In contrast, a significantly lower yield (0.36 ± 0.03%) was recorded in Mascara by Bourakna *et al*. (2022).

Similar yields have also been reported in other countries for *E. globulus* EO: 0.95 to 1.32 % in Ethiopia (Shiferaw *et al*., 2019), 1.05 - 1.1 % in India (Joshi *et al*., 2016), and lower yield 0.235% was obtained by hydrodistillation method in Iran (Madreseh-Ghahfarokhi *et al*., 2018). A higher yield 2.68 % was obtained in Argentina (Viturro *et al*., 2003). This variation in EO yield among *Eucalyptus* species could be attributed to genotype, age, seasonal variation, environmental factors, time of collection and extraction method (Gilles *et al*., 2010; Mahumane, 2016).

E. globulus as well as *E. radiata* are well-known species that produce EOs. The chromatographic analysis of *E. globulus EO* showed a high percentage of several major compounds including eucalyptol also known as 1,8-cineole (72.053%), α-pinene (8.572%), pinocarveol (3.022%) and (+)-aromadendrene (3.014%).

Table 5. Protein ligand interaction of selected ligand against protein 18GS

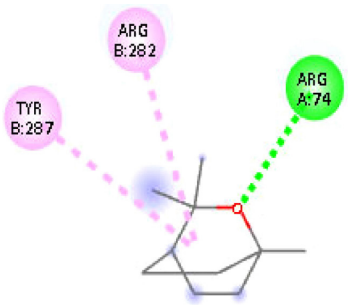
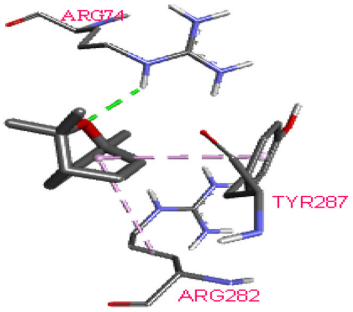
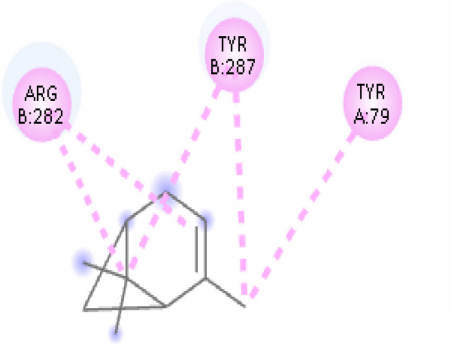
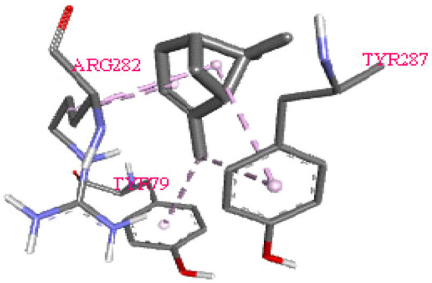
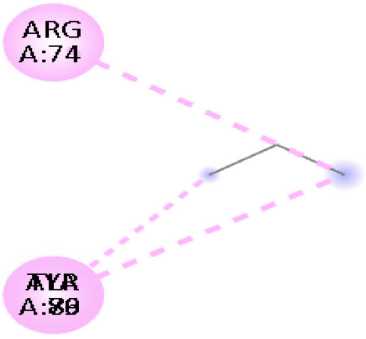
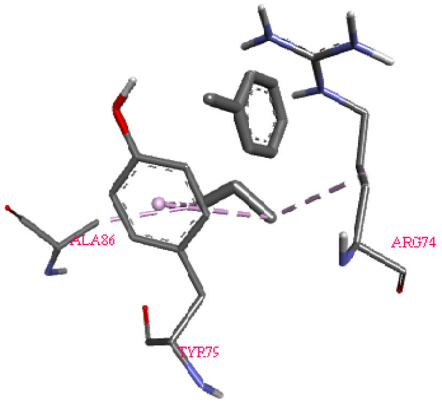
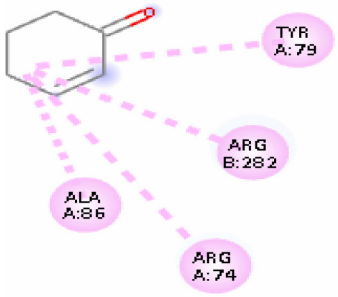
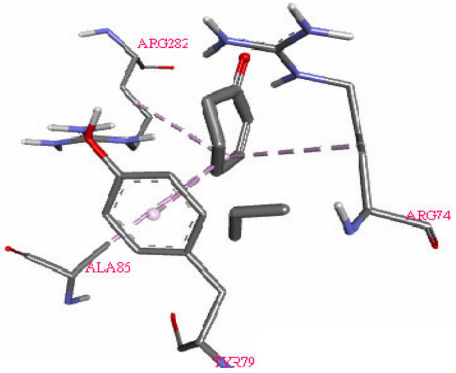
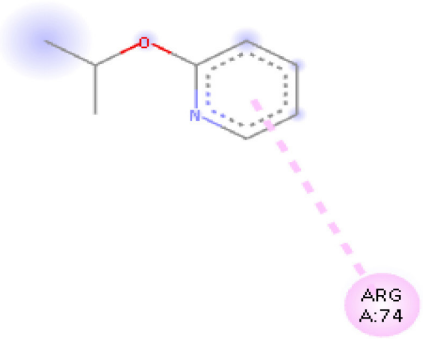
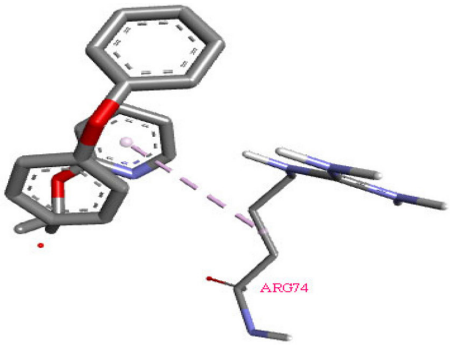
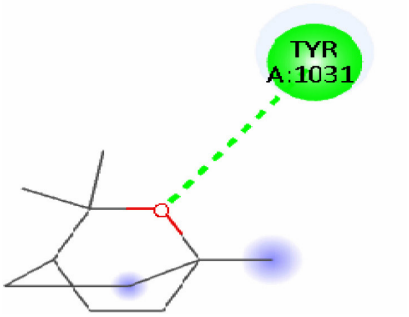
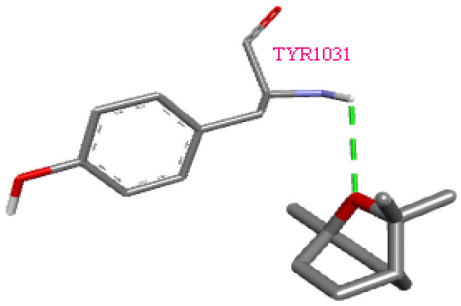
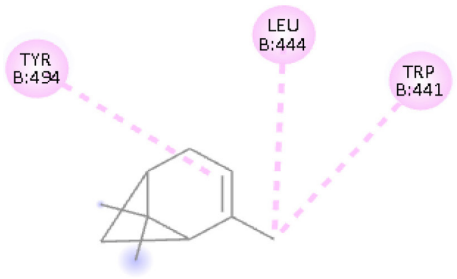
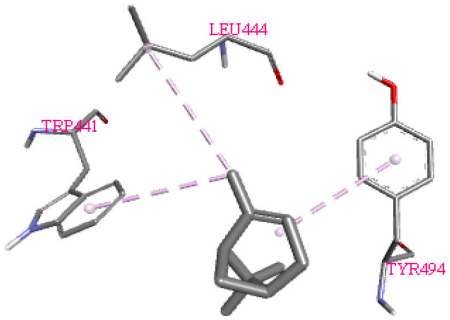
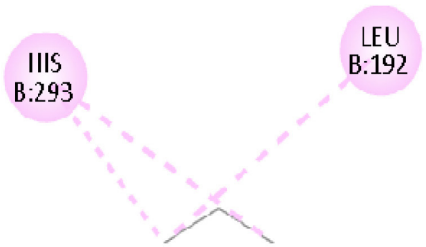
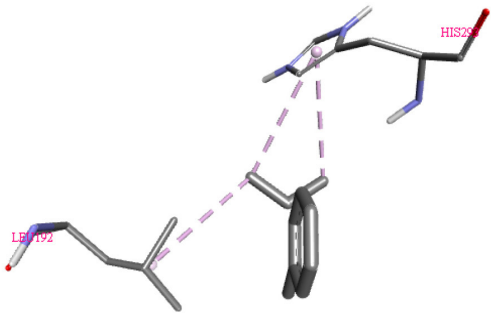
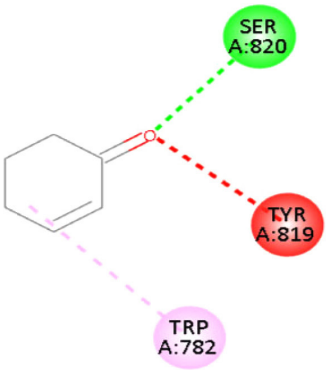
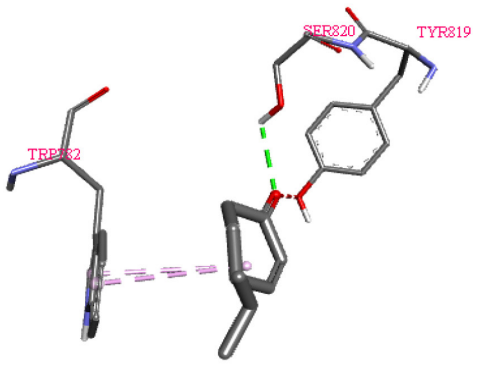
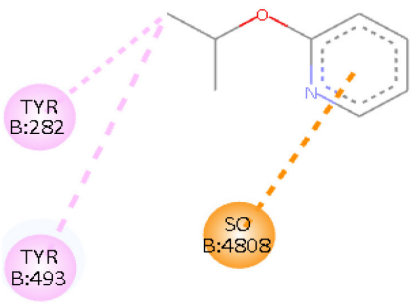
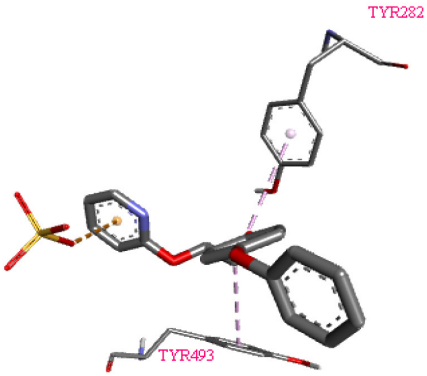
Compound	2D Structure	3D Structure
1,8 cineole		
α-pinene		
O-cymene		
Cryptone		
Pyriproxyfen		

