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Pesticidal Activities of *Callicarpa* and *Premna* Essential Oils From Vietnam

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Abstract

Mosquito-borne diseases are a consistent problem in Vietnam. Additionally, freshwater snail species are agricultural pests and are known to be intermediate hosts for several parasitic worms. There is a need for new and complementary botanical pesticidal agents for controlling these pests and essential oils have shown promise. In this work, essential oils from 2 species of *Callicarpa* (*C. rubella* and *C. sinuata*) and 4 species of *Premna* (*P. chevalieri, P. corymbosa, P. maclurei*, and *P. tomentosa*) were screened for mosquito larvicidal activity against *Aedes albopictus* and *Culex quinquefasciatus* and for molluscicidal activity against 3 freshwater snail species, *Gyraulus convexiusculus, Pomacea canaliculata,* and *Tarebia granifera*. *Callicarpa rubella* essential oil showed exceptional larvicidal activity against *Cx. quinquefasciatus* with 24-h LC_{50} of 9.8 μg/mL. In addition to *C. rubella*, the essential oils of *P. chevalieri* and *P. tomentosa* showed notable molluscicidal activities against *P. canaliculata* with LC_{90} values ≤ 20 μg/mL. These *Callicarpa* and *Premna* essential oils were all rich in sesquiterpenes and should be considered for continued investigation as botanical pesticidal agents.

Keywords

molluscicidal, larvicidal, lamiaceae, sesquiterpenes, essential oils

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Introduction

Synthetic pesticides have played an important role in controlling mosquitos and snails for decades. However, synthetic pesticides are harmful to the environment and human health, the most serious is the development of pesticide resistant target organism populations.[1-4] In recent years, essential oils have emerged as a biodegradable, nontoxic, and environmentally friendly source of pesticides.[5,6] Essential oils are rich and complex in chemical composition, which make it difficult for target organisms to become resistant to the essential oils.[6,7]

Some freshwater snail species are important as intermediate hosts that cause diseases in humans or animals. *Pomacea canaliculata* (Lamarck) is native to South America.[8] In many Southeast Asian countries, *P. canaliculata* is of particular interest as a troublesome invasive species and considered one of the most harmful pests to rice. Moreover, *P. canaliculata* is an intermediate host for human parasites such as *Angiostrongylus cantonensis*, which leads to eosinophilic meningitis,[9] *Gnathostoma spinigerum*, which is the cause of gnathostomiasis,[10] and *Angiostrongylus vasorum* which leads to eosinophilic encephalitis,[11] and the intestinal fluke *Echinostoma ilocanum*.[12]

Gyraulus convexiusculus (Hutton) is an intermediate host for several trematode parasites[13,14] including *Echinostoma revolutum*, *Australapatemon burti*,[15] *Artyfechinostomum malayanum* (intestinal flukes),[16] *Sanguinicola armata* (blood fluke),[17] and *Cercaria* sp.[18] In addition, *G convexiusculus* is an intermediate host of *Olveria indica*, an amphistome parasite.[19]

Tarebia granifera (Lamarck) is a host of several parasitic trematodes including *Paragonimus westermani* (the oriental lung fluke) as well as the intestinal flukes *Haplorchis taichui*,[20] *Centrocestus*

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formosanus, and *Haplorchis pumilio*.[21] This species is also considered to be an intermediate host of *Philophthalmus gralli*, which is the cause of oriental eye-fluke in birds.[22]

The Asian tiger mosquito, *Aedes albopictus* (Skuse) is distributed throughout the world. This species acts as a vector of viruses such as the dengue viruses, chikungunya virus, [23] and Zika virus.[24] The mosquito *Culex quinquefasciatus* (Say) is one of mosquito vectors transmitting lymphatic filariasis, including Saint Louis encephalitis virus,[25] West Nile virus,[26] and Zika virus.[27] Lymphatic filariasis affects over 120 million people in 81 countries throughout the tropics and sub-tropics of Asia, Africa, the western Pacific, and parts of the Caribbean and South America; and an estimated 1.34 billion live in areas where filariasis is endemic and are at risk of infection.[28]

