Research Article

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Larvicidal properties of essential oils of three *Artemisia* species against the chemically insecticide-resistant Nile fever vector *Culex pipiens* (L.) (Diptera: Culicidae): *In vitro* and *in silico* studies

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Abstract: The objective of this study is to determine the larvicidal activity of essential oils (EOs) extracted from three plants of the genus *Artemisia* against the mosquito *Culex pipiens* (*C. pipiens*) using *in vitro* and *in silico* studies. A total number of 20 third- and fourth-instar larvae were exposed to various concentrations of the three plants. The LC_{50} and LC_{90} values of the tested Artemisia EOs were determined using Probit analysis. In addition, the sensitivity of *C. pipiens* to these EOs was determined and compared against a standard insecticide, temephos, under laboratory conditions. Furthermore, *in silico* assessments were carried out on the major constituents to help understand and explain the acquired *in vivo* results. Gas chromatography analysis identified the major compounds as D-limonene and β-pinene for *Artemisia flahaultii*, camphor and borneol for *Artemisia. aragonensis*, and artemisia

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ketone and caryophyllene for *Artemisia annua*. *A. flahaultii* oil showed the highest efficacy against *C. pipiens* larvae, followed by *A. annua* oil with average larvicidal activity. In contrast, *A. aragonensis* EO, composed of a high percentage of monoterpenes, was the least active. Docking simulation indicated that several studied ligands had promising binding scores within the receptor's binding site compared to the reference insecticide temephos. The obtained results allow us to conclude that *A. flahaultii*, a species endemic to Morocco, is an excellent means of controlling *C. pipiens*.

Keywords: *Artemisia* species, essential oil, *Culex pipiens*, vector, bioassay larvicide, lethal concentration, endemic, *in silico*

1 Introduction

West Nile virus is an example of an emerging arbovirus transmitted by an arthropod vector (Culex sp.) to birds that causes West Nile fever [1,2]. It is one of the world's major public health problems [3]. The disease is endemic and is widely distributed in sub-Saharan Africa [4]. Although its impact on human health has never been measured in sub-Saharan Africa, the West Nile virus has long been considered a low pathogenic arbovirus, with no significant public health consequences [5]. However, over the last 10 years or so, epidemics of several dozens to hundreds of cases have been reported in the Mediterranean basin [6]. Above all, following its introduction into the New World, the West Nile virus has spread in just a few years from Canada to Argentina [7]. In this new ecosystem, it has found vectors and hosts, enabling it to establish sustainable transmission cycles. Since then, it has caused thousands of cases every year, mainly in the United States [8].

In Morocco, the West Nile virus was first described in 1990, causing human diseases and equine epizootics for several successive years [9]. After 6 years of apparent absence, it caused a large-scale epizootic in 1996, 2003, and 2010, circulating regularly along the Mediterranean coast [10]. Horses are the main species affected by this virus. Birds, particularly crows and sparrows (on their way from Africa to Europe), are considered amplifying hosts, and mosquitoes of the Culex genus are vectors [11,12].

Culex pipiens Linnaeus, 1758 (*C. pipiens*) (Diptera: Culicidae) is the main biting species involved in the transmission of West Nile virus [13]. Its high density, which coincides in time and space with the date of detection of equine cases, makes it the most likely vector [14]. At present, this virus is not a public health problem in Morocco, but the American example prompts us to take the health risk associated with the West Nile virus seriously [15]. On the other hand, it poses a major veterinary problem in a region where cultural and tourist activities associated with horses are essential [16,17].

The World Health Organization (WHO, 2016) encourages the development of different approaches to achieve successful and sustainable vector control [18], including the use of biological technologies, physical and chemical, have been implemented [19]. A program of vector control is crucial for the prevention of certain vector-borne diseases [20]. In fact, synthetic pesticides with insecticidal properties are most frequently used to control *C. pipiens* [21,22].

However, these chemical pesticides have harmful effects on the environment, non-target organisms, and human health [23,24]. In addition, overexploitation of these chemicals induces the risk of Culicidae insects developing resistance to the insecticides used, which significantly reduces and diminishes the treatment efficacy [25,26]. Due to the enormous damage caused by synthetic insecticides, there is now a need to discover alternative mosquito control methods that have little or no negative impact on the environment or human health [27–29]. As part of its traditional medicine program, the WHO welcomes opportunities to collaborate with countries and scientific researchers to develop new alternative treatments and encourages this collaboration to develop safe and effective therapies that can be used in most African countries and other parts of the world [30,31].

This research also aims to investigate the *in silico* assessments on the major constituents in the essential oil (EO) studied to help understand and explain the acquired *in vivo* results, using molecular dynamics simulations. The EOs tested are from the genus of *Artemisia*: therein, two species are spontaneous, wild, and endemic to Morocco, *A. flahaultii* and *A. aragonensis*, while *A. annua* is cultivated.

2 Materials and methods

2.1 Plant collection and EO extraction of the *Artemisia* species studied

A. flahaultii, *A. annua*, and *A. aragonensis* plants were harvested between October and April 2021 in the Eastern Middle Atlas Mountains, Morocco. After harvesting, they were dried in the shade, away from light, in a dry, well-ventilated place. To extract the EOs, 100 g of leaves from each plant was ground to a powder and subjected to hydrodistillation for 2–3 h using a Clevenger apparatus. The EOs

obtained after distillation were separated by decantation and then dried using anhydrous sodium sulfate to remove any residual moisture. Finally, the EOs were stored in small opaque bottles at a temperature of 4 ± 2°C.

2.2 Chemical study and identification of compounds of EOs

Chemical analysis of the EOs was carried out by gas chromatography coupled with mass spectrometry (GC/MS), enabling both chromatographic analysis of each oil and qualitative determination of the majority of compounds.

GC/MS was performed on a Shimadzu (Tokyo, Japan) GCMS-TQ8040 NX Triple Quadrupole GC/MS apparatus to determine the chemical components of the produced EOs. Helium was applied as a carrier gas during the analysis, which was conducted through an apolar capillary column (RTxi-5 Sil MS-30 m x 0.25 mm ID x 0.25 m). In our earlier research, the analytical settings were described in great detail.

A homologous sequence of *n*-alkanes was used to calculate the Kovats retention index (RI), which was then utilized to determine the compounds. Importantly, the estimated Kovats RI values were compared to those from the NIST 98 collection and the Adams database in order to identify the constituents [32].

2.3 Characteristics of the larval site

C. pipiens larvae were collected from a breeding site located in the urban area of the city of Fez in a small tributary of the El-Gaada dam (altitude, 407 m; 34 01 155 N and 004 57 213 W).

This site is established by a very high density of Culicidae larvae. It is located near an animal farm, which favors the proliferation of *C. pipiens* larvae.

2.4 Collecting larvae of *C. pipiens*

A rectangular plastic tray that was 45° angled toward the water's surface was used to gather the larvae because the tension force it creates draws the plate toward the larvae. In the Faunistic Laboratory of the Scientific University of Fez, the obtained larvae were kept in breeding in rectangular trays at an average temperature of 22.6°C ± 2°C and a relative humidity of 70% ± 5%.

2.5 Identification of larvae

The Moroccan Culicidae identification key was used to identify the morphological characteristics of the larvae [33], and the Mediterranean African arthropod identification software was used to determine the scientific name of the vector to be tested (mosquitoes) [34].