Table 6. Protein ligand interaction of selected ligand against protein 5X61

Compound	2D Structure	3D Structure
1,8 cineole		
α-pinene		
O-cymene		
Cryptone		
Pyriproxyfen		

In Algeria, several studies align with our findings, showing that the terpene 1,8-cineole is the major compound in the EO of *E. globulus*. For example, studies carried out by Atmani-Merabet *et al*. (2018) and Djenane *et al*. (2011) reported contents of 78.45% and 81.70% respectively. Similar results were obtained from studies of other countries in the world such as Brazil (Maciel *et al*., 2010), Argentina (Lucia *et al*., 2008) and India (Vivekanandhan *et al*., 2020).

Despite the majority of *Eucalyptus* EO in the world have been reported to contain 1,8-cineole as the major constituent (Goldbeck *et al*., 2014), our results showed that this compound was absent in the leaves oil of *E. radiata*. This finding is consistent with the results of Luís *et al*. (2016) and Nishimura *et al*. (1980), who reported limonene (68.51%) and α-pinene as the principal components, respectively. The major constituent found in this research is the monoterpene, o-cymene (32.229%), followed by cryptone (9.685%), spathulenol (13.109%), 4-terpineol (4.981%), and 3-carene (5.525%). Constituents such as *p*-Cymene, Spathulenol and 4-terpineol were also reported by Mulyaningsih *et al.* (2011).

According to Bourakna *et al*. (2022) the qualitative and quantitative variations in the composition of EOs from the same botanical species can be explained by the influence of several factors by numerous factors, notably the plant organ and its age, the condition of the plant material used (whether fresh or dried), geographical and environmental factors, climate and sunlight exposure, soil composition, the season and time of harvest, as well as the extraction techniques and experimental conditions applied. These factors influence the plant's biosynthetic pathways, thereby altering the relative proportions of the main characteristic compounds. As a result, different chemotypes emerge, which differentiate EOs based on their origins (Djenane *et al*., 2011).

The larvicidal activity reveals a direct relationship between larval mortality and both concentration and exposure time. This finding was confirmed by Yahia *et al*. (2023). This supports our results, where the mortality increases from 45±1.15% to 68±1.67% mortality for *E. globulus* EO, and from 30±1.35% and 55±1.45% for *E. radiata* EO after 24 and 48 hours, respectively.