The essential oil compositions and larvicidal activities against *Aedes aegypti* have been reported for *Callicarpa rubella*, *C. sinuata*,[29] *Premna chevalieri*, *P. corymbosa*, *P. maclurei*, and *P. tomentosa*.[30] In this present work, we have extended the pesticidal bioassays to include mosquito larvicidal activities against *Aedes albopictus* and *Culex quinquefasciatus* as well as molluscicidal activities against 3 freshwater snail species, *Gyraulus convexiusculus*, *Pomacea canaliculata*, and *Tarebia granifera*. In addition, we have screened these essential oils for insecticidal activity against the non-target water bug, *Diplonychus rusticus*.

Table 1. Major Components of the Leaf Essential Oils of *Callicarpa* and *Premna* Species From Vietnam[a].

Plant essential oil	Major components	Ref
Callicarpa nudiflora	β-Pinene (34.2%), caryophyllene oxide (20.1%), α-pinene (8.1%), myrtenal (6.8%)	29
Callicarpa petelotii	α-Humulene (53.8%), α-selinene (12.8%), humulene epoxide II (8.1%)	29
Callicarpa rubella	(*E*)-Caryophyllene (18.0%), α-cubebene (17.4%), δ-cadinene (4.6%), bicyclogermacrene (4.6%), α-copaene (4.6%)	29
Callicarpa sinuata	α-Humulene (24.8%), α-copaene (12.6%), humulene epoxide II (6.7%), spathulenol (5.9%)	29
Premna cambodiana	α-Copaene (23.3%), (*E*)-caryophyllene (12.8%), α-gurjunene (11.3%), δ-cadinene (5.5%)	30
Premna chevalieri	(*E*)-Caryophyllene (31.5%), β-pinene (16.8%), α-pinene (12.2%), α-humulene (7.5%), caryophyllene oxide (5.3%)	30
Premna corymbosa	*allo*-Aromadendrene (39.7%), (*E*)-caryophyllene (13.3%), α-copaene (8.1%)	30
Premna maclurei	(*E*)-Caryophyllene (30.7%), caryophyllene oxide (12.3%), δ-cadinene (8.4%), spathulenol (6.8%), α-humulene (5.3%)	30
Premna mekongensis (Chu Mom Ray)	Bicyclogermacrene (11.9%), (*E*)-nerolidol (7.5%), germacrene D (5.6%), viridiflorol (5.6%)	30
Premna mekongensis (Ngoc Linh)	α-Pinene (66.9%), (*E*)-caryophyllene (14.7%)	30
Premna tomentosa	(*E*)-Caryophyllene (22.0%), germacrene D (11.4%), ledol (6.1%), α-selinene (5.5%), α-gurjunene (5.2%), *trans*-β-elemene (5.0%)	30

[a]Photographs and botanical descriptions of the plants described in this study are shown in Supplemental Table S1.

Results and Discussion

Essential Oil Compositions

The major components of the essential oils for the *Callicarpa* and *Premna* species, previously published, are summarized in Table 1.

Mosquito Larvicidal Activity

The *C. rubella*, *C. sinuata*, *P. chevalieri*, *P. corymbosa*, *P. maclurei*, and *P. tomentosa* leaf essential oils were screened for mosquito larvicidal activity against *Aedes albopictus* and *Culex quinquefasciatus* (Table 2). In addition, the essential oils were tested for insecticidal activity on the non-target water bug, *Diplonychus rusticus* (Figure 1).

Dias and Moraes [31] have suggested that essential oils with 24-h LC_{50} less than 100 μg/mL should be considered to be "active" while LC_{50} above 100 μg/mL should be "inactive." Using this criterion, all of the *Callicarpa* and *Premna* essential oils could be considered to be active. Based on our experience,[29,30,32,33] however, we suggest amending the definition of active larvicidal essential oils. Essential oils with 24-h LC_{50} <10 μg/mL should be considered "exceptionally active," those with 24-h LC_{50} between 10 μg/mL and 50 μg/mL "very active," those with 24-h LC_{50} between 50 μg/mL and 100 μg/mL "moderately active, and LC_{50} > 100 μg/mL are "inactive." In this case, *C. rubella* leaf essential oil is exceptionally active against *Cx. quinquefasciatus*, but only marginally active on *Ae. albopictus* larvae. In a previous study, *C. rubella* essential oil showed a 24-h LC_{50} of 26.0 μg/mL (*ie*, very active) against *Ae. aegypti* .[29]