2.6 Biocide tests

The biological tests were done following the standard protocol proposed by the WHO (2005, 2016) with slight modifications [35,36]. A stock solution (10%) of each EO was prepared by dissolving the particular EO in ethanol and diluting it with ethanol to obtain a range of concentrations: 7.812, 15.625, 31.25, 62.5, and 125 ppm for *A. flahaultii*; 62.5, 125, 250, 500, and 1,000 ppm for *A. annua*; 250, 500, 1,000, 3,000, and 6,000 ppm for *A. aragonensis*. A positive control (temephos) was also prepared with a range of concentrations: 0.0006, 0.0012, 0.0025, 0.05, and 0.0625 ppm. A total number of 20 third-instar larvae were placed in plastic beakers filled with distilled water at the designated concentration. For each concentration, three replicates were performed, and three controls were prepared for each test. The negative control comprised 99 ml of distilled water plus 1 ml of ethanol and received the same number of larvae. Mortality was counted after 24 h. LC_{50} and LC_{90} values were obtained using Log-Probit software. Three replicates were executed for each dilution and for the two controls. The beakers containing the larvae were placed in the laboratory under standard conditions (temperature, 26 ± 3°C; humidity, 70–80%). After 24 h of contact, live and dead larvae were counted.

The results of the bioassay test were expressed as % mortality in relation to the concentrations of EOs (biological insecticides) and controls used. If the % mortality in the controls was higher than 5%, the % mortality in the larvae exposed to the EO was corrected by the Abbott formula equation (1) [37]:

$$\%\text{Corrected mortality} = \left[\frac{\%\text{Observed mortality} - \%\text{Control mortality}}{100 - \%\text{Control mortality}}\right] \times 100. \quad (1)$$

The test must be repeated if the control mortality exceeds 20%.

Table 1: Phytochemical components identified in *A. flahaultii*, *A. aragonensis*, and *A. annua* EOs by GC/MS

RI	Compound name	*A. flahaultii*		*A. aragonensis*		*A. annua*	
		Peak	Area (%)	Peak	Area (%)	Peak	Area (%)
933	α-Pinene	1	2.18	1	1.53	–	—
949	Camphene	—	—	2	3.10	–	—
952	Benzaldehyde	2	1.65	—	—	–	—
979	β-Pinene	3	15.22	3	1.43	–	—
999	Yamogi alcohol	—	—	4	2.75	–	—
1026	Cymene	4	11.72	5	0.69	–	—
1029	D-Limonene	5	22.09	—	—	—	—
1032	1,8-Cineole	—	—	6	10.88	1	3.21
1045	(*E*)-β-Ocimene	6	2.18	—	—	—	—
1048	Artemisia ketone	—	—	—	—	2	43.19
1068	Artemisia alcohol	—	—	—	—	3	1.48
1017	Terpinene	7	1.77	7	0.48	—	—
1139	*neo*-allo-ocimene	8	0.62	—	—	—	—
1086	Fenchone	—	—	8	10.20	—	—
1102	α-Thujone	—	—	9	0.51	—	—
1146	Camphor	—	—	10	24.97	4	4.41
1147	*trans*-pinocarveol	—	—	11	0.52	—	—
1164	Pinocarvone	—	—	12	0.44	—	—
1169	Borneol	—	—	13	13.20	5	3.07
1082	Terpinen-4-ol	—	—	14	1.39	—	—
1173	Artemisia acetate	—	—	15	1.0	—	—
1133	α-Terpineol	—	—	16	0.69	—	—
1198	Myrtenol	—	—	17	2.73	—	—
1206	Benzene, 2,4-pentadiynyl-	9	9.06	—	—	—	—
1216	*trans*-Carveol	—	—	18	0.42	—	—
1221	Copaene	—	—	—	—	6	3.52
1237	Pulegone	—	—	19	1.44	—	—
1243	Cyclohexasiloxane, dodecamethyl-	10	0.71	—	—	—	—
1288	Bornyl acetate	—	—	20	2.33	—	—
1326	Myrtenyl acetate	—	—	21	0.83	—	—
1335	Δ-Elemene	11	3.82	—	—	—	—
1373	α-Copaene	—	—	22	0.75	—	—
1403	Methyleugenol	12	0.93	—	—	—	—
1435	γ-Cadinene	—	—	—	—	7	1.25
1447	Cinnamic acid, methyl ester	13	0.96	—	—	—	—
1464	Caryophyllene	14	0.62	—	—	8	15.75
1442	β-Farnesene	—	—	—	—	9	2.62
1446	Cycloheptasiloxane, tetradecamethyl-	15	0.43	—	—	—	—
1485	Germacrene D	—	—	23	0.71	10	9.56
1490	β-Selinene	16	1.02	—	—	11	10.32
1439	β-Vinylnaphthalene	17	10.47	—	—	—	—
1555	2,4-Di-*tert*-butylphenol	18	1.07	—	—	—	—
1556	Elemicin	19	3.59	—	—	—	—
1570	Caryophyllene oxide	20	2.52	25	1.26	12	1.62
1578	Spathulenol	21	0.53	24	1.26	–	—
1624	Isospathulenol	22	4.25	26	0.50	–	—
1554	Cyclooctasiloxane, hexadecamethyl-	23	0.38	—	—	–	—
1632	γ-Eudesmo	—	—	27	2.20	–	—
1637	Capillin	24	1.56	—	—	–	—
1600	Ledol	25	0.77	—	—	–	—
1640	Cadinol	—	—	28	0.51	–	—
1650	β-Eudesmo	—	—	29	1.30	–	—
1658	Bisabolol oxide B	—	—	30	0.45	-	—
1685	Bisabolone oxide A		—	31	5.63	–	—

(Continued)

Table 1: *Continued*

RI	Compound name	*A. flahaultii*		*A. aragonensis*		*A. annua*	
		Peak	Area (%)	Peak	Area (%)	Peak	Area (%)
1749	α-Bisabolol oxide A	—	—	32	0.56	–	—
2500	Pentacosane	—	—	34	1.63	–	—
2800	Octacosane	—	—	33	1.33	–	—
Monoterpene (C_{10})			56.74%		77.37%		55.35%
Sesquiterpene (C_{15})			13.43%		15.13%		44.65%
Other compounds			29.83%		7.12%		00%
Total compounds identified			100%		99.62%		100%

2.7 Data processing

The data were processed using Log-Probit analysis software (Windl, version 2.0). This software was developed by CIRAD-CA/MABIS (October 1999) [38]. ANOVA tests were also used to determine the analysis of mean values and standard deviation.

2.8 Insecticide-likeness

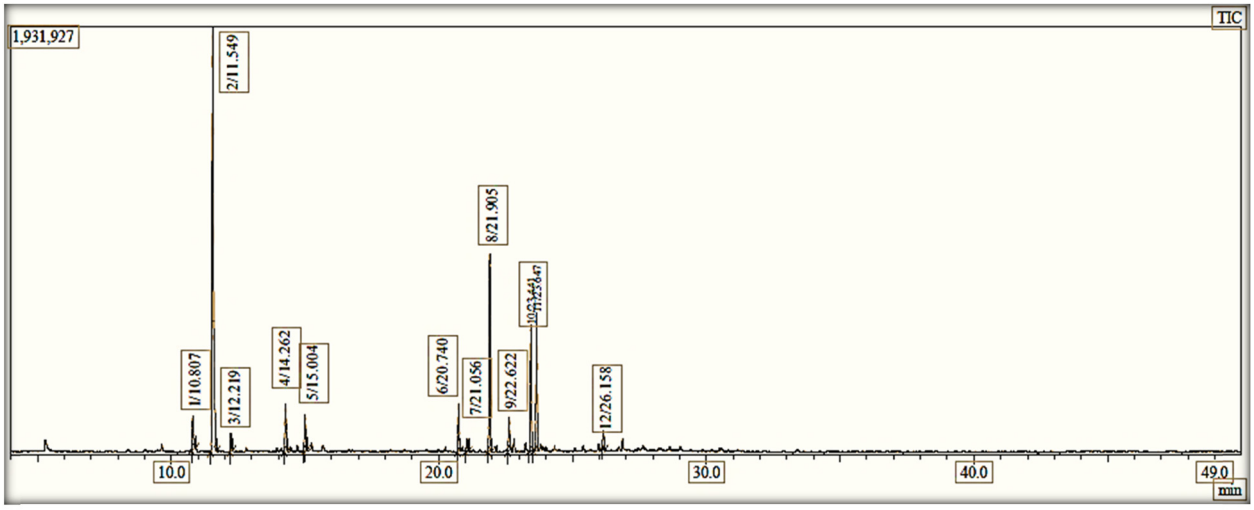
The insecticide-likeness of the compounds under analysis was evaluated by assessing various descriptors, including molecular weight (M_W), hydrophobicity (log P), hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), rotatable bonds (RBs), and aromatic rings [39–41]. These descriptors, as recommended in previous studies, have specific ranges of values: an M_W ranging from 150 to 500, <12 RBs, 1–8 HBAs, fewer than 2 HBDs, and a log P value < 5 [42,43]. The compounds that meet these criteria are deemed to possess significant potential for insecticidal activity. These descriptors were computed with the aid of the Molinspiration online tool [44]. The quantitative estimate of the insecticide-likeness (QEI) was calculated using QEPest, a free Java program addressing the field of agrochemicals. It allows the scoring of our molecules based on a function of previously reported descriptors [45].

2.9 Molecular docking study

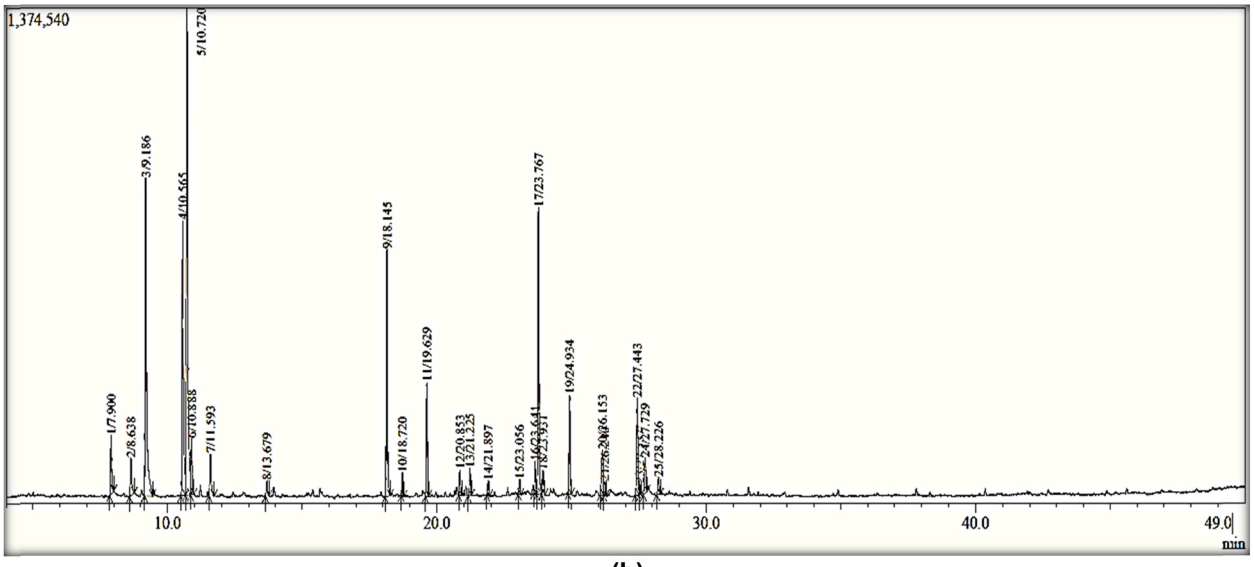
The molecular docking analysis involved docking the studied ligands into the desired receptor using AutoDock Vina [46] to determine their optimal binding poses within the protein's binding site [47]. The crystal structure of acetylcholinesterase from *C. pipiens*, obtained from the Protein Data Bank (PDB ID: 6v4c) [48], was prepared by subtracting water molecules, adding hydrogen bonds, and regulating the Kollman charges, following procedures outlined in our previous studies [49]. Additionally, a reference insecticide (temephos), known for its larvicidal activity, was docked to allow for a comparison of the interactions formed. Ligands were generated using Chem3D software, and optimized under the MMFF94 force field using Avogadro software [50]. A grid box with dimensions of 40 Å, positioned at coordinates x = 16.68 Å; y = −2.99 Å; and z = −27.44 Å, was employed for the docking simulations. The interactions between the ligands and the receptor were subsequently analyzed using Discovery Studio Visualizer [51].

2.10 Molecular dynamics simulations

The selected complexes from the docking results were subjected to 100 ns molecular dynamics simulations by using Maestro software [52]. Water molecules were excluded from all systems, and hydrogen atoms were added to the simulation beforehand [53]. The systems were then optimized and underwent energy minimization using the OPLS3e force field. Solvation was carried out using an orthorhombic box of TIP3P water molecules, and the systems were equilibrated with sodium and chloride ions [53]. The simulations began with a 1 ns run under NVT (constant number of particles, volume, and temperature) conditions, followed by a 100 ns run under NPT (constant number of particles, pressure, and temperature) conditions [54]. Throughout the simulations, parameters such as RMSD (root-mean-square deviation), RMSF (root-mean-squared fluctuation), and protein–ligand contacts were monitored to evaluate the stability of the complexes [55]. Additionally, the uncomplexed protein was simulated to compare structural changes upon binding with the docked ligands.



(a)



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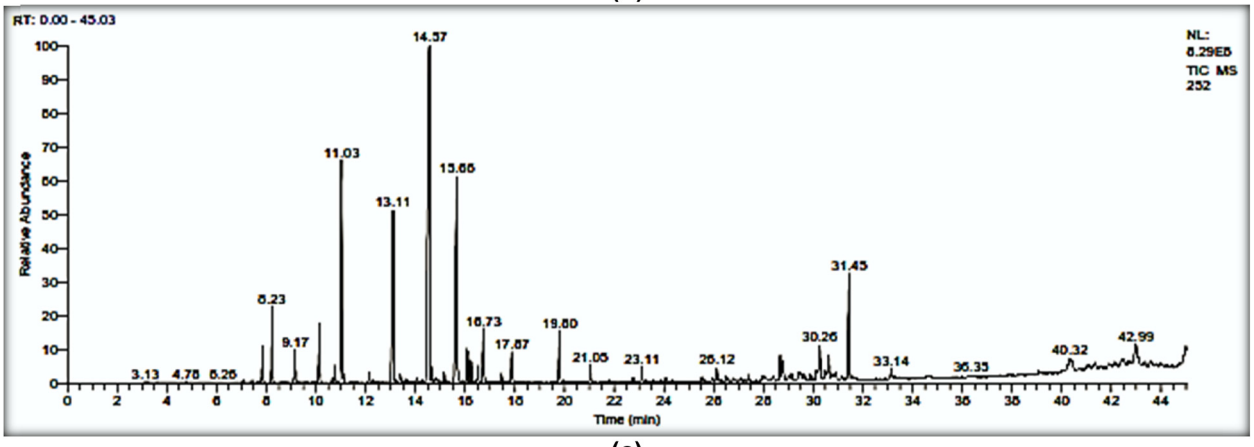


(c)

Figure 1: Chromatographic profiles of *A. annua* (a), *A. flahaultii* (b), and *A. aragonensis* (c) EOs obtained by GC/MS [56–58].

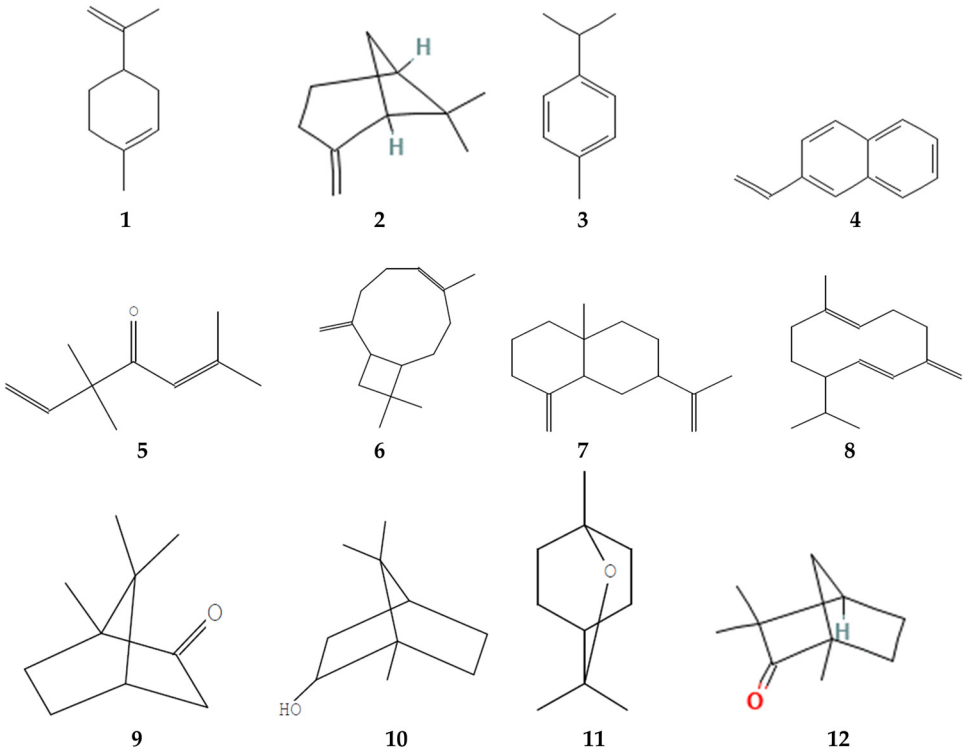


Figure 2: Chemical structure of the main components: (1) D-limonene, (2) *β*-pinene, (3) *p*-cymene, (4) *β*-vinylnaphthalene, (5) artemisia ketone, (6) caryophyllene, (7) *β*-selinene, (8) germacrene D, (9) camphor, (10) borneol, (11) 1,8-cineole, and (12) fenchone.

3 Results and discussion

3.1 Chemical analysis

After hydrodistillation, yellowish-brown oils were obtained in yields of 0.25% (w/w) (*A. flahaultii*), 0.30% (*A. annua*), and 1.19% (*A. aragonensis*), with a strong characteristic aroma. The composition of the EOs is presented in Table 1 and Figure 1, and chemical structures of the major components are shown in Figure 2. Based on the obtained findings, 25 chemical molecules were identified in *A. flahaultii* oil, making up 99.98% of the total oil. The *A. flahaultii* oil was dominated by D-limonene, which accounted for 22.09% of the total oil; the other main metabolites of the oil are β-pinene (15.22%), cymene (11.72%), and β-vinylnaphthalene (10.47%). A total of 12 compounds were identified in the *A. annua* oil, representing 100% of the oil. The most abundant constituents of *A. annua* were artemisia ketone (43.19%), caryophyllene (15.75%), β-selinene (10.32%), germacrene D (9.56%), and camphor (4.41%). In the *A. aragonensis* oil, 34 chemical molecules were identified by GC/MS. It was characterized by a predominance of monoterpenes (77.37%) with a low percentage of sesquiterpenes (15.13%), the main components of the oil being camphor (24.97%), borneol (13.20%), 1,8-cineole (10.88%), and fenchone (10.20%). The *A. flahaultii* oil had a medium monoterpene content (56.74%) and sesquiterpenes were also present in small amounts (13.43%), while *A. annua* oil continued to have a medium content of monoterpenes (55.35%) and hydrocarbon sesquiterpenes (43.03%) and was lower in oxygenated sesquiterpenes (1.62%) (Table 1).

3.2 Larvicidal activity of the EO of Artemisia plant on *C. pipiens*

The use of EOs from aromatic, medicinal, and biocide plants in vector control is an alternative method to minimize the side effects of chemical pesticides in the environment [59,60]. In recent research, some secondary plant metabolites have been found to act as botanical insecticides [61,62]. According to the biological results of this susceptibility test, the three EOs of Artemisia exerted significant larvicidal potential against *C. pipiens*. Figure 3 shows that the mortality rate of *C. pipiens* larvae increases with the concentration of EOs used. For example, with *A. flahaultii*, the mortality rate reaches 98.33% of larvae eliminated at 125 ppm. For *A. annua*, it rises from 11.67% mortality at 62.5 ppm to 98.33% mortality with a concentration

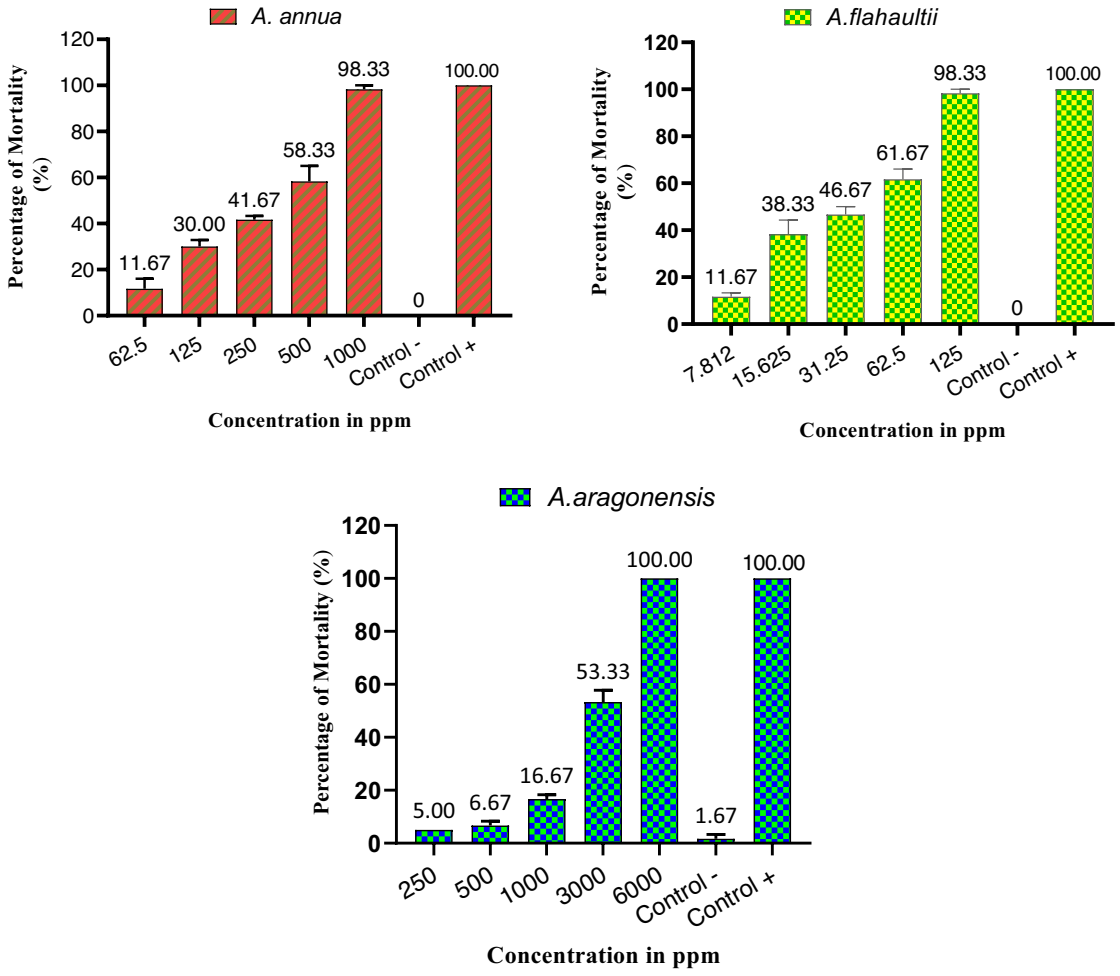


Figure 3: Mortality of *C. pipiens* larvae (%) due to the effect of EOs of *A. flahaultii, A. annua*, and *A. aragonensis* at different concentrations during 24 h of evaluation (negative control: ethanol; positive control: temephos).

of 1,000 ppm. For *A. aragonensis*, the percentage of mortality rises from 5% with a concentration of 250 ppm after 100% of larvae eliminated to a very high concentration of 6,000 ppm compared with the other two oils tested.

The LC_{50} [confidence intervals] values at the end of larval stage were 29.328 ± 0.316 [2.892; 297.414] ppm, 278.539 ± 0.187 [70.387; 1102.67] ppm, and 2373.75 ± 0.989 [1150.37; 4898.16] ppm for *A. flahaultii, A. annua*, and *A. aragonensis* oils, respectively (Table 2). Moreover, their LC_{90} [confidence intervals] values were 127.996 ± 0.175 [35.42; 462.525] ppm, 1089.21 ± 0.172 [307.572 ± 3857.21] ppm, and 5202.7 ± 0.12 [2150.43; 12587.3] ppm, respectively (Table 2). The Chi-square test was not significant at 5% for both EOs, indicating a good model fit.

These results indicate that *A. flahaultii* EOs are very toxic to *C. pipiens* larvae, with an LC_{50} value below 100 ppm (Table 2). Previous studies by Cheng et al. and also by Dias and Moraes stated that a bio-insecticide product with an LC_{50} below 100 ppm was considered a promising product for mosquito control [63–65]. These toxicological parameters could be due to the chemical molecule of the EO of *A. flahaultii* composed mainly of limonene, belonging to the monoterpenes famous for their insecticidal effect against several species of insects [66,67].

Previous studies have indicated that the chemical molecule pinene (LC_{50} values between 36.53 and 66.52 ppm) and the chemical molecule limonene (LC_{50} values between 50.36 and 43.71 ppm) have a medium level of toxicity. In contrast, cymene is highly toxic, with an LD_{50} value close to 20 ppm, and terpinen-4-ol is non-toxic (LC_{50} value > 200 ppm). This reinforces our result, especially for *A. flahaulti*, which possesses all these molecules (15.22% pinenes, 22.09% limonene, and 11.72% cymene) [68,69].

To test and confirm the sensitivity of *C. pipiens* species to the EOs tested, we used temephos (positive control), which is considered as the most widely used insecticide locally (Morocco) in the control of mosquito larvae [70].

Table 2: Lethal concentrations (LC_{50} and LC_{90}) of *A. flahaultii, A. annua,* and *A. argonensis* oils after 24-h exposure

Essential oil	Concentrations (ppm)	LC_{50} (ppm) CI	LC_{90} (ppm) CI	Regression line equation	Calculated Chi^2 (χ^2)	df
A. flahaultii	(7.812; 15.625; 31.25; 62.5; 125)	29.328 ± 0.316 [2.892; 297.414]	127.996 ± 0.175 [35.42; 462.525]	2.00298 × *X* − 2.93895	15.092	3
A. annua	(62.5; 125; 250; 500; 1000)	278.539 ± 0.187 [70.387; 1102.67]	1089.21 ± 0.172 [307.572 ± 3857.21]	2.16457 × *X* − 5.29232	15.016	3
A. aragonensis	(250; 500; 1000; 3000; 6000)	2373.75 ± 0.989 [1150.37; 4898.16]	5202.7 ± 0.12 [2150.43; 12587.3]	3.76101 × *X* − 12.69505	18.979	3
Temephos (positive control)	(0.0006; 0.0012; 0.0025; 0.05; 0.0625)	0.0055 ± 0.219 [0.0032; 0.212]	0.097 ± 0.41 [0.0149; 0.42]	1.03254 × *X* + 2.32702	22.368	3

CI: 95% confidence intervals; df: degree of freedom; LC_{90}: lethal concentration that kills 90% of exposed larvae; LC_{50} lethal concentration that kills 50% of exposed larvae.

Table 2 shows that temephos has a very high insecticidal power compared to our oils, and it has the lowest LC_{50} which was 0.0055 ± 0.219 [0.0032; 0.212] ppm and a LC_{90} of 0.097 ± 0.41 [0.0149; 0.42] ppm. But the problem is that the *C. pipiens* species acquired resistance against this insecticide (temephos) and other insecticides such as malathion, fenitrothion, and fenthion [70,71].

Despite the differences in LC_{50} and LC_{90} between EOs and temephos (chemical insecticide) in relation to *C. pipiens* larvae, the larvicidal effect of EOs could be of great interest in the field of vector control. This is due to the problems associated with the use of synthetic insecticides (environmental pollution, resistance, risks to human health, etc.).

In general, EOs can be used in insecticides to target disease-carrying insects while having little or no impact on the surrounding insect population [72]. The potential biological activities of different EOs vary according to the plant, its origin, and its composition [73,74].

Regarding our results on larvicidal activity at stages 3 and 4, there were differences between the three EOs tested, with variations in LC_{50} and LC_{90} values. The data shown in Table 2 and Figure 3 show that the examined EOs have effective control over *C. pipiens* larvae, and the effect of sensitivity may be attributed to the interaction of many chemical molecules present in each essential oil, minor compounds, or mainly major components.

The secondary plant compounds in the EOs of *A. flahaultii, A. annua,* and *A. aragonensis* may act synergistically rather than individually, as previous studies have shown that the botanical insecticidal activity of EOs is stronger than that of their compounds studied individually [75].

Numerous studies have extracted and purified chemical compounds from the EOs of plants in the genus *Artemisia* to identify effective bioinsecticides with specific active components. For instance, EO extracts from *A. absinthium, A. spicigera*, and *A. santonicum*, containing 1,8-cineole, terpinen-4-ol, camphor, and alpha-terpineol, have proven effective against *Lasioderma serricorne* [76,77]. The EOs of *A. negrei* and *A. aragonensis* composed of camphor, β-thujone, 1,8-cineole, and borneol exhibit notable insecticidal potential against the stored product pest *Callosobruchus maculatus* Fab [75]. Additionally, *A. rupestris* contains α-terpinyl, α-terpineol, 4-terpineol, and linalool, which show high contact toxicity against *Liposcelis bostrychophila.* Furthermore, *A. argyi* and *A. rupestris*, composed of camphor, β-pinene, β-caryophyllene, eucalyptol, α-terpinyl acetate, and 4-terpineol, demonstrated significant toxicity against adult *Lasioderma serricorne* [78,79]. Collectively, these findings confirm that the chemical compounds present in the EOs of the *Artemisia* genus can serve as bioinsecticides, offering a safer alternative to harmful chemicals for the environment and human health.

Table 3: Calculated descriptors of all compounds to determine the insecticide likeness by using Molinspiration

Name	M_W	MLOGP	nHBA	nHBD	nRB	nAR	QEI
β-Pinene	136.23	3.33	0	0	0	0	0.4043
Cymene	134.22	3.9	0	0	1	1	0.4189
D-Limone	136.23	3.62	0	0	1	0	0.427
Benzene	140.18	2.87	0	0	1	1	0.4176
β-Vinylnaphthalene	154.21	3.97	0	0	1	2	0.4279
Isospathulenol	220.35	3.96	1	1	0	0	0.5988
1,8-Cineole	154.25	2.72	1	0	0	0	0.4994
Fenchone	152.23	2.16	1	0	0	0	0.4795
Camphor	152.23	2.16	1	0	0	0	0.4795
Borneol	154.25	2.35	1	1	0	0	0.4081
Bisabolone oxide A	236.35	3.22	2	0	1	0	0.7606
Camphene	136.23	3.33	0	0	0	0	0.4043
Artemisia ketone	152.23	2.96	1	0	3	0	0.572
Caryophyllene	204.35	5.17	0	0	0	0	0.5974
Germacrene D	208.38	5.3	0	0	1	0	0.6326
β-Selinene	204.35	5.02	0	0	1	0	0.6275
Copaene	204.35	5.75	0	0	1	0	0.6107

Table 4: Binding score of the complexes obtained by molecular docking

Plants	Main compounds	Score (kcal/mol)
A. annua	Artemisia ketone	−6.3
	Camphor	−5.1
	Caryophyllene	−9.4
	Copaene	−8
	Germacrene D	−6.1
	β-Selinene	−7.6
A. aragonensis	1,8-Cineole	−5.8
	Bisabolone oxide A	−7.2
	Borneol	−5.1
	Camphene	−6
	Camphor	−5.1
	Fenchone	−6.4
A. flahaultii	Benzene, 2,4-pentadiynyl	−7.1
	Cymene	−6.3
	D-Limonene	−6
	Isospathulenol	−9.3
	β-Pinene	−6.2
	β-Vinylnaphthalene	−7.7
(Standard insecticide)	Temephos	−6.7

The larvicidal activity of the chemical substances listed as main components of the tested EOs was examined to provide a solution to this final query, and the findings are shown in Table 2.

3.3 *In silico* studies

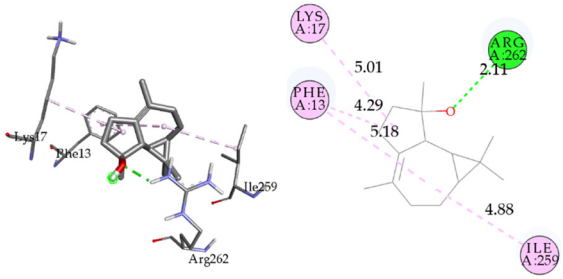
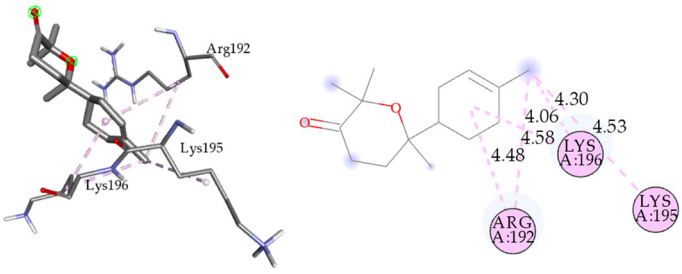
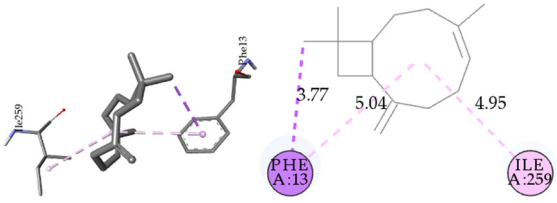
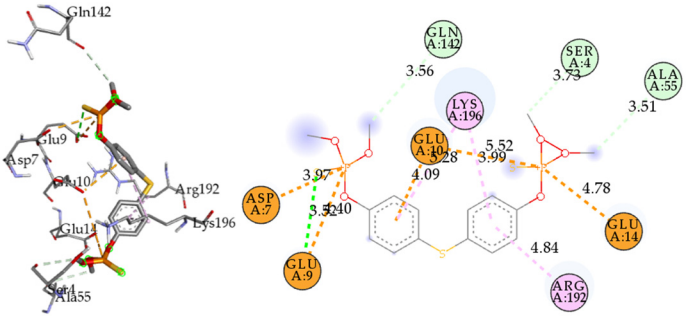
3.3.1 Insecticide-like activity of the major compounds

The outlined criteria for predicting insecticide-like compounds include a M_W between 150 and 500, log P between 0 and 5, HBD ≤ 2, HBA 1–8, and RB ≤ 12. These criteria were applied to assess the insecticide potential of the analyzed compounds (Table 3). β-Pinene, cymene, D-limonene, benzene, 2,4-pentadiynyl, and camphene were disqualified due to their molecular weights being less than 150 and the absence of HBAs. Additionally, four other compounds had log P values greater than 5 and no HBAs; on the other hand, compounds like isospathulenol, 1,8-cineole, fenchone, camphor, borneol, bisabolone oxide A, and artemisia ketone met all the recommended criteria. However, the only exception was β-vinylnaphthalene, which had fewer than 1 HBA, violating one criterion. The quantitative estimate of the insecticide-likeness QEI of all compounds was calculated, and seven top-ranked compounds were suggested. These findings suggest that isospathulenol and bisabolone oxide A are promising insecticide candidates.

3.3.2 Molecular docking study

As shown in Table 4, the docking results indicated that several studied ligands had promising binding scores within the receptor's binding site compared to the reference insecticide

Table 5: 2D and 3D visualization of the best selected complexes, the created interactions, distances, and the participated residues

Complex	Residues	Interaction type	Distances (Å)
6v4c–isospathulenol	Arg262	Conventional hydrogen bond	2.11
	Ile25	Alkyl	4.88
	Phe13	Pi–Alkyl	4.29
			
6v4c–bisabolone oxide A	Arg192	Alkyl	4.58
	Lys195	Alkyl	4.53
	Lys196	Alkyl	4.29
	Arg192	Alkyl	4.48
	Lys196	Alkyl	4.06
			
6v4c–caryophyllene	Phe13	Pi–Sigma	3.77
	Ile259	Alkyl	4.95
			
6v4c–temephos	Asp7	Attractive charge	3.97
	Glu9	Attractive charge	4.40
	Glu14	Attractive charge	4.78
	Glu9	Conventional hydrogen bond	3.52
	Gln142	Carbon–hydrogen bond	3.56
	Ser4	Carbon–hydrogen bond	3.72
	Ala55	Carbon–hydrogen bond	3.50
	Glu10	Pi–anion	4.09
	Arg192	Pi–alkyl	4.84
	Lys196	Pi–alkyl	3.99
			

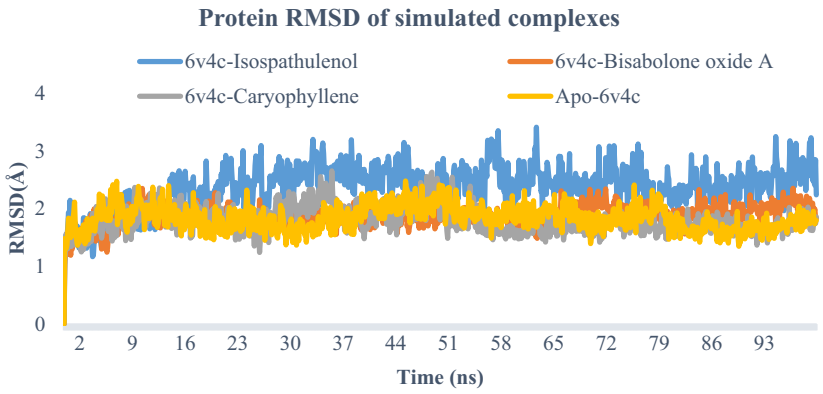


Figure 4: Protein RMSD plot of the best selected complexes in comparison with the apo-protein.

temephos. Three compounds extracted from *A. annua* (caryophyllene, copaene, and β-selinene) were predicted to have a higher binding affinity than the temephos. From *A. aragonensis*, bisabolone oxide A was the only compound that showed a higher binding score than temephos. Additionally, compounds from *A. flahaultii*, including isospathulenol, benzene, 2,4-pentadiynyl, and β-vinylnaphthalene, exhibited good binding affinity compared to temephos.

Three lead compounds were chosen based on the outcomes of molecular docking and the insecticide-likeness prediction. Their molecular interactions with key protein residues are detailed in Table 5 and were analyzed to determine their influence on the desired activity.

For the caryophyllene–6v4c complex, the interactions included a conventional hydrogen bond with Arg262 at a distance of 2.11 Å, an alkyl interaction with Ile25 at 4.88 Å, and a pi–alkyl interaction with Phe13 at 4.29 Å. In the 6v4c–bisabolone oxide A complex, six alkyl bonds were observed with Arg192, Lys195, and Lys196 at distances ranging from 4.06 to 4.58 Å.

The 6v4c–isospathulenol complex featured Pi–sigma and alkyl bonds with Phe13 and Ile259 at distances of 3.77 and 4.95 Å, respectively.

The standard insecticide formed multiple interactions: attractive charges, conventional hydrogen bonds, carbon–hydrogen bonds, Pi–anion, and Pi–alkyl interactions with residues Asp7, Glu9, Glu14, Gln142, Ser4, Ala55, Glu10, Arg192, and Lys196, at distances ranging from 3.50 to 4.84 Å.

3.3.3 Molecular dynamics simulations

Molecular dynamics simulations were done on the three best-chosen compounds to provide insights into the structural changes affecting the protein and ligands upon complex formation, as well as the molecular interactions created between them. Key parameters such as RMSD, RMSF, and protein–ligand contacts were analyzed.

The RMSD values for both the protein and ligands in each system were calculated and plotted to facilitate the

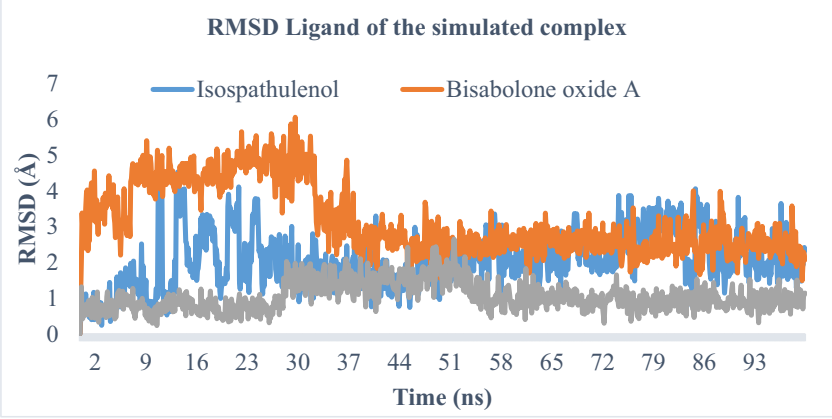


Figure 5: Ligand RMSD of the best selected complexes during the 100 ns of simulation.

interpretation of the results. Figure 4 shows the protein RMSD of the three best-selected complexes in addition to the apo-protein, indicating stability throughout the entire simulation period. Conversely, Figure 5, which presents the ligand RMSD of the analyzed complexes, exhibited significant deviations during the first 30 ns of the simulation, followed by stability for the remainder of the simulation.

The RMSF of the protein, calculated and plotted in Figure 6, demonstrated minor fluctuations, in the protein residues for all complexes. These results indicate that the protein maintained its structural integrity throughout the simulations, and the ligands achieved stable binding after an initial adjustment period.

3.3.4 Protein–ligand contact

The created molecular interactions between the protein and candidate ligands are presented in Figure 7 to display their influences on the stability of the obtained complexes. In the 6v4c–caryophyllene complex, only hydrophobic bonds were detected with the following residues: Phe12, Ala16, Met191, Ala204, Ile259, and Tyr266. While in the 6v4c–bisabolone oxide A complex, two hydrophobic bonds were observed with Ile150 and Phe153, along with two hydrogen bonds with Gln157 and Arg189. Additionally, water bridges were formed with Asp7, Gln142, Lys144, Gln157, and Arg189 residues. Meanwhile, the isospathulenol complex formed hydrogen bonds with Glu20, Arg207, and Arg262, hydrophobic bonds with Phe13, Ala16, Ile259, and Tyr266, and water bridges with Ala16, Glu20, Arg262, Asn265, and Tyr268 residues.

4 Conclusions

To sum up, this study reports the chemical composition and larvicidal activity of the EOs extracted from three plants of the genus *Artemisia*. The extracted EOs were yellowish-brown in color, with yields of 0.25% for *A. flahaultii*, 0.30% for *A. annua*, and 1.19% for *A. aragonensis*, all possessing a strong characteristic aroma. The EOs exhibited

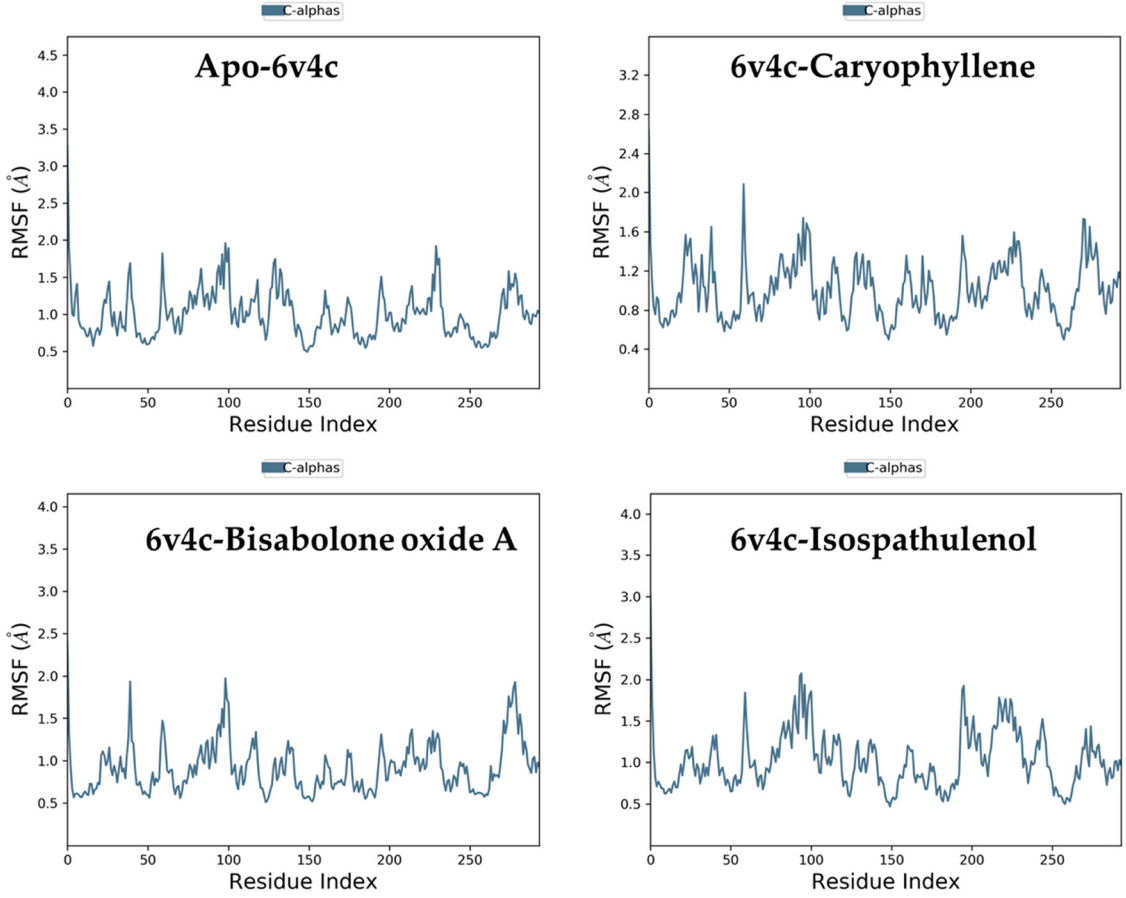


Figure 6: Protein RMSF plot of the simulated complexes.

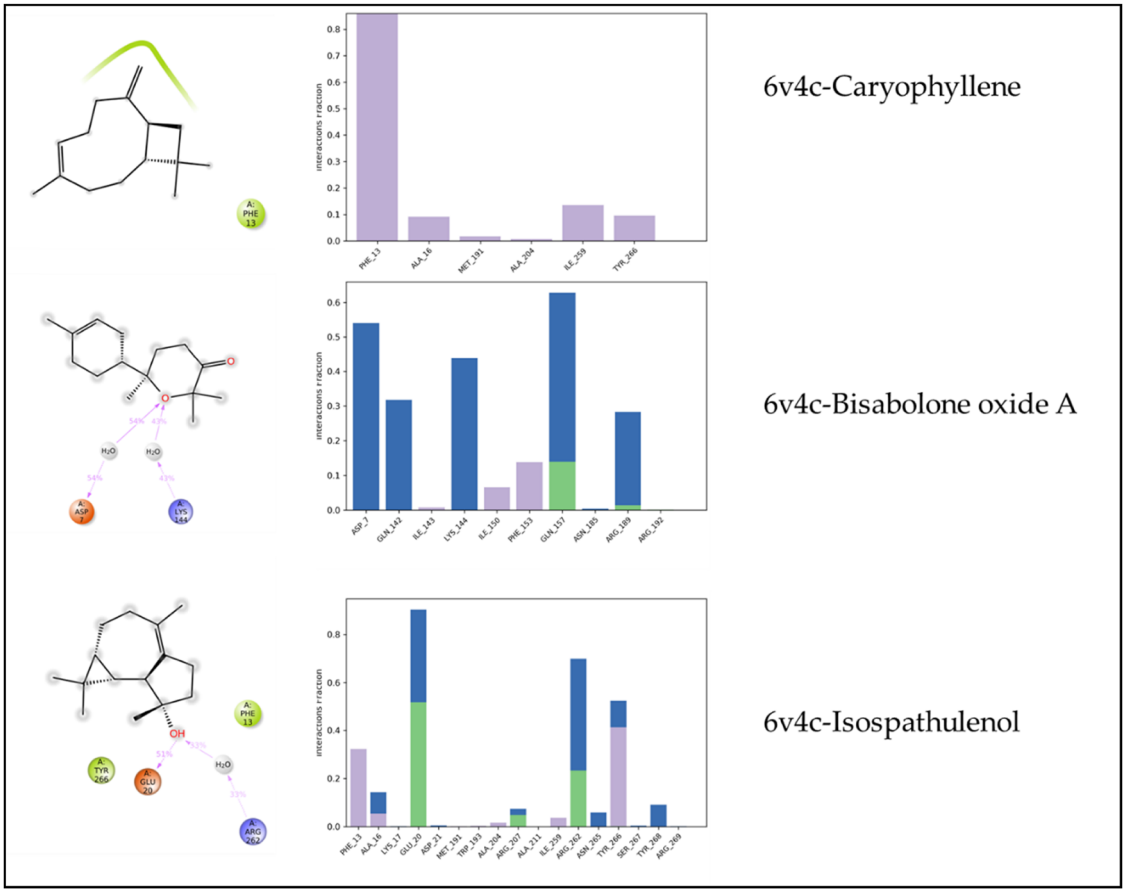


Figure 7: Protein–ligand contact of the best selected compounds (violet: hydrophobic bonds, green: hydrogen bonds, and blue: water bridges).

varying compositions of mono- and sesquiterpenes, as determined by GC/MS analysis. The EOs of the three *Artemisia* species show good larvicidal efficacy against the *C. pipiens*. In addition, we examined, using *in silico* tools, the larvicidal activity of the major chemical compounds identified in the EOs of plants of *Artemisia*.

Molecular docking analysis and molecular dynamics simulations revealed that the major chemical compounds of the studied EOs exhibited strong interactions with the target proteins, suggesting their potential role in insecticidal activity. Also, ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies of the main chemical compounds indicated acceptable drug-like properties. Altogether, the present study shows the importance of using EOs of *A. flahaultii*, *A. annua*, and *A. aragonensis* in the control of *C. pipiens* mosquitoes, vectors of the West Nile virus, due to their larvicidal properties. It could therefore represent a less costly alternative for its application in the production of bioinsecticides.

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Ethical approval: The conducted research is not related to either human or animal use.

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