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RESEARCH ARTICLE

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Environmentally-Friendly Pesticidal Activities of *Callicarpa* and *Karomia* Essential Oils from Vietnam and Their Microemulsions

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There is an ongoing interest to identify alternative pesticidal agents to avoid the chronic problems associated with synthetic pesticides. Essential oils have shown promise as botanical pest control agents. In the present study, the essential oils of four members of the Lamiaceae (*Callicarpa candicans*, *C. erioclona*, *C. macrophylla*, and *Karomia fragrans*; Vietnamese names: Nàng nàng, Tu châu lông mem, Tu châu lá to and Cà diện, respectively), obtained from wild populations in Vietnam, have been obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The essential oils were formulated into microemulsions and the essential oils and their microemulsions were screened for mosquito larvicidal activity against *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, and for molluscicidal activity against *Pomacea canaliculata*. Atractylone and (*E*)-caryophyllene dominated the volatiles of *C. candicans* (CCEO) and *C. erioclona* (CEEO), while the major component in *C. macrophylla* (CMEO) and *K. fragrans* (KFEO) was (*E*)-caryophyllene. The essential oils and microemulsions of both *C. candicans* and *C. erioclona* exhibited excellent larvicidal activity against all three mosquito species (*Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*) with LC_{50} values < 10 μg/mL. Additionally, the larvicidal activity of the microemulsions were significantly improved compared with their free essential oils, especially for *C. candicans* and *C. erioclona*. All four essential oils and their microemulsions showed excellent molluscicidal activity with $LC_{50} < 10$ μg/mL. In most cases, the essential oils and microemulsions showed greater pesticidal activity against target organisms than the non-target freshwater fish, *Oreochromis niloticus*. The *in silico* studies on physicochemical and ADMET properties of the major components in the studied essential oils were also investigated and most of the compounds possessed a favorable ADMET profile. Computational modeling studies of the studied compounds demonstrated a favorable binding interaction with the mosquito odorant-binding protein target and support atractylone, β-selinene, and caryophyllene oxide as potential inhibitors. Based on the observed pesticidal activities of the essential oils and their microemulsions, the *Callicarpa* species

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and *K. fragrans* should be considered for potential cultivation and further exploration as botanical pesticidal agents.

Keywords: *Callicarpa macrophylla*, *Calliarpa erioclona*, *Callicarpa candicans*, *Karomia fragrans*, larvicidal, molluscicidal.

Introduction

According to the WHO, more than 17% of all infectious diseases are vector-borne diseases, which cause more than 700,000 deaths annually.[1] Both *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (Culicidae) are transmitters of yellow fever virus, chikungunya virus, dengue virus, and Zika virus,[2,3] as well as Mayaro virus,[4,5] Phasi Charoen-like virus,[6] and many other pathogenic viruses.[2,7] *Culex quinquefasciatus* (Say) (Culicidae) is an intermediate host of the parasitic nematode *Wuchereria bancrofti* (Cobbold) Seurat (Onchocercidae), which is the causative agent of lymphatic filariasis in humans. Lymphatic filariasis is including in the list of one of the most important neglected tropical diseases and is the second leading cause of permanent and long-term disability in the world.[8] In addition, *Cx. quinquefasciatus* is a vector of several pathogenic viruses, including Western equine encephalitis virus, Zika virus and West Nile virus,[9] Japanese encephalitis virus,[10] and Usutu virus.[11]

Pomacea canaliculata (Lamarck) (Ampullariidae) acts as a transitional vector for parasitic nematodes such as *Angiostrongylus cantonensis* (Chen) (Angiostrongylidae),[12–14] *Angiostrongylus vasorum* (Baillet) (Kamensky) (Angiostrongylidae),[15] *Gnathostoma spinigerum* Levinsen (Gnathostomatidae),[16] as well as the intestinal trematode *Echinostoma ilocanum* (Garrison) (Echinostomatidae).[17–19] In addition, *P. canaliculata* has adversely affected agricultural production of rice in many countries of Southeast Asia, including Vietnam.[20–22]

In recent years, essential oils (EOs) have been emerging as promising alternatives to synthetic pesticides. Essential oils generally have rich and complex chemical compositions, which make them difficult for target organisms to develop resistance to them.[23,24] In addition, essential oils are biodegradable, nontoxic, and environmentally friendly.[25] However, EOs are unstable, volatile, and insoluble in water, which limits their use for new pesticide formulations. These disadvantages can be solved by encapsulating the EOs into suitable formulations such as nanoemulsions or EO-loaded nanoparticles.[26,27]

Callicarpa macrophylla Vahl (Lamiaceae) is an herbal folk medicine in Asian countries. The plant is used as a remedy for gastrointestinal diseases,[28] relieve pain, stop bleeding, and eliminate stasis to subdue swelling.[29] In Vietnam, the root is employed as a rheumatism treatment and to prepare a tonic to improve appetite.[30] *Callicarpa erioclona* Schauer (Vietnamese name: Tu châu lông mem) was employed in Vietnamese traditional medicine for gastrointestinal bleeding, to treat gonorrhea, as an insecticide, and to poison fish.[30] *Karomia fragrans* Dop (Lamiaceae) (Vietnamese name: Cà diện) is apparently endemic exclusively to Vietnam.[30] Prior to this work, the only reported occurrence was in South Vietnam, specifically in Phan Rang, Ninh Thuan province. The tree grows scattered in the forest, flowers in July-August. The tree produces good wood, which is used in construction.

In this study, we present the results of the chemical compositions of EOs from *C. macrophylla*, *C. erioclona* and *K. fragrans* growing wild in Vietnam. The study also presents a method of preparing microemulsion (ME) formulations that are sustainable, improve pesticide activity, and are environmentally friendly. We evaluated the pesticide activity of the EOs and their MEs against *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, *P. canaliculata*, and the toxicity to the non-target fish *Oreochromis niloticus* (Linnaeus) (Cichlidae). Furthermore, we tested larvicidal activity against *Ae. aegypti* in a small-scale field trial of the MEs that had shown larvicidal activity excellence in laboratory conditions in the hope of identifying potential renewable sources of botanical pesticides. In addition, *in silico* evaluation of physicochemical and ADMET properties as well as molecular target investigation of major components of studied essential oils samples were conducted.

Results and Discussion

Chemical Composition of the Essential Oils

The major components and yields of the essential oils of *C. candicans*, *C. erioclona*, *C. macrophylla*, and *K. fragrans* are summarized in *Table 1*. Atractylone and (*E*)-

Table 1. Major essential oil components of *Callicarpa* spp. And *Karomia fragrans* essential oils.