In addition to the breakdown based on lethality of the essential oils against the target organisms, we can propose a "selectivity index" (SI, see Table 2) based on the lethality of each essential oil against target organisms compared to its lethality against non-targets (in this case, *D. rusticus*). That is, not only are larvicidal activities important, but also selectivity for the target species is also important. In this study, *C. rubella* essential oil showed the best overall selective lethality on *Cx. quinquefasciatus* compared to *D. rusticus*. Of course, the selectivity index will depend on the non-target organism(s) chosen and it remains to be determined what constitutes a "good SI."

Most of the *Callicarpa* and *Premna* essential oils in this study were dominated by sesquiterpenes. It is tempting to suggest that sesquiterpenes are responsible for the larvicidal activities observed. The major sesquiterpene components in *C. rubella*

Table 2. Mosquito Larvicidal Activities of *Callicarpa* and *Premna* Essential Oils[a].

Plant essential oil	LC_{50} (fiducial limits)	LC_{90} (fiducial limits)	χ^2	*P*	SI [b]
		Aedes albopictus, 24-h			
Callicarpa nudiflora	33.00 (30.49-35.80)	48.40 (44.64-53.38)	1.36	.715	---
Callicarpa petelotii	37.70 (34.48-41.16)	60.31 (53.89-70.42)	1.61	.807	---
Callicarpa rubella	50.48 (43.91-58.98)	157.9 (123.1-223.2)	7.57	.056	2.7
Callicarpa sinuata	41.26 (37.78-45.46)	64.66 (57.86-74.37)	0.909	.823	2.6
Premna chevalieri	46.61 (43.44-50.42)	64.19 (59.02-71.86)	0.679	.878	1.7
Premna cambodiana	75.38 (65.47-89.81)	202.1 (154.4-304.0)	8.34	.080	---
Premna corymbosa	45.88 (42.67-49.76)	64.26 (58.95-72.06)	1.99	.575	2.5
Premna maclurei	94.72 (86.13-105.17)	154.1 (138.8-175.4)	20.3	.000	---
Premna mekongensis (Chu Mom Ray)	37.70 (34.48-41.16)	60.31 (53.89-70.42)	1.61	.807	2.2
Premna mekongensis (Ngoc Linh)	25.67 (23.20-28.40)	51.11 (44.51-61.22)	2.94	.569	5.1
Premna tomentosa	39.23 (36.52-42.22)	54.97 (51.04-60.24)	0.958	.812	2.7
Permethrin control	0.0024 (0.0021-0.0026)	0.0042 (0.0038-0.0049)	4.64	.031	---
		Culex quinquefasciatus, 24-h			
Callicarpa nudiflora		Not tested [c]			
Callicarpa petelotii		Not tested [c]			
Callicarpa rubella	9.842 (8.649-11.158)	26.61 (22.22-33.77)	15.9	.003	13.7
Callicarpa sinuata	46.67 (41.24-53.29)	123.5 (100.8-162.5)	2.02	.569	2.3
Premna cambodiana	41.64 (36.82-47.58)	110.0 (89.9-143.8)	42.8	.000	---
Premna chevalieri	75.89 (69.19-84.05)	123.7 (111.7-140.3)	11.3	.010	1.0