Insecticides derived from plant sources tend to be more specific in targeting pests and easily broken down by natural processes which makes them safer for humans and animals and less harmful, to the environment compared to artificial chemicals (Jeyabalan *et al*., 2003). Previous reports have demonstrated the insecticidal properties of *Eucalyptus* EOs (Lucia *et al*., 2012; Yahia *et al*., 2023). *A comparative study was conducted by* Kaura *et al*. (2019) and confirmed that *Eucalyptus* oil was found to be more effective than Neem against larvae (LC_{50}= 93.3 ppm) and pupae (LC_{50}= 144.5 ppm).

According to Batish *et al*. (2008) *Eucalyptus* oil can directly act as a natural insect repellent to provide protection against mosquitoes and other harmful arthropods. Moreover, *Eucalyptus* EOs are used as the active substances in non-toxic repellent products that are recommended for children and can be used also in sensitive areas, such as homes, schools, restaurants, and hospitals.

In comparison with *E. radiata*, *E. globulus* EO exhibited a much lower LC_{50} (23.74 ppm). In Algeria, this low LC_{50} value was in good agreement with previous reports of Yahia *et al*. (2023) in which *E. globulus* EO was able to induce 50% mortality of fourth instar larvae of *C. longiareolata* at the concentration of 24.23 ppm after 24 h of treatment while LC_{90} value was 46.13 ppm. A slightly higher result was recorded by Vivekanandhan *et al.* (2020) where the EO extracted from *E. globulus* in India showed LC_{50} and LC_{90} values of 30.198 ppm and 103.389 ppm, respectively, against *Anopheles stephensi* after 24 h. Whereas, too much lower values were obtanied by the same authors when evaluating the larvicidal activity against *Aedes aegypti* (13.578 ppm, 106.755 ppm), *Culex quinquefasciatus* (7.469 ppm, 32.454 ppm). After 48 h post treatment, LC_{50} and LC_{90} values were 12.576, 49.380 ppm for *Anopheles stephensi,* 7.926, 34.470 ppm for *Aedes aegypti* and 4.408, 21.048 ppm for *Culex quinquefasciatus.* Significant insecticidal activity of *E. globulus* EO against *Culex theileri* (Theobald) has also been observed, its effectiveness being particularly notable at high concentrations (Madreseh-Ghahfarokhi *et al*., 2018).

Regarding *E. radiata*, despite its wide distribution in Algeria, to the best of the authors' knowledge and current date, there are currently no available data about insecticidal activity of its EO against *C. pipiens*, except studies conducted in Argentina by Lucia *et al*. (2012) who evaluate the larvicidal and adulticidal activities of several *Eucalyptus* EOs including *E. radiata ssp radiata* against another mosquito spieces, *Aedes aegypti*. According to the same authors, the results confirmed the importance of the major components in the biological activity of *Eucalyptus* EOs on *A. aegypti.* Recently Duarte *et al*. (2024b), demonstrated that nanoemulsion formulations containing cymene, the major compound of *E. radiata* in this work, have a significant efficacy as repellents against the dengue vector mosquito, *Aedes aegypti*. However, other studies were interrested about fumigant and insecticidal activities of the same plant against other insects *Blattella germanica*. Results showed a high fumigant toxicity against adult male German cockroaches *Blattella germanica* at a concentration of 7.5 mg/liter air concentration and a strong insecticidal activity against adult male and female of the same insect (Yeom *et al*., 2013).

The observed differences in larvicidal activity between the two EOs were probably due to the variations in their chemical composition, highlighting the influence of chemotypic variation on larvicidal potency. The higher efficacy of *E. globulus* EO may be attributed to its dominant 1,8-cineole chemotype (72.05%), a monoterpene oxide known for strong neurotoxic effects on mosquito larvae. In contrast, *E. radiata* EO, characterized by an o-cymene rich chemotype (32.23%) with lower oxygenated compound content, exhibited comparatively weaker activity. Since oxygenated monoterpenes such as 1,8-cineole are often associated with enhanced larvicidal and enzyme inhibitory effects, these compositional differences may account for the higher efficacy of *E. globulus*. The same result was found in the species *E. globulus* which exhibited a strong insecticidal effect on mosquito larvae and a chemical composition rich in 1,8-cineole (Elzayyat *et al*., 2018).

The observed activity could be attributed also to the synergistic interactions among the constituents with higher content. According to literature data, the larvicidal activity of *Eucalyptus* EOs could be estimated from the relative concentration of two main components (*p*-cymene and 1,8-cineole) (Lucia *et al*., 2012). It is noteworthy that o-cymene is frequently present in 1,8-cineole-rich oils, including *Eucalyptus* EOs (Mulyaningsih *et al*., 2011). An interesting mechanism for synergistic effects using a metabolic model was proposed by Scalerandi *et al*. (2018). Their findings demonstrated that, within a mixture, the insect's detoxification system primarily targets the major component, allowing the minor component to intoxicate the insect more effectively. Consequently, the minor component exhibits higher toxicity than when tested alone, as the detoxification system is preoccupied with the main component.

Regarding the mode of action, several mechanisms through which EOs produce neurotoxic effects on insects, leading to paralysis and death by interfering with their nervous system, primarily through the acting on acetylcholinesterase, GABA receptors or octopamine receptors (Jankowska *et al*., 2017).

To maintain normal physiological functions, insects produce detoxification enzymes such as Acetylcholinesterase and S-glutathione transferase as part of their defense mechanisms (Enayati *et al*., 2005). Acetylcholinesterase is a promising molecular target for EOs with larvicidal activity (Seo *et al*., 2015) and Glutathione-S-transferases (GSTs) are multifunctional enzymes involved in the detoxification of diverse endogenous and xenobiotic substances through the catalytic conjugation of these compounds with the thiol moiety of glutathione (GSH) (Türkan *et al*., 2019; Turkan *et al*., 2020).

Therefore primary compounds in EOs of *E. globulus* (1,8-cineole and α-pinene) and *E. radiata* (o-cymene and cryptone) were further studied along with pyriproxyfen as standard compound for their insecticidal properties using *in silico* molecular-docking studies against acetylcholinesterase (AChE, PDB: 5X61) and S-glutathione transferase (GST, PDB: 18GS).

For S-glutathione transferase (PDB ID:18GS), the docking results reveal distinct interaction patterns for the tested compounds, with binding energies ranging from -5.2 to -6.4 kcal/mol. 1,8-cineole from *E. globulus* showed a binding energy of -5.2 kcal/mol, interacting through a conventional hydrogen bond with Arg74, a hydrophobic alkyl bond with Arg282, and a hydrophobic Pi-alkyl bond with Tyr287. This combination of hydrogen and hydrophobic interactions suggests a balanced binding mode, potentially contributing to moderate inhibitory activity. α-Pinene, also from *E. globulus* with a slightly better binding energy of -5.3 kcal/mol, formed several hydrophobic interactions, including two Pi-alkyl bonds with Tyr287, two alkyl bonds with Arg282, and one Pi-alkyl bond with Tyr79.

For *E. radiata*, o-cymene and cryptone each showed a binding energy of (-5.3 kcal/mol). O-cymene engaged in hydrophobic alkyl interactions with Arg282 and Arg74, as well as Pi-alkyl interactions with Ala86 and Tyr79, indicating a strong hydrophobic binding affinity. On the other hand, cryptone formed just one hydrophobic Pi-alkyl bond with Arg74, suggesting a weaker interaction network that could reduce its effectiveness as an inhibitor.

Glutathione S transferases (GSTs) detoxify xenobiotics (insecticides or toxic phytochemicals) through conjugation with glutathione (GSH). If a chemical is known to bind to the active site of GST, especially those which have interaction with residues of the GSH binding site or the electrophile binding "substrate site", they inhibit substrate binding (competitive or noncompetitive inhibition) or catalytic turnover. This will reduce the capacity of the larvae to detoxify the chemical and, therefore, increase toxicity.

For instance, Khan *et al*. (2013) demonstrated that cantharidin inhibits GST activity in the midgut of *Helicoverpa armigera* with potence by binding to dominant catalytic residues such as ALA12 and TYR108. Cantharidin, as their study illustrated, triggers hydrogen bonding in the active site, which leads to conformation alterations and enzyme function inhibition as well as detoxification disruption.

1,8-cineole demonstrated a binding energy of -6.4 kcal/mol to acetylcholinesterase, whereas it formed only one conventional hydrogen with Tyr1031. The value indicates a stable but not complex mechanism of interaction that may result in moderate inhibitory activity. The binding energy with acetylcholinesterase for α-pinene is slightly lower at -6.2 kcal/mol, but it formed many hydrophobic interactions, such as Pi-alkyl with Tyr494 and Pi-alkyl and alkyl with Trp441 and Leu444, respectively. O-cymene and cryptone exhibited 5.8 kcal/mol binding energy to acetylcholinesterase.

For o-cymene and cryptone both displayed a binding energy of -5.8 kcal/mol. O-cymene formed a conventional hydrogen bond with Ser820, two hydrophobic Pi-alkyl bonds with Trp782, and an unfavorable interaction with Tyr819, suggesting a mixed binding profile that may reduce its overall efficacy. On the other hand, cryptone, etablished hydrophobic Pi-alkyl bonds with Tyr282 and Tyr493, indicating a reliance on hydrophobic interactions for binding.

Docking calculations reveal that hydrogen bonding between ligands and key catalytic residues of acetylcholinesterase (AChE) (Ser, His, or oxyanion hole residues) may physically block substrate access or destabilize the catalytic triad, and thus inhibit acetylcholine hydrolysis. Hydrophobic interactions (π–π stacking and van der Waals) contact with aromatic residues at the active or peripheral anionic sites may also help to stabilize ligand binding and enhance inhibitory activity. Such findings are supported by recent studies. For instance, the study on noncovalent AChE inhibitors in *Aedes aegypti* and *Anopheles gambiae* shows how specific patterns of substitution influence catalytic as well as peripheral site binding with correlation to improved biological efficacy (Vidal-Albalat *et al*., 2023). Similarly, another study on phosphonate group-containing nereistoxin derivatives reports that hydrogen bonding with Ser, His, or Tyr residues is correlated with stronger AChE inhibition *in vitro* (Yan *et al*., 2024).

Compared to these, pyriproxyfen displays superior binding to both targets (-6.4 and -8.1 kcal/mol), through hydrophobic Pi-alkyl bonds interactions with key aromatic residues (Arg74 in 18GS, Tyr282 and Tyr493 in 5X61), confirming its strong hydrophobic stabilization and high ligand-protein affinity.

In contrast, EO compounds though less potent show diverse interaction networks, combining hydrophobic and polar contacts, particularly for 1,8-cineole, which outperforms others in binding strength and mode. Thus, EO major constituents demonstrate relevant insecticidal potential, with *E. globulus* compounds showing slightly better affinities and interaction depth, suggesting their promise as eco-friendly alternatives to chemical insecticides.

Although plant-based bioinsecticides are considered safer and less toxic alternatives to synthetic insecticides, their efficacy may be limited by factors such as the potential development of mosquito resistance (Şengül Demirak & Canpolat, 2022). In this context, despite the well-known effectiveness of terpenes in mosquito control, the study conducted by Duarte *et al*. (2024a) reported that the monoterpenes myrcene and p-cymene exhibited moderate larvicidal activity against *Aedes aegypti* larvae. The same study compared the efficacy of free terpenes and terpene-based nanoemulsions, showing that the nanoemulsions did not enhance the larvicidal activity of either myrcene or p-cymene.

Limitations and future work

Despite the valuable information provided by this study, some limitations must be acknowledged. Current work focused on *C. pipiens* as the most representative vector species in Algeria; however, further studies should include other mosquito vectors such as *Aedes* or *Anopheles* species to confirm the generality of our results. Moreover, since docking analysis provides predictive information, experimental enzyme inhibition tests are needed to validate the proposed mechanisms. Finally, future *in silico* studies should consider other major compounds such as spathulenol in *E. radiata* to better understand their contribution to larvicidal activity.

CONCLUSION

The present study demonstrates the first comparative larvicidal effect of two EOs (*E. globulus* and *E. radiata*) against the most abondunt mosquito species in Algeria *C. pipiens*. Both EOs were found to be very effective in order to fight against these mosquitoes. However, *E. globulus* EO seems to be potentially more effective than *E. radiata* EO with LC_{50} respectively values 23.74 ppm and 53.42 ppm at 24h. The major compounds of studied EOs exhibit promising insecticidal potential through diverse hydrophobic and polar interactions with acetylcholinesterase and glutathione S-transferase, as demonstrated by molecular docking. Overall, this work contributes novel insights into the larvicidal and molecular mechanisms of *Eucalyptus* essential oils and supports their potential as safe, sustainable, and eco-friendly alternatives to synthetic insecticides for mosquito control.

Conflict of interest

The author declares that they have no conflict of interests

ACKNOWLEDGEMENT

The authors express their gratitude to Mrs. Kessab Samira for her assistance with plant collection and oil extraction.

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