RI[a]	Compound	*C. candicans*[b] (Yield: 0.17%)	*C. erioclona*[c] (Yield: 0.19%)	*C. macrophylla*[d] (Yield: 0.24%)	*K. fragrans*[e] (Yield: 0.12%)
973	Sabinene	nd[f]	tr[g]	7.9	0.3
1026	*p*-Cymene	tr	tr	2.8	7.5
1417	(*E*)-Caryophyllene	15.3	11.1	25.2	26.5
1452	α-Humulene	1.9	1.2	1.4	10.1
1484	Germacrene D	0.5	0.2	4.8	0.7
1486	β-Selinene	4.5	5.1	0.7	0.4
1493	Curzerene	5.3	3.2	nd	nd
1519	δ-Cadinene	0.1	0.1	3.5	5.2
1558	Germacrene B	5.1	4.0	nd	0.2
1580	Caryophyllene oxide	3.4	5.9	6.4	10.5
1662	Atractylone	42.4	34.6	nd	nd
	Monoterpene compounds	0.4	2.3	23.8	19.8
	Sesquiterpene compounds	95.0	75.4	73.9	73.1

[a] RI = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5 ms column. [b] The chemical composition of *C. candicans* essential oil has been published.[31] [c] The complete chemical composition of *C. ericlona* essential oil can be found in *Supplementary Table S1*. [d] The complete chemical composition of *C. macrophylla* essential oil can be found in *Supplementary Table S2*. [e] The complete chemical composition of *K. fragrans* essential oil can be found in *Supplementary Table S3*. [f] nd = not detected. [g] tr = trace (< 0.05%).

caryophyllene dominated the essential oils of *C. candicans* and *C. erioclona*, followed by β-selinene, curzerene, germacrene B, and caryophyllene oxide. The major component in *C. macrophylla* and *K. fragrans* was (*E*)-caryophyllene. Other major contributors to the essential oil composition of *C. macrophylla* were germacrene D, sabinene, and caryophyllene oxide, while *K. fragrans* showed significant quantities of caryophyllene oxide, α-humulene, and *p*-cymene.

In addition to *C. candicans* and *C. ericlona*, atractylone is a major component of several essential oils. *Atractylodes macrocephala* Koidz. (Asteraceae) rhizome essential oil has shown 39.2% atractylone.[32] The compound is also found in appreciable quantities in the essential oils from leaves of *Eugenia uniflora* L. (Myrtaceae),[16,33] leaves of *Siparuna guianensis* Aubl. (Siparunaceae),[34] and aerial parts of *Siparuna muricata* (Ruiz & Pav.) A. DC.[35] Several *Callicarpa* spp. have shown considerable concentrations of (*E*)-caryophyllene and/or caryophyllene oxide.[31]

Characterization of Microemulsions

Microemulsions (MEs) are homogeneous and isotropic nanodispersions endowed with low viscosity, optical transparency, thermodynamic stability and dispersed phase sizes in the range of 10–200 nm.[36] The use of alcohols in combination with surfactants for the preparation of MEs has been described in several previously published studies.[37–40] Ethanol has been reported to reduce droplet size[41] and improve oil solubilization[42] in oil-water microemulsions. Awad and co-workers evaluated the characteristics in model oil-in-water emulsions containing Tween 80 and medium chain triglycerides (MCT), and observed that ME droplets of nearly uniform size in the aqueous phase were obtained when the concentration of MCTs was below 1%.[43]

The results of the measurement of the particle size distribution of the MEs are shown in *Figure 1*. The sizes of the MEs at time T01 were around 14.92 nm and 17.51 nm and their polydispersity index (PDI) varied between 0.121–0.269. At 45 days (T45), *C. erioclona* ME appeared to peak at 174.1 nm with 35% in intensity, the mean size of ME was 17.27 nm (PDI = 0.447). Meanwhile, at 45 days (T45), *C. candicans* ME appeared to peak at 217.6 nm with 19.5% in intensity, the mean size of ME was 14.86 nm (PDI = 0.341). The intensity of the light scattered is proportional to the diameter to the sixth power; a small number of large particles in the sample can skew the results more than a population of small particles.[44,45]

Toxicity of Essential Oils and Microemulsions

The larvicidal activity results for the EOs and their MEs against the three mosquito species are presented in *Tables 2* and *3*. The EOs and MEs of both *C. candicans* and *C. erioclona* exhibited excellent larvicidal activity against all three mosquito species (*Ae. aegypti*, *Ae. al-*

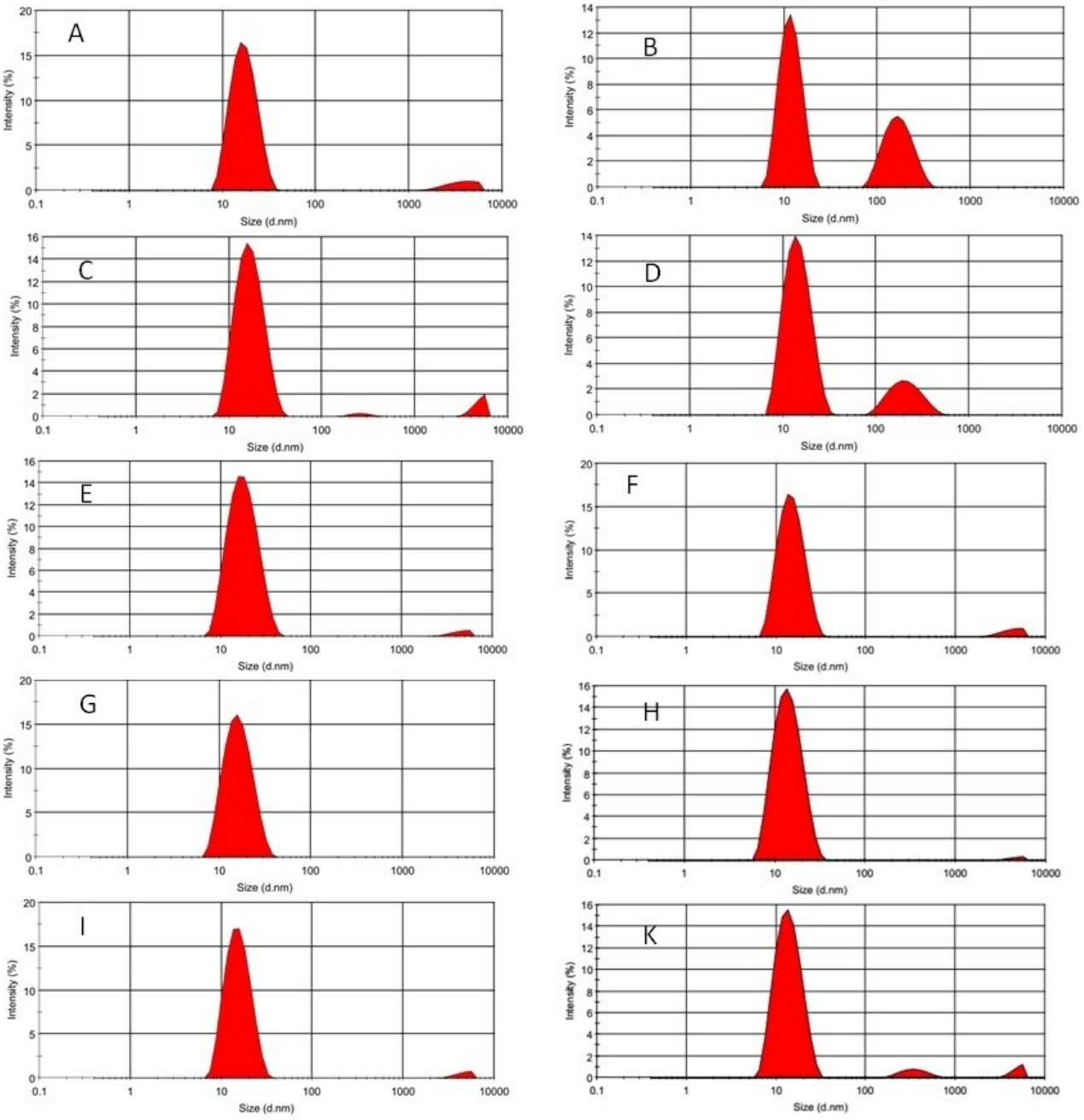

Figure 1. Dynamic light scattering (DLS) traces of MEs at different timepoints (t1 and t45 days). A) CEEO ME at 01 day; B) CEEO ME at 45 days; C) CCEO ME at 01 day; D) CCEO ME at 45 days; E) CMEO831 ME at 01 day; F) CMEO831 ME at 45 days; G) CMEO830 ME at 01 day; H) CMEO830 ME at 45 days; I) ME at 01 day; K) KFEO ME at 45 days.

bopictus, and *Cx. quinquefasciatus*) with LC_{50} values <10 μg/mL. The *Callicarpa macrophylla* essential oil, the *Karomia fragrans* essential oil and their MEs all exhibited good larvicidal activity against all three mosquito species with LC_{50} values <100 μg/mL. Notably, the larvicidal activities of the MEs were significantly improved compared with their free EOs, especially for *C. candicans* and *C. erioclona*, but not for *K. fragrans*. Similar results were observed for *P. canaliculata* (*Table 4*) and *O. niloticus* (*Table 5*). The higher toxicity of MEs may be their small droplet size. The small size of the MEs may increase the surface area in contact with the organism that improves a better penetration into organism tissues and an effective

Table 2. Twenty-four-hour larvicidal activity of essential oils and their microemulsions against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*.[a]

Tested material	LC_{50} (μg/mL)	LC_{90} (μg/mL)	χ^2	*p*
	Aedes aegypti			
Callicarpa candicans essential oil	9.675 (9.045–10.323)	12.78 (11.99–13.81)	0.398	0.995
Callicarpa candicans microemulsion	2.547 (2.272–2.852)	4.595 (4.115–5.285)	4.38	0.223
Callicarpa erioclona essential oil	4.438 (4.060–4.856)	6.927 (6.318–7.794)	4.89	0.430
Callicarpa erioclona microemulsion	0.6019 (0.5162–0.6979)	2.602 (2.114–3.356)	33.5	0.000
Callicarpa macrophylla essential oil	28.20 (26.04–30.85)	39.76 (36.29–44.72)	1.28	0.865
Callicarpa macrophylla microemulsion	23.28 (21.12–25.86)	38.84 (35.02–44.11)	8.09	0.088
Karomia fragrans essential oil	21.48 (19.51–22.91)	28.46 (26.34–31.91)	12.4	0.015
Karomia fragrans microemulsion	24.81 (22.65–27.45)	38.84 (35.09–44.07)	16.5	0.002
Negative control	No mortality			
Positive control (permethrin)	0.000643 (0.000551–0.00753)	0.00246 (0.00192–0.00344)	12.5	0.006
	Aedes albopictus			
Callicarpa candicans essential oil	8.536 (7.904–9.221)	12.13 (11.23–13.35)	1.15	0.887
Callicarpa candicans microemulsion	0.5775 (0.5056–0.6499)	1.306 (1.112–1.640)	47.7	0.000
Callicarpa erioclona essential oil	7.240 (6.594–7.940)	12.96 (11.46–15.26)	3.67	0.597
Callicarpa erioclona microemulsion	0.7407 (0.6072–0.8760)	2.918 (2.393–3.762)	66.5	0.000
Callicarpa macrophylla essential oil	65.61 (60.93–71.24)	86.10 (79.31–95.80)	0.926	0.921
Callicarpa macrophylla microemulsion	56.97 (52.80–62.27)	77.75 (70.97–87.93)	2.90	0.574
Karomia fragrans essential oil	31.89 (29.56–34.52)	43.95 (40.57–48.66)	2.75	0.252
Karomia fragrans microemulsion	50.60 (47.07–55.22)	69.00 (62.90–78.63)	0.845	0.932
Negative control	No mortality			
Positive control (permethrin)	0.0024 (0.0021–0.0026)	0.0042 (0.0038–0.0049)	4.64	0.031
	Culex quinquefasciatus			
Callicarpa candicans essential oil	3.924 (3.554–4.327)	7.134 (6.246–8.566)	12.7	0.026
Callicarpa candicans microemulsion	8.164 (7.121–9.367)	29.18 (23.99–37.07)	16.4	0.012
Callicarpa erioclona essential oil	7.508 (6.730–8.370)	16.86 (14.52-20.41)	6.24	0.283
Callicarpa erioclona microemulsion	4.843 (4.252–5.514)	15.21 (12.67–19.07)	11.2	0.084
Callicarpa macrophylla essential oil	49.22 (45.11–54.13)	76.29 (69.46–85.49)	13.0	0.011
Callicarpa macrophylla microemulsion	42.04 (38.68–46.10)	63.74 (58.07–71.56)	4.69	0.321
Karomia fragrans essential oil	44.59 (41.08–48.86)	65.92 (60.08–74.02)	7.00	0.136
Karomia fragrans microemulsion	63.77 (58.31–70.19)	84.79 (77.52–94.43)	19.0	0.001
Negative control	No mortality			
Positive control (permethrin)	0.0165 (0.0149–0.0181)	0.0305 (0.0266–0.0367)	5.24	0.073

[a] Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from eight independent experiments carried out in quadruplicate, after 24 h of treatment.

distribution of the active ingredient, enhancing the pesticidal activity.[46,47] Improvement in the toxicity of MEs has also been reported previously. Montefuscoli et al. obtained a significantly higher improvement of geranium EO ME than free geranium EO against fourth-instar larvae of *Culex pipiens pipiens*.[48] Okonogi and Wantida found that the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibitory activities of *Zingiber montanum* (J. Koenig) Link ex A. Dietr. (Zingiberaceae) EO-based MEs were twenty and twenty-five times higher than free essential oils, respectively.[49]

The larvicidal activity of *C. candicans* and *C. erioclona* essential oils and microemulsions can probably be attributed to the high concentrations of atractylone. In addition to numerous pharmacological effects,[50] atractylone has shown acaricidal,[51] and insecticidal[52] activities.

Toxicity of EOs and their MEs against the fresh water snail, *P. canaliculata*, is shown in *Table 4*. According to the WHO, agents are considered to have

Table 3. Forty-eight-hour larvicidal activity of essential oils and their microemulsions against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*.[a]

Tested material	LC_{50} (μg/mL)	LC_{90} (μg/mL)	χ^2	*p*
	Aedes aegypti			
Callicarpa candicans essential oil	6.506 (5.920–7.205)	10.40 (9.39–11.81)	5.97	0.309
Callicarpa candicans microemulsion	1.945 (1.626–2.200)	3.475 (3.097–4.141)	0.0132	0.993
Callicarpa erioclona essential oil	3.591 (3.250–3.952)	5.818 (5.298–6.548)	5.86	0.320
Callicarpa erioclona microemulsion	0.3121 (0.2626–0.3652)	1.352 (1.100–1.747)	36.1	0.000
Callicarpa macrophylla essential oil	27.65 (25.51–30.27)	39.37 (35.91–44.31)	1.76	0.780
Callicarpa macrophylla microemulsion	18.20 (15.90–20.81)	38.45 (33.94–44.93)	5.73	0.220
Karomia fragrans essential oil	21.01 (19.25–22.51)	28.06 (26.19–31.40)	5.43	0.246
Karomia fragrans microemulsion	22.51 (20.36–25.09)	38.27 (34.36–43.75)	29.7	0.000
Negative control	No mortality			
	Aedes albopictus			
Callicarpa candicans essential oil	7.328 (6.768–7.984)	10.349 (9.475–11.622)	3.01	0.556
Callicarpa candicans microemulsion	0.4844 (0.4498–0.5282)	0.6682 (0.5996–0.7926)	36.6	0.000
Callicarpa erioclona essential oil	6.473 (5.931–7.045)	10.59 (9.49–12.28)	6.75	0.240
Callicarpa erioclona microemulsion	0.5633 (0.4777–0.6467)	1.455 (1.242–1.786)	75.2	0.000
Callicarpa macrophylla essential oil	43.65 (39.69–48.50)	71.89 (64.77–81.71)	38.3	0.000
Callicarpa macrophylla microemulsion	45.08 (40.77–50.32)	77.67 (69.81–88.40)	34.9	0.000
Karomia fragrans essential oil	29.43 (27.24–32.17)	40.33 (36.78–45.79)	0.852	0.653
Karomia fragrans microemulsion	48.22 (44.85–52.43)	66.72 (61.07–75.21)	1.51	0.824
Negative control	No mortality			
	Culex quinquefasciatus			
Callicarpa candicans essential oil	1.897 (1.596–2.183)	4.943 (4.136–6.372)	11.2	0.048
Callicarpa candicans microemulsion	2.122 (1.902–2.362)	4.654 (4.014–5.647)	13.3	0.039
Callicarpa erioclona essential oil	4.190 (3.700–4.716)	10.49 (8.96–12.80)	36.8	0.000
Callicarpa erioclona microemulsion	1.269 (1.052–1.485)	4.317 (3.561–5.573)	1.51	0.959
Callicarpa macrophylla essential oil	41.53 (38.16–45.51)	64.02 (58.41–71.58)	13.4	0.010
Callicarpa macrophylla microemulsion	39.85 (36.71–43.57)	60.59 (55.37–67.68)	5.74	0.220
Karomia fragrans essential oil	34.03 (31.32–37.17)	52.10 (47.78–57.77)	5.27	0.261
Karomia fragrans microemulsion	48.99 (44.37–55.10)	70.30 (62.79–81.76)	20.3	0.000
Negative control	No mortality			

[a] Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from eight independent experiments carried out in quadruplicate, after 48 h of treatment.

Table 4. Molluscicidal activity of essential oils and their microemulsions against *Pomacea canaliculata* after 24 h exposure and 24 h recovery.[a]

Material tested	LC_{50} (μg/mL)	LC_{90} (μg/mL)	χ^2	*p*
Callicarpa candicans essential oil	8.201 (7.615–8.905)	10.78 (9.83–12.01)	0.841	0.657
Callicarpa candicans microemulsion	4.452 (4.149–4.759)	5.745 (5.389–6.212)	0	1.000
Callicarpa erioclona essential oil	4.237 (3.811–4.637)	6.479 (5.925–7.326)	9.32×10^{-5}	1.000
Callicarpa erioclona microemulsion	2.323 (2.105–2.539)	3.544 (3.226–4.046)	1.51×10^{-3}	1.000
Callicarpa macrophylla essential oil	5.654 (5.298–6.032)	7.475 (6.955–8.291)	5.78×10^{-5}	1.000
Callicarpa macrophylla microemulsion	5.294 (4.923–5.600)	6.605 (6.272–7.060)	0	1.000
Karomia fragrans essential oil	6.280 (5.694–6.955)	9.938 (8.938–11.470)	3.92	0.270
Karomia fragrans microemulsion	5.658 (5.169–6.165)	9.187 (8.247–10.620)	9.81	0.020
Negative control	No mortality			
Positive control (tea saponin)	24.78 (23.26–26.72)	32.62 (29.98–37.10)	0.1301	0.988

[a] Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from five independent experiments carried out in quadruplicate, after 24 h of treatment with an additional 24 h recovery time.

Table 5. Toxicity of essential oils and their microemulsions to non-target organism *Oreochromis niloticus* after 48 h exposure.[a]

Material tested	LC_{50}	LC_{90}	χ^2	*p*
Callicarpa candicans essential oil	10.96 (10.13–11.90)	15.66 (14.28–17.90)	5.32×10^{-3}	0.997
Callicarpa candicans microemulsion	8.244 (7.498–8.968)	12.03 (11.08–13.42)	6.0×10^{-7}	1.000
Callicarpa erioclona essential oil	15.83 (14.67–17.14)	21.90 (20.20–24.26)	3.09	0.213
Callicarpa erioclona microemulsion	12.43 (11.40–13.72)	18.34 (16.49–21.38)	0.462	0.794
Callicarpa macrophylla (Pù Hoạt) EO	116.5 (106.4–127.5)	201.7 (179.0–236.5)	1.57	0.667
Callicarpa macrophylla (Pù Hoạt) μE	61.15 (56.09–66.96)	96.66 (88.46–107.46)	11.5	0.021
Karomia fragrans essential oil	19.51 (17.82–21.36)	33.99 (30.06–40.12)	3.49	0.480
Karomia fragrans microemulsion	73.15 (68.22–78.42)	99.02 (92.46–107.56)	1.33	0.857
Positive control	No mortality			

[a] Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from six independent experiments carried out in quadruplicate, after 48 h of treatment.

molluscicidal activity when LC_{90} or $LC_{100} < 100$ μg/mL or $LC_{50} < 40$ μg/mL.[53] Thus, all of the essential oils and microemulsions derived from them in this investigation showed excellent molluscicidal activity, with significantly stronger toxicity against *P. canaliculata* than the positive control, tea saponin.

There have been several publications on the toxicity of essential oils, extracts and individual phytochemicals on *P. canaliculata*. For example, the cardiac glycoside fraction from *Nerium oleander* L. (Apocynaceae) showed a 24 h LC_{50} of 80.8 μg/mL.[54] The methanol extract of leaves and twigs of *Aglaia odorata* Lour. (Meliaceae) displayed an LC_{50} of 33.4 μg/mL, and naringenin trimethyl ether, isolated from the extract showed an LC_{50} of 3.9 μg/mL.[55] A petroleum ether extract of *Solidago canadensis* L. (Asteraceae) showed a 48 h LC_{50} value of 0.18 mg/mL.[56] Vulgarone B, a sesquiterpene from *Artemisia douglasiana* Besser ex Besser (Asteraceae) has shown toxicity with a 24 h LC_{50} value of 6.54 μg/mL).[57] Two saponins, pedunsaponin A and pedunsaponin C, isolated from the root of *Pueraria peduncularis* (Benth.) Benth. (Fabaceae) exhibited 72 h LC_{50} values of 3.893 and 4.252 μg/mL, respectively.[58] Extract of soapnut, *Sapindus mukorossi* Gaertn. (Sapindaceae), showed 48 h LC_{50} value of 22 μg/mL, and saponins isolated from this extract caused mortality from 70 to 100% at concentrations of 10 μg/mL.[59] The leaf essential oils of *Lantana camara* L. (Verbenaceae) showed LC_{50} values of 23.6–40.2 μg/mL, while the essential oil components (*E*)-caryophyllene, α-humulene, and caryophyllene oxide displayed molluscicidal activities of 16.7, 19.0, and 17.8 μg/mL, respectively.[60] Based on comparison with previously published activities of botanical molluscicides, *Callicarpa* and *Karomia* essential oils and their microemulsions show excellent promise.

(*E*)-Caryophyllene and caryophyllene oxide are both relatively abundant in the *Callicarpa* and *Karomia* essential oils. Both of these compounds showed toxicity against *P. canaliculata* with LC_{50} values of 16.7 μg/mL and 17.8 μg/mL, respectively.[60] Synergistic effects between these components and minor constituents are also likely to be important.

In order to assess the toxicity of *Callicarpa* and *Karomia* essential oils and their microemulsions on a non-target aquatic organism, the materials were screened for lethality against the freshwater fish *Oreochromis niloticus* (Linnaeus) (*Table 5*).

Compared to lethality against *O. niloticus*, *C. candicans* and *C. erioclona* microemulsions showed excellent selectivity for larvicidal activity against both *Aedes* mosquito species. For example, the 48 h LC_{99} for *C. candicans* microemulsion on *Ae. albopictus* was 4.722 (4.076–5.943) μg/mL. At that concentration, it is expected to kill only 10% of *O. niloticus* [$LC_{10} = 4.455$ (2.967–5.461)]. Likewise, the 48 h LC_{99} for *C. erioclona* microemulsion on *Ae. albopictus* was 3.154 (2.445–4.531) μg/mL, while at that concentration, it would be expected to kill only 2% of *O. niloticus* [$LC_2 = 2.957$ (0.000–4.851)]. Furthermore, the 48 h LC_{99} for *C. erioclona* microemulsion on *Ae. aegypti* was 4.466 (3.204–6.905) μg/mL. At that concentration, it is expected to kill only 4% of *O. niloticus* [$LC_4 = 4.355$ (1.706–5.996)].

The essential oil microemulsion of the *Callicarpa* species as well as *K. fragrans* exhibited selective molluscicidal activity against *P. canaliculata*. In particular, *K. fragrans* microemulsion had a LC_{99} of 13.64 (11.62–17.18) μg/mL, and at that concentration would be expected to kill less than 1% of *O. niloticus* [$LC_1 =$ 26.20 (15.81–33.93) μg/mL].

Small-Scale Field Trials

A small-scale field trial was conducted using the essential oil microemulsions of *C. candicans* and *C. erioclona* against *Ae. aegypti* as a preliminary investigation of the residual larvicidal effectiveness. Each essential oil was screened using three different concentrations over a three-day period. It is likely that that the microemulsions have degraded over the time period and the essential oil evaporated. The larvicidal activities are summarized in *Figure 2*. Both essential oils showed a significant decline in larvicidal activity over the three-day period. In a laboratory-based residual effect determination, Faustino and co-investigators showed that the nanoemulsion of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) resin essential oil not only showed excellent larvicidal activity against *Ae. aegypti* ($LC_{50} = 2.91$ µg/mL), but also that the nanoemulsions retained some activity after 72 h.[61] Similarly, Firoozlyan et al. found *Cinnamomum verum* J. Presl (Lauraceae) essential oil microemulsion to have better larvicidal activity on *Anopheles stephensi* (Liston) and longer residual effectiveness compared to the essential oil itself.[62]

In Silico *ADMET Analysis*

In this study, the physicochemical and ADMET properties of the studied compounds are shown in *Table 6*. The compounds were classified to two major groups including sesquiterpene hydrocarbons and oxygenated sesquiterpenoids.

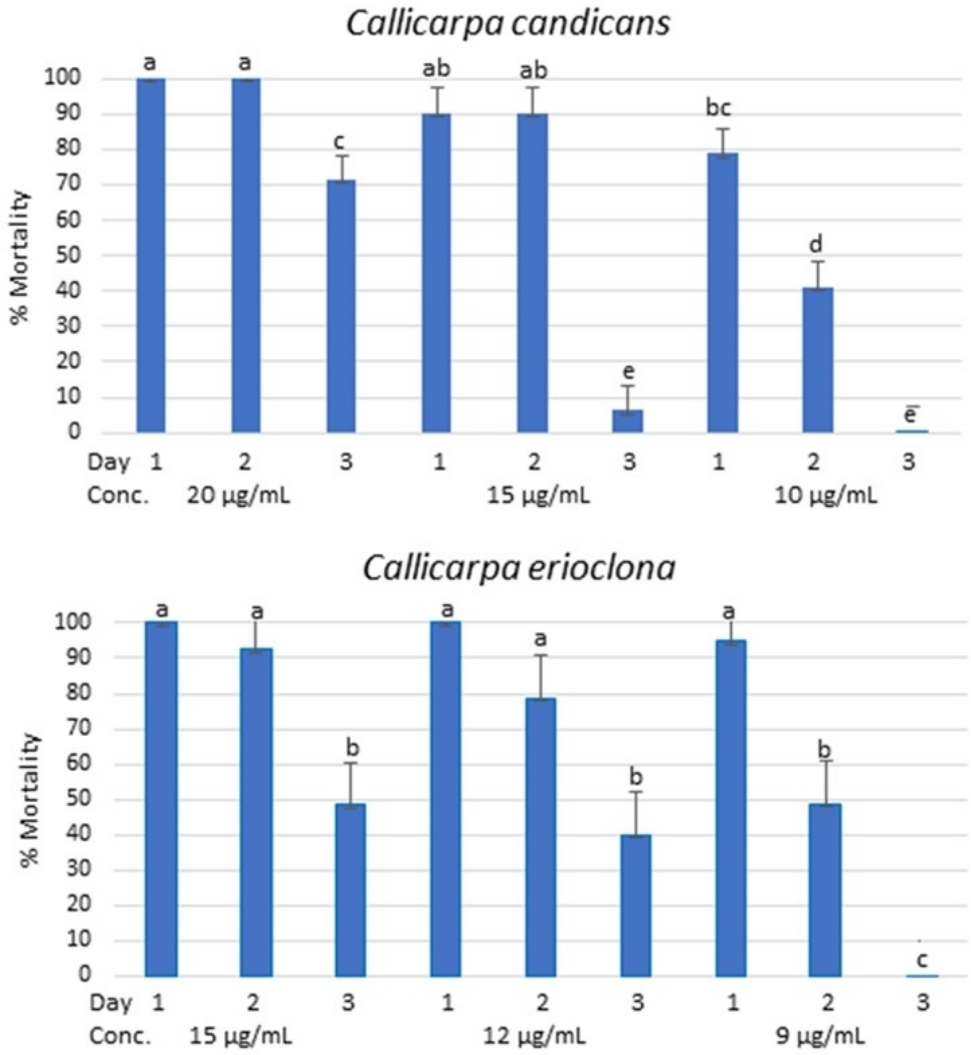

Figure 2. Small-scale field residual larvicidal activity of *Callicarpa candicans* and *Callicarpa erioclona* essential oil microemulsions against *Aedes aegypti*. Bars with the same letters are not significantly different at $p \leq 0.05$.

Table 6. *In silico* ADMET properties of studied compounds.

Compound	MW	HBD	HBA	BBB	HIA	Hepatotoxicity	CYP450 inhibition	LogP
(*E*)-Caryophyllene	204.36	0	0	0.728	95.083	No	No	4.72
Germacrene D	204.36	0	0	0.714	94.621	No	No	4.89
α-Humulene	204.36	0	0	0.660	94.430	No	No	5.03
β-Selinene	204.36	0	0	0.816	95.937	No	No	4.72
Sabinene	136.24	0	0	0.837	95.135	No	No	2.99
p-Cymene	134.22	0	0	0.456	94.253	No	No	3.11
δ-Cadinene	204.36	0	0	0.771	94.908	No	No	4.72
Germacrene B	204.36	0	0	0.665	94.409	No	No	5.17
Caryophyllene oxide	220.36	0	1	0.654	95.880	No	No	3.93
Atractylone	216.32	0	1	0.675	96.359	No	No	3.81
Curzerene	216.32	0	1	0.662	94.615	No	No	3.83

Note: MW: Molecular weight; HBD: Number of hydrogen bond donors; HBA: Number of hydrogen bond acceptors; BBB level (blood-brain barrier); HIA level (human intestinal absorption); LogP: Hydrophobicity factor (octanol/water partition coefficient).

The success of a compound as a possible insecticide is determined not only by good efficacy against its target but also by acceptable physicochemical and ADMET profile.[63] The obtained data show that none of the compounds have more than 1 violation of Lipinski's Rule of Five, thus suggesting that these compounds possess high theoretical bioavailability. It should be noted that the sesquiterpene hydrocarbons do not contain HBA or HBD while this type of bond does exist in the oxygenated compounds. Most of the compounds exhibited LogP value in a range of 2.99–4.89 (octanol/water partition coefficient) except α-humulene and germacrene B, these values are considered as favorable for the penetration and reaching to the target site in the living organisms. Blood-brain barrier value analysis (BBB) indicated that atractylone, β-selinene, and caryophyllene oxide might readily cross the brain cell membrane and interact with the central nervous system (CNS). As human intestinal absorption descriptor of all studied compounds exhibited high values (ranged from 94.253 to 96.359), they were predicted to be absorbed through the intestine to reach the bloodstream circulation and be transported to the desired molecular target. Regarding drug metabolism aspect, no inhibition against Cytochrome P450 (CYP450) was predicted for all the compounds, indicating favorable metabolic stability against CYP450 enzymes. No compound demonstrated toxicity toward liver cells through hepatotoxicity indicator.

In this study, odorant binding protein (OBP) and acetylcholinesterase (AChE) were chosen as targets to investigate the possible inhibitory activities of the studied compounds. The mosquito odorant binding protein has been a traditional drug target for insecticides. This protein transports the odorants to olfactory receptors, which plays in major activities of host seeking.[64] On the other hand, AChE is a key enzyme in biological nerve conduction, and it can degrade acetylcholine and terminate the nerve impulse in cholinergic synapses.[65] For the reason above, AChE is the target enzyme of many insecticides used in the world.

According to Gowthaman et al., when the RMSD of dock pose of the co-crystallized ligand is less than 2.0 Å in relation to the native crystallographic pose, the docking validation is considered satisfactory.[66] Retrieving the dock pose of co-crystallized ligands, it was possible to validate the docking protocol (*Figure 3*).

AutoDock4, an open-source program, is commonly utilized for calculating binding free energy and docked poses. Given the good mosquito larvicidal and molluscicidal activities of the studied essential oils, we continue to investigate the binding mode and mechanism of action of potential inhibitors against odorant binding protein and acetylcholinesterase receptor. It was reported by Gohlke et al. that ligand calculated partial charge with the B3LYP/6-31g(d,p) method has been shown to greatly increase docking accuracy and cluster population of the most accurate docking.[67] According to the ranking criteria of AutoDock, the more negative value of docking score, the better binding affinity of compound towards targeted receptor.[68] Docking results are presented in *Table 7*.

The obtained docking scores of two reference inhibitors, permethrin and galantamine, were −10.60 kcal/mol for 3OGN and −12.47 kcal/mol for 4EY6, respectively. Thus, any molecules whose docking energies are close to this threshold would be viewed

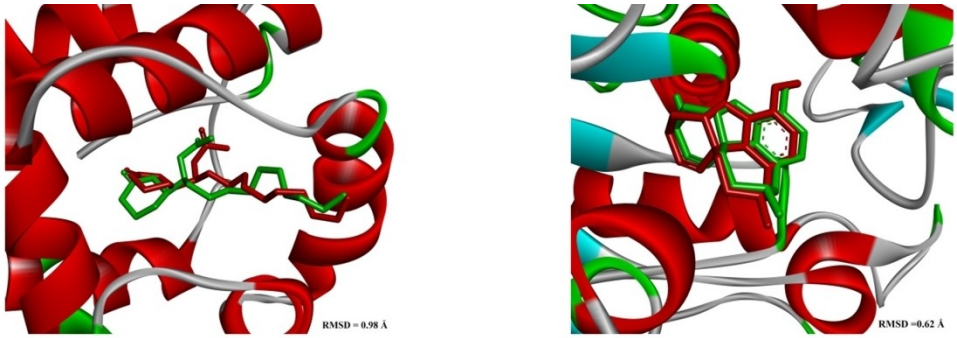

Figure 3. Dock pose overlay of crystallographic ligands (in green) with the calculated shape (in red): (A) (1*S*)-1-[(2*R*)-6-oxotetrahydro-2*H*-pyran-2-yl]undecyl acetate (3GO) docked with odorant binding protein (PDB 3OGN) and (B) galantamine (GNT) docked with acetylcholinesterase (AChE) (PDB 4EY6).

Table 7. Docking results of studied compounds and key interacting residues.

Compound	Dock score (kcal/mol)		Interacting residues with odorant binding protein (PDB ID: 3OGN)
	3OGN	4EY6	
(*E*)-Caryophyllene	−8.93	−7.77	Ala88, Met91, Trp114
Germacrene D	−7.70	−7.58	Ala88, Met91, Trp114
α-Humulene	−7.62	−7.37	Met91, His111, Trp114, Tyr122
β-Selinene	−8.88	−7.66	Leu80, Ala88, Met91, Trp114, Phe123
Sabinene	−6.11	−5.80	Ala88, Met91, Trp114
p-Cymene	−5.98	−5.54	Leu76, Ala88, Met91, Trp114
δ-Cadinene	−7.66	−7.71	Leu76, Leu80, Ala88, Met91, His111, Trp114, Tyr122, Phe123
Germacrene B	−7.26	−7.47	Phe123
Caryophyllene oxide	−8.55	−7.54	Met91, His111, Trp114
Atractylone	−9.62	−7.31	Leu76, Leu80, Ala88, Met91, Trp114, Phe123
Curzerene	−7.61	−6.67	Leu19, Met84, Met91, His121, Phe123, Leu124
Permethrin	−10.60	–	Leu15, Leu19, Leu73, Leu76, His77, Ala88, Met91, Gly92, His111, Trp114, Phe123, Leu124
Galantamine	–	−12.47	–

as potential inhibitors of targeted proteins in the virtual screening stage. Docking results from *Table 7* indicated that all the studied compounds showed significantly lower binding affinity toward acetylcholinesterase with dock score values ranged from −5.54 to −7.77 kcal/mol than those of galantamine. Therefore, the hypothesis that these compounds exhibited pesticidal activities through inhibition of AChE function could be excluded. Regarding the mosquito odorant binding protein, atractylone, (*E*)-caryophyllene, β-selinene and caryophyllene oxide were ranked as the top four with their docking scores close to those of permethrin. Binding conformation of potential inhibitors in the active site of mosquito odorant protein suggested by molecular docking studies are shown in *Figure 4*.

Conclusions

The essential oils of *Callicarpa candicans*, *C. erioclona*, *C. macrophylla*, and *Karomia fragrans* have shown promising mosquito larvicidal and molluscicidal activities. Furthermore, microemulsions of these essential oils generally showed enhanced pesticidal activities. The essential oils and their microemulsions are generally less toxic to a non-target freshwater fish. Small-scale field trials of *C. candicans* and *C. erioclona* microemulsions shows potential for residual larvicidal effectiveness. On the basis of docking results and ADMET profile analysis, it could be suggested that atractylone, β-selinene and caryophyllene oxide were predicted to be highly penetrant molecules toward the cell brain membrane and act on the CNS of larvae, these compounds exhibit larvicidal activity as potent mosquito odorant binding protein inhibitors. Therefore, these essential oils should be examined further

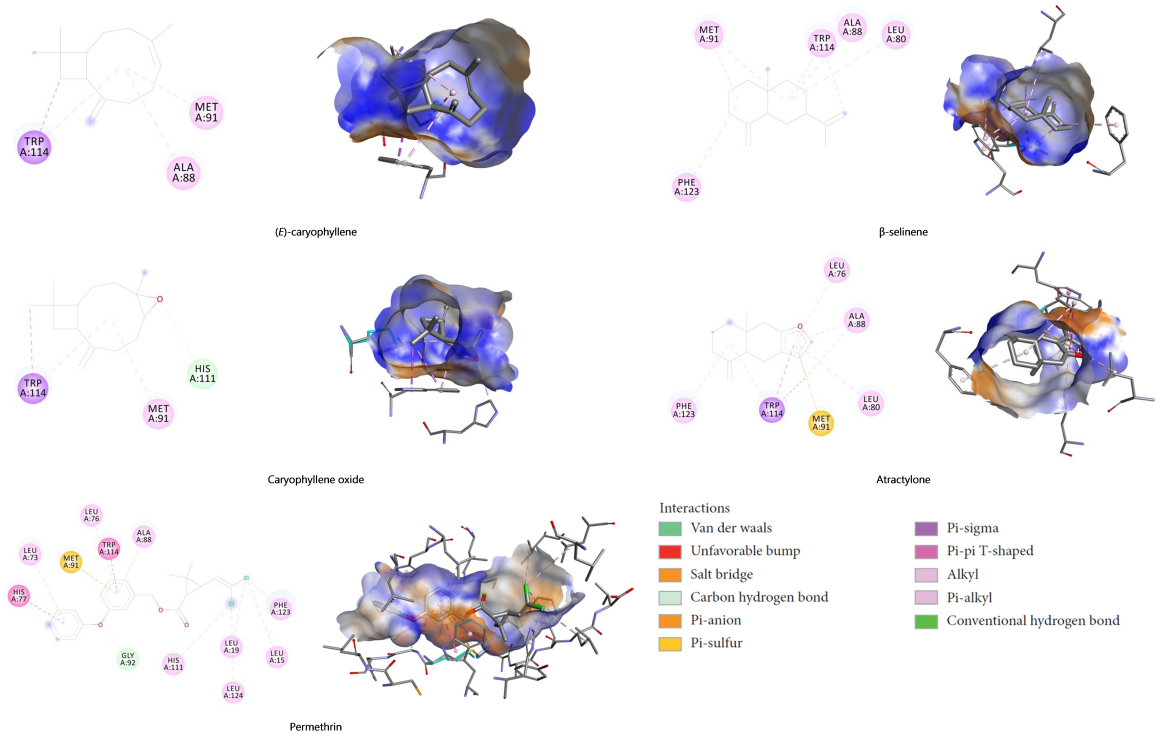

Figure 4. Interaction of potential inhibitors in the binding site of mosquito odorant binding protein suggested by molecular docking studies.

for possible cultivation and potential industrial applications and commercialization as botanical pesticidal agents. Additional microencapsulation methods should be explored and more extensive field trials should be carried out.

Experimental Section

Chemicals

Polysorbate 80 (Tween 80) surfactant was purchased from Croda Singapore Pte Ltd (Singapore). Coconut oil (MCT) was purchased from Sternchemie GmbH & Co. KG (Germany). Ethanol ACS, ISO [Scharlau ET00052500] was made in Spain. Permethrin and DMSO were purchased from Merck Vietnam (Ho Chi Minh City, Vietnam). Tea saponin (90 % purity) was purchased from Zhejiang Orient Tea Co., Ltd. (China).

Plant Material

Callicarpa erioclona Schauer and *Karomia fragrans* Dop were collected in Nui Chua National Park, Ninh Thuan Provence in March 2021 (*C. erioclona*, 11°43′27″ N, 109°11′32″ E, 8 m elevation, voucher numbers DND 832; *K. fragrans*, 11°41′38″ N, 109°09′50″ E, 75 m elevation, voucher number DND 833). *Callicarpa macrophylla* Vah was collected in March 2021 (voucher numbers DND 831) in Pu Mat National Park, Nghe An Province (19°02′19″ N, 104°54′32″ E, 30 m elevation). *Callicarpa candicans* (Burm.f.) Hochr. was collected in September 2019 (voucher number NHH 57) Hoa Vang district, Da Nang city (16°01′0.6″ N, 108°4′25.6″ E; 28 m elevation). Dr. Do Ngoc Dai and Dr. Le Thi Huong identified the plants and deposited voucher specimens with Vinh University School of Natural Science Education.

Hydrodistillation

The freshly-collected leaves of plants (5.0 kg each) were chopped and subjected to hydrodistillation with a Clevenger apparatus (Witeg Labortechnik, Wertheim, Germany) for 6 h. The EOs were dried over anhydrous Na_2SO_4 and stored at 4 °C until use.

Gas Chromatographic – Mass Spectral (GC/MS) Analysis

Each of the EOs was subjected to GC/MS analysis as previously described:[31,69] Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD, USA); electron impact mode (electron energy = 70 eV),

scan range = 40–400 amu, 3.0 scans/s scan rate, ZB-5 ms GC column (Phenomenex, Torrance, CA, USA) (5% phenyl)-polymethylsiloxane stationary phase (60 m length ×0.25 mm internal diameter, 0.25 μm film thickness), He carrier gas, column head pressure = 208 kPa, flow rate = 2.00 mL/min; injector temperature = 260 °C, ion source temperature = 260 °C; GC oven temperature program: 50 °C initial temperature, increased at 2 °C/min to 260 °C; 0.1 μL of a 5% w/v solution in CH_2Cl_2 injected, splitting mode = 24:1.

The essential oil components were identified based on their calculated retention indices (based on a homologous series of *n*-alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the databases.[70–73] The percentages of the components were calculated from total ion current.

Preparation and Characterization of Microemulsions

The MEs were prepared by the emulsion phase inversion (EPI) method.[74,75] The EO, ethanol, and coconut oil (MCT) (ratio 3%:1%:1% v/v/v), in the respective order, were combined and then the mixture was stirred with a magnetic stirrer H3770-HS (Benchmark Digital Hotplate Stirrer) for 15 min. Next, polysorbate 80 (10% v/v) was added to the mixture and stirring was continued for an additional 30 min. Distilled water (85% v/v) was added to the mixture at a rate of 3 mL/min and stirred until transparent and homogeneous MEs were obtained. The MEs were contained in transparent vials which were stored at 25 °C and 12 h light, 12 h dark cycle. The particle size distributions of the samples were determined on a Zetasizer-Nano ZS instrument (Malvern, UK) by dynamic laser scattering method. The MEs were evaluated for droplet size distribution at two time points of 01 day and 45 days.

Molluscicidal Assay

Pomacea canaliculata eggs were collected from a rice field (Da Nang City, Hoa Vang District, 16°01′02.4″ N, 108°06′34.8″ E) and were identified by Dr. Nguyen Huy Hung. Eggs were incubated under laboratory conditions: temperature = 25 ± 2 °C, relative humidity = 70 ± 5%. After hatching, the snails were raised in an aquarium at 25 ± 2 °C with a 720 min/720 min light/dark cycle and fed on fresh leaves of *Ipomoea aquatica* Forssk. Seven-day-old juvenile snails with shell lengths of 3.0–4.0 mm were used for screening.

The molluscicidal assays were carried out following the procedure of Ding et al.[76] with minor modifications. For each test, 20 snails and 150 mL of water were added to 250 mL beakers. Aliquots of the EOs or MEs, which were prepared in ethanol (1% stock solution) were subsequently added. Tea saponin, dissolved in DMSO, was used to prepare the positive control solutions. The solution containing ethanol, coconut oil (MCT), polysorbate 80 and H_2O (ratio 1%:1%:13%:85% v/v) was stirred for 30 min and served as the negative control. Each test was conducted in quadruplicate with five concentrations (50, 25, 12.5, 6.25 and 3.13 μg/mL). After 24 h of exposure, the snails were transferred to another container with 150 mL of distilled water to allow for recovery. Snails that did not recover after the additional 24 h were determined to be dead. During the experiment, the laboratory temperature was maintained at 25 ± 2 °C with a 720 min/720 min light/dark cycle.

Mosquito Larvicidal Assay

Aedes aegypti eggs were obtained from the Vietnam Academy of Science and Technology – Institute of Biotechnology and were used to raise the larvae. Adult *Ae. albopictus* mosquitoes were collected from the wild and larvae raised as described previously.[77] Wild *Cx. quinquefasciatus* first and second instar larvae were collected from car tires containing the fruit of *Ficus racemosa* L. The larvae were continuously fed on the fruit of *F. racemosa* in the laboratory until the third – early fourth instar. Dr. Nguyen Huy Hung identified the mosquitoes. The developing larval stages were maintained at 25 ± 2 °C and 65–75% relative humidity with a 720 min/720 min light/dark cycle.

Lethality screening of the EOs and MEs against the mosquito larvae were carried out as described previously:[31,69] Quadruplicate assays, 20 fourth-instar mosquito larvae, 25 ± 2 °C, eight EO concentrations (100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.781 μg/mL), positive control was permethrin, larval death was ascertained at 24 h after exposure and again at 48 h. A solution containing ethanol, coconut oil (MCT), polysorbate 80 and H_2O (ratio 1%:1%:13%:85% v/v) was stirred for 30 min and was used as a negative control.

Toxicity on Non-Target Organism

Young Nile tilapia, *Oreochromis niloticus* (Linnaeus), were collected from rice fields (Da Nang City, Hoa Vang District, 16°01′02.4″ N, 108°06′34.8″ E). They

were raised in net cages that had been placed in a cement pond (7.5 m long, 2.5 m wide and 1.5 m deep of water) and were fed on Koi fish food. Every week, 20% of the water in the pond was replaced. The fish (size 10 mm) were transferred to culture in the laboratory conditions during 7 days before they were used for testing.

Twenty fish were transferred to 600 mL glass containers with 250 mL of tap water left overnight. Each test was conducted in quadruplicate with six concentrations (200, 100, 50, 25, 12.5, and 6.25 μg/mL), mortality was recorded after 48 h of exposure. During the experiment, the laboratory temperature was maintained at 25 ± 2°C with a 720 min/720 min light/dark cycle. The solution containing ethanol, coconut oil (MCT), polysorbate 80 and H_2O (ratio 1%:1%:13%:85% v/v) was stirred for 30 min and was used as a negative control.

Small-Scale Field Trials

Small-scale field residual larvicide activity was carried out using 2.5 L plastic containers. The containers were filled with 2 L of tap water and were kept in the condition of the natural environment for 24 h, after which 20 third-instar *Ae. aegypti* larvae were placed into each container. After an acclimatization period of 2 h, CEEO ME was added to each container at concentrations 9 μg/mL, 12 μg/mL and 15 μg/mL (3, 4, and 5 times the LC_{90} value of CEEO ME against *Ae. aegypti* larvae, respectively). For CCEO ME, the final concentrations were 10, 15, and 20 μg/mL, respectively (2, 3, and 4 times the LC_{90} value of CCEO ME against *Ae. aegypti* larvae, respectively). Four replicates for each concentration and negative control were carried out. The test containers were placed in the shade under the roof. During the test, it did not rain, the temperature varied from 26 to 36°C, the relative humidity was over 60% (Weather conditions were obtained from the National center for hydro-meteorological forecasting.). The solution containing ethanol, coconut oil (MCT), polysorbate 80 and H_2O (ratio 1%:1%:13%:85% v/v) was stirred for 30 min and was used as a negative control. The dead larvae were recorded after every 24 h of treatment, and the entire batch of larvae, living and dead, was replaced each 24 h period.

In Silico *ADMET Studies*

ADMET properties of the studied compounds were predicted using open bioactivity prediction online server Molinspiration (https://www.molinspiration.com/cgi-bin/properties. Date accessed: February 10, 2022.) and admetSAR (http://lmmd.ecust.edu.cn:8000, accessed on DATE). ADMET-related parameters such as drug-likeness, permeability, intestine absorption, liver toxicity and CYP450 inhibition were investigated.

Molecular Docking

The major components in the essential oil of four studied species were selected for the docking study. The three-dimensional structures of studied compounds were prepared using MarvinSketch 19.27.0 and PyMOL version 1.3r1.[78] Energy minimization of studied ligands were conducted using MM2 force field and quantum chemical calculations were performed at the B3LYP/6-31g(d,p) level implemented in Gaussian 09.[79]

The X-ray crystal structure of mosquito odorant binding protein (PDB ID: 3OGN)[80] and acetylcholinesterase receptor (PDB ID: 4EY6)[81] were downloaded from the Protein Data Bank archive. To validate the docking procedure, the co-crystallized ligand was re-docked to ensure proper binding interactions with respect to those reported in the original state. Permethrin and galantamine, two known inhibitors of the mosquito odorant binding protein and acetylcholinesterase, were chosen as reference. The protein structures were prepared in order to obtain the correct ionization and tautomeric states of amino acid residues. Further, the water molecules were removed and polar hydrogen atoms were added. Then, the Kollman united atom partial charges and salvation parameters were assigned. The protein preparation process resulted in a PDBQT file that contained the atomic coordinates of the protein in a format that was necessary to execute AutoGrid and AutoDock.[82]

The location and dimensions of the grid box for each protein were chosen such that they incorporate the amino acid domain involved in binding with the reference compound, which was enclosed in a box with the number of grid points in x×y×z directions and a grid spacing of 0.375 Å. In particular, the grid box parameter for 3OGN comprised 70×60×60 points and 66×60×66 for 4EY6. The precalculated binding affinity of each ligand's atom type was prepared using

Autogrid. AutoDock 4.2 was utilized for the molecular docking simulation. The parameters of the Lamarckian Genetic Algorithm (LGA) were, 50 runs; elitism of 1; a mutation rate of 0.02; a population size of 300; a crossover rate of 0.80; number of generations of 27,000; the energy evaluations of 10,000,000; and the root-mean-square (rms) cluster tolerance was set to 2.0 Å in each run. The ligand conformation with the lowest free energy of binding, chosen from the most favored cluster, was selected for the further analysis. The outputs from AutoDock modeling studies were analyzed using PyMOL and Discovery Studio Visualizer.

Data Analysis

Mortality data were analyzed by log-probit analysis[83] to acquire LC_{50} and LC_{90} values as well as 95% confidence limits using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA). Analysis of variance was conducted by one-way ANOVA followed by the Tukey test using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA). Differences at $p < 0.05$ were considered to be statistically significant.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution Statement

Conceptualization: Nguyen Huy Hung; Data curation: William N. Setzer; Formal analysis: Prabodh Satyal, William N. Setzer; Funding acquisition: Nguyen Huy Hung; Investigation: Nguyen Huy Hung, Do Ngoc Dai, Le Thi Huong, Prabodh Satyal, William N. Setzer, Pham Minh Quan, Le Duc Giang, Le Thanh Hung; Methodology: Nguyen Huy Hung, Prabodh Satyal, William N. Setzer, Pham Minh Quan; Project administration: Nguyen Huy Hung; Resources: Nguyen Huy Hung, Do Ngoc Dai; Software: Prabodh Satyal, William N. Setzer, Pham Minh Quan; Supervision: Nguyen Huy Hung; Validation, William N. Setzer; Visualization, Nguyen Huy Hung, William N. Setzer; Roles/Writing – original draft: William N. Setzer, Nguyen Huy Hung, Pham Minh Quan; Writing – review & editing: William N. Setzer, Nguyen Huy Hung, Pham Minh Quan.

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