Premna corymbosa	37.07 (33.88-40.83)	59.61 (54.05-67.37)	8.89	.031	3.1
Premna maclurei	67.53 (58.83-79.63)	184.2 (142.7-267.0)	0.245	.970	2.0
Premna mekongensis (Chu Mom Ray)	26.67 (27.02-32.56)	53.39 (47.16-62.96)	3.23	.520	3.1
Premna mekongensis (Ngoc Linh)	43.10 (39.60-47.19)	65.73 (59.94-73.77)	9.02	.061	3.0
Premna tomentosa	52.03 (47.81-57.33)	79.05 (70.99-90.26)	9.47	.024	2.1
Permethrin control	0.0165 (0.0149-0.0181)	0.0305 (0.0266-0.0367)	5.24	.073	---
		Diplonychus rusticus, 24-h			
Callicarpa nudiflora		Not tested [c]			---
Callicarpa petelotii		Not tested [c]			---
Callicarpa rubella	134.4 (124.8-145.0)	214.7 (198.0-237.0)	38.1	.000	---
Callicarpa sinuata	105.5 (96.3-116.6)	164.7 (149.2-185.8)	12.6	.006	---
Premna cambodiana		Not tested [c]			---
Premna chevalieri	79.12 (76.60-81.94)	91.40 (87.66-97.26)	10.6	.014	---
Premna corymbosa	116.1 (106.4-127.7)	176.2 (160.4-197.4)	19.5	.000	---
Premna maclurei		Not tested [c]			---
Premna mekongensis (Chu Mom Ray)	82.90 (80.54-85.77)	92.68 (89.23-97.94)	0.00064	.98	---
Premna mekongensis (Ngoc Linh)	130.0 (124.7-135.4)	158.0 (151.1-167.1)	0.797	.85	---
Premna tomentosa	107.2 (97.8-118.6)	169.6 (153.7-191.3)	23.4	.000	---
		Aedes albopictus, 48-h			
Callicarpa nudiflora	31.64 (29.14-34.47)	47.59 (43.72-52.74)	2.53	.469	---
Callicarpa petelotii	30.41 (27.78-33.36)	51.36 (45.33-61.13)	3.88	.422	---
Callicarpa rubella	45.14 (39.30-52.48)	143.7 (113.0-199.8)	8.18	.042	2.6
Callicarpa sinuata	37.10 (33.44-41.49)	64.23 (58.19-72.75)	1.91	.591	2.3
Premna cambodiana	62.94 (55.82-72.05)	149.5 (121.5-201.3)	3.27	.514	---
Premna chevalieri	45.65 (42.53-49.35)	63.24 (58.21-70.58)	0.407	.939	1.4
Premna corymbosa	35.57 (32.65-38.93)	55.47 (50.66-62.03)	3.81	.282	2.7
Premna maclurei	72.27 (65.22-80.65)	126.0 (112.9-144.7)	14.0	.003	---
Premna mekongensis (Chu Mom Ray)	35.04 (32.06-38.32)	56.43 (50.27-66.17)	4.92	.296	---
Premna mekongensis (Ngoc Linh)	23.79 (21.51-26.32)	47.23 (41.16-56.51)	2.54	.638	5.0
Premna tomentosa	36.21 (33.60-39.09)	51.84 (48.02-56.89)	0.859	.835	1.8
		Culex quinquefasciatus, 48-h			
Callicarpa nudiflora		Not tested [c]			---
Callicarpa rubella	4.495 (3.995-5.003)	8.983 (7.760-11.073)	21.6	.000	25.7
Callicarpa petelotii		Not tested [c]			---
Callicarpa sinuata	35.48 (31.39-40.23)	95.27 (79.00-121.90)	10.7	.013	2.4
Premna cambodiana	20.78 (18.74-23.02)	42.37 (36.84-50.83)	4.13	.388	---
Premna chevalieri	56.63 (50.17-64.32)	113.3 (100.3-131.9)	27.9	.000	1.2
Premna corymbosa	30.95 (27.95-34.36)	54.13 (48.83-61.53)	15.8	.001	3.1

(Continued)

Table 2. Continued

Plant essential oil	LC_{50} (fiducial limits)	LC_{90} (fiducial limits)	χ^2	*P*	SI [b]
Premna maclurei	43.14 (36.81-51.60)	175.1 (129.3-268.5)	16.1	.001	---
Premna mekongensis (Chu Mom Ray)	23.75 (21.33-26.42)	51.48 (44.47-62.17)	2.95	.566	---
Premna mekongensis (Ngoc Linh)	32.77 (29.27-36.79)	77.55 (65.63-96.40)	12.18	.016	3.6
Premna tomentosa	46.20 (41.76-51.55)	76.50 (69.33-86.79)	16.7	.001	1.4
		Diplonychus rusticus, 48-h			
Callicarpa nudiflora		Not tested [c]			---
Callicarpa petelotii		Not tested [c]			---
Callicarpa rubella	115.6 (106.1-125.8)	200.0 (183.7-221.9)	21.9	.000	---
Callicarpa sinuata	84.28 (76.60-93.67)	137.3 (123.5-156.8)	10.4	.016	---
Premna cambodiana		Not tested [c]			---
Premna chevalieri	65.80 (63.15-68.29)	77.16 (74.28-80.99)	0.00466	1.000	---
Premna corymbosa	96.77 (87.65-107.75)	162.9 (146.6-185.3)	17.8	.001	---
Premna maclurei		Not tested [c]			---
Premna mekongensis (Chu Mom Ray)		Not tested [c]			---
Premna mekongensis (Ngoc Linh)	117.8 (112.1-123.8)	154.8 (145.1-168.9)	6.60	.086	---
Premna tomentosa	66.46 (60.71-73.07)	107.1 (97.5-120.3)	7.94	.047	---

[a]Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from 6 independent experiments carried out in quadruplicate, after 24-h and 48-h of treatment.
[b]SI = selectivity index = LC_{50} (non-target species)/LC_{50} (mosquito larvae).
[c]Not tested due to insufficient essential oil.

essential oil were (*E*)-caryophyllene (18.0%) and α-cubebene (17.4%). The high concentration of (*E*)-caryophyllene in *C. rubella* oil cannot account for the exceptional larvicidal activity of that essential oil on *Cx. quinquefasciatus*, however. The sesquiterpenes (*E*)-caryophyllene, α-humulene, and caryophyllene oxide showed only weak activity against *Cx. quinquefasciatus* with 24-h LC_{50} values of 161, 108, and 99 μg/mL, respectively (see Supplemental Table S2).[34] Nevertheless, (*E*)-caryophyllene was present in most essential oils with strong larvicidal activity.[7] Essential oils characterized by (*E*)-caryophyllene and caryophyllene oxide exhibited larvicidal activity against *Ae. aegypti*.[35,36] *Piper purusanum* essential oil is characterized by (*E*)-caryophyllene, α-humulene, and germacrene D, and has shown strong larvicidal activity against *Ae. aegypti* and *Ae. albopictus*.[37] The unripe fruit peel of *Hymenaea courbaril* essential oil was characterized by germacrene D (31.9%) and (*E*)-caryophyllene (27.1%) and exhibited strong larvicidal activity against *Ae aegypti*.[38] Sesquiterpene hydrocarbons and oxygenated sesquiterpenoids dominated the essential oil of *Callicarpa candicans*, which showed exceptional mosquito larvicidal activities against *Ae. aegypti* and *Cx. quinquefasciatus* (24-h LC_{50} = 2.7 and 1.2 μg/mL, respectively).[29] Interestingly, *Premna flavescens*, with 92.2% sesquiterpene hydrocarbons, was relatively inactive against *Ae. aegypti* and *Ae. albopictus* larvae with 24-h LC_{50} values of 64.7 and 90.0 μg/mL, respectively.[30] Apparently, synergistic and/or antagonistic effects of essential oil components play a role in the larvicidal activities.[39]

Callicarpa nudiflora essential oil was dominated by β-pinene (34.2%) and the very good larvicidal activity of *C. nudiflora* against *Ae. albopictus* (24-h LC_{50} = 33.00 μg/mL) can be attributed to the larvicidal activity of β-pinene against *Ae. albopictus* (24-h LC_{50} = 8.51 μg/mL, see Supplemental Table S2). Likewise, *P. mekongensis* essential oil from Ngoc Linh, an α-pinene-rich chemotype (66.9% α-pinene) also showed good larvicidal activity against *Ae. albopictus* with 24-h LC_{50} of 25.67 μg/mL and SI of 5.1. Essential oils are complex mixtures of many compounds, and synergistic effects between the essential oil components are likely responsible for the enhanced larvicidal activities compared to the individual components that were tested. Essential oils containing high levels of α-pinene showed strong larvicidal activity against *Culex pipiens*[40-42] and *Ae. albopictus*.[43]

Molluscicidal Activity

The *Callicarpa* and *Premna* leaf oils were screened for molluscicidal activity against the freshwater snails, *Gyraulus convexiusculus*, *Pomacea canaliculata*, and *Tarebia granifera* (Table 3).

The World Health Organization (WHO) has defined "active" molluscicidal botanicals as those that have $LC_{90} \leq$ 20 μg/mL.[44] Based on this criterion, *P chevalieri* essential oil was active against *G. convexiusculus* and *P. canaliculata*, while *C. rubella* and *P. tomentosa* essential oils were active against *P. canaliculata*. In addition to these lethality data, *C. rubella*, *P. chevalieri*, and *P. tomentosa* essential oils were relatively selective for *P. canaliculata* compared to the non-target water bug with selectivity indices > 10.

In contrast to larvicidal activities, (*E*)-caryophyllene, α-humulene, and caryophyllene oxide have shown molluscicidal LC_{90} values around 20 μg/mL on *P canaliculata* (see Supplemental Table S3),[34] and likely contributed to the molluscicidal activities of *C. rubella*, *P. chevalieri*, and *P. tomentosa* essential oils against *P. canaliculata*. The sesquiterpenoid-rich *P. mekongensis* essential oil (from Chu Mom Ray) showed excellent molluscicidal activity against *G. convexiusculus* and *P. canaliculata* with LC_{90} values of 12.10 and 11.61 μg/mL, respectively, and SI values of 9.0 and 17.1, respectively. The role of

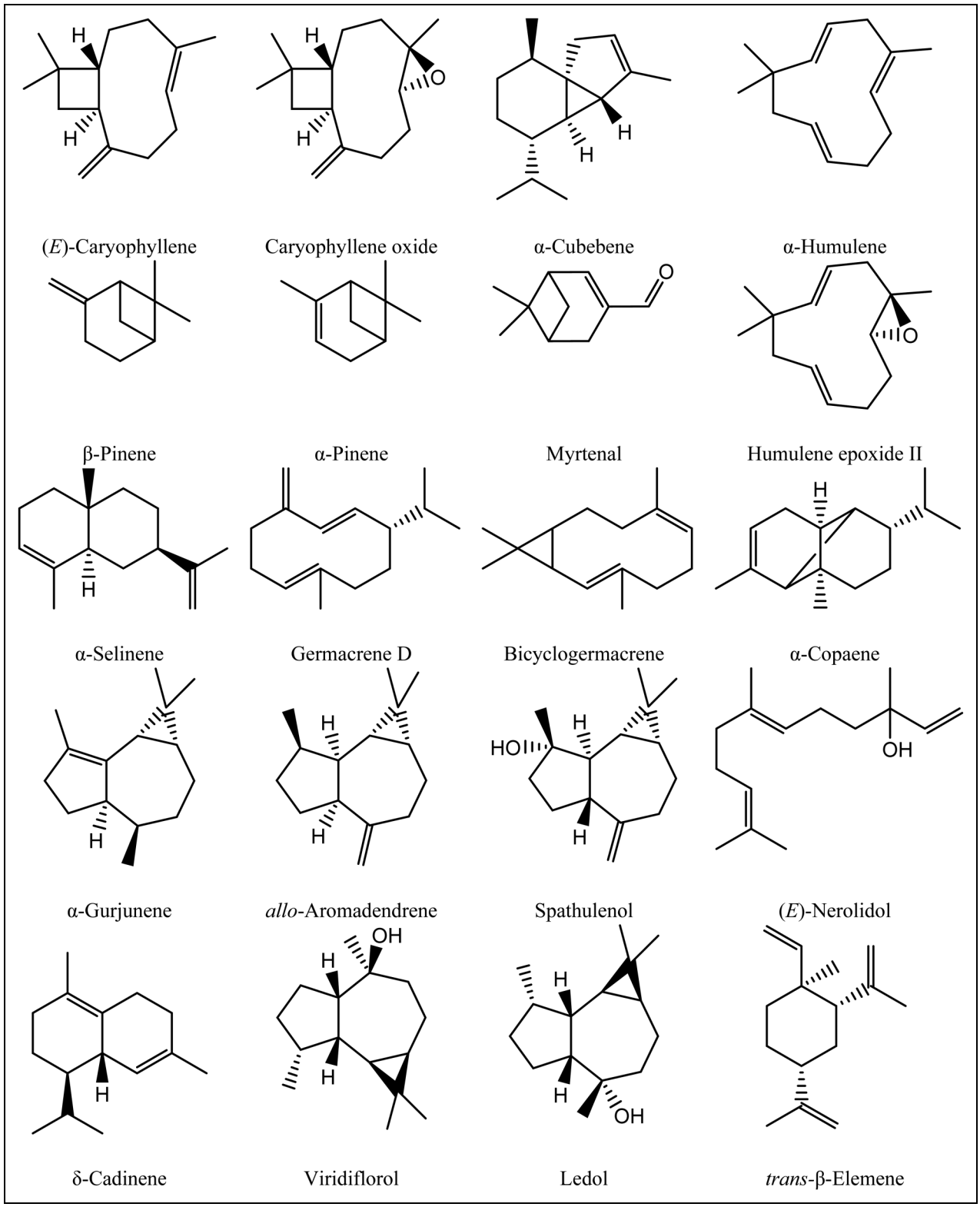

Figure 1. Chemical structures of the main compounds of essential oils *Callicarpa* and *Premna* species from Vietnam.

sesquiterpenoids in molluscicidal activity has also been observed in previous studies. The essential oil of *Xylopia langsdorffiana*, characterized by germacrene D (22.9%), *trans*-β-guaiene (22.6%), and (*E*)-caryophyllene (15.7%), exhibited strong molluscicidal activity against *Biomphalaria glabrata* with an LC_{50} value of 5.6 μg/mL.[45] *Schinus terebinthifolius* essential oil, containing sabinene (14.9%),

Table 3. Molluscicidal Activities of *Callicarpa* and *Premna* Essential Oils[a].

Plant essential oil	LC_{50} (fiducial limits)	LC_{90} (fiducial limits)	χ^2	*P*	SI [b]
		Gyraulus convexiusculus			
Callicarpa nudiflora	17.60 (15.88-19.49)	35.45 (30.89-42.37)	0.261	.992	---
Callicarpa petelotii	25.30 (23.25-27.54)	40.72 (36.40-47.47)	0.595	.964	---
Callicarpa rubella	20.10 (18.12-22.29)	40.92 (35.51-49.34)	5.61	.061	5.2
Callicarpa sinuata	14.69 (12.00-17.41)	39.23 (34.28-46.49)	16.8	.001	4.2
Premna cambodiana	41.85 (38.14-45.89)	71.49 (63.17-84.97)	6.59	.159	---
Premna chevalieri	11.54 (10.47-12.70)	21.62 (19.05-25.50)	7.01	.072	4.2
Premna corymbosa	20.73 (17.82-23.74)	44.38 (39.35-51.69)	2.05	.359	4.0
Premna maclurei	19.16 (17.16-21.41)	33.69 (30.14-38.92)	4.01	.261	---
Premna mekongensis (Chu Mom Ray)	9.190 (8.553-9.850)	12.10 (11.32-13.12)	0.322	.956	9.0
Premna mekongensis (Ngoc Linh)	8.456 (7.460-9.536)	21.84 (18.53-26.99)	11.64	.020	15.4
Premna tomentosa	27.89 (24.83-31.47)	54.39 (48.46-62.69)	16.9	.001	3.1
		Pomacea canaliculata			
Callicarpa nudiflora	Not tested [c]				---
Callicarpa petelotii	Not tested [c]				---
Callicarpa rubella	16.33 (15.18-17.78)	21.14 (19.42-23.74)	0.371	.831	10.2
Callicarpa sinuata	34.86 (32.41-37.68)	44.31 (41.07-48.77)	9.39	.009	3.7
Premna cambodiana	15.89 (14.64-17.46)	21.59 (19.65-24.53)	23.1	.000	---
Premna chevalieri	6.018 (5.518-6.684)	8.803 (7.865-10.431)	0.169	.919	10.4
Premna corymbosa	28.67 (26.50-31.60)	39.00 (35.31-44.88)	1.59	.451	4.5
Premna maclurei	Not tested [c]				---
Premna mekongensis (Chu Mom Ray)	4.844 (4.222-5.473)	11.61 (9.88-14.43)	0.285	.963	17.1
Premna mekongensis (Ngoc Linh)	11.84 (10.64-13.16)	23.82 (20.63-28.89)	39.63	.000	11.0
Premna tomentosa	10.34 (9.68-10.95)	13.01 (12.33-13.90)	0.0217	.999	13.0
		Tarebia granifera			
Callicarpa nudiflora	37.08 (33.52-41.10)	74.21 (64.31-89.66)	5.67	.225	---
Callicarpa petelotii	24.13 (21.55-27.02)	57.24 (48.87-70.10)	6.75	.150	---
Callicarpa rubella	47.54 (42.72-53.05)	87.06 (78.47-98.68)	13.1	.004	2.5
Callicarpa sinuata	26.72 (23.46-30.45)	79.80 (65.47-103.53)	23.1	.000	2.1
Premna cambodiana	Not tested [c]				---
Premna chevalieri	35.90 (32.54-39.89)	60.74 (54.65-69.34)	5.93	.115	1.5
Premna corymbosa	50.28 (45.92-55.45)	80.03 (72.66-90.06)	9.05	.029	2.2
Premna maclurei	57.68 (53.04-63.31)	84.18 (76.73-94.45)	8.55	.036	---
Premna mekongensis (Chu Mom Ray)	29.45 (26.05-33.43)	80.85 (67.09-102.89)	11.54	.021	2.8
Premna mekongensis (Ngoc Linh)	80.69 (73.51-87.96)	145.2 (130.3-166.8)	18.20	.006	1.6
Premna tomentosa	32.14 (28.99-35.79)	56.43 (50.73-64.44)	2.89	.409	3.0

[a]Data are presented as LC_{50} and LC_{90} values with 95% confidence limits (log-probit analysis) obtained from 5 independent experiments carried out in quadruplicate, after 24-h of treatment with an additional 24-h recovery time.
[b]SI = selectivity index = LC_{50} (non-target species)/LC_{50} (snails).
[c]Not tested due to insufficient essential oil.

γ-elemene (13.2%), and β-elemene (6.6%), was active against *Theba pisana* with an LC_{50} value of 8.36 μg/mL. Meanwhile, *Vitex agnuscastus* essential oil consisting of (*E*)-caryophyllene (15.2%), 1,8-cineole (13.0%), and bicyclogermacrene (7.3%) exhibited an LC_{50} value of 20.72 μg/mL.[46] Other studies that support this trend include the essential oil from the stem bark of *Ocotea bracteosa* [47] and the leaf essential oil of *Eugenia uniflora*.[48]

Materials and Methods

Isolation of Essential Oils

The essential oils were extracted from the fresh leaves of the plants by hydrodistillation. The leaves were shredded and hydrodistilled using a Clevenger type apparatus (Witeg Labortechnik). The essential oils were dried over anhydrous Na_2SO_4 and stored in sealed glass vials at 4 °C until used for analysis and bioactivity assays.

Preparation of Test Solutions

For all experiments, a 1% stock solution of the essential oils was prepared by dissolving each essential oil using ethanol. Different volumes of stock solution were introduced into test beakers containing 150 mL of distilled water to obtain test solutions with the desired concentrations.

Mosquito Larvicidal Assays

Adult *Aedes albopictus* mosquitoes were maintained continuously under laboratory conditions such as relative humidity 75%, temperature 25 ± 2 °C, cycles 12-h light and 12-h dark. *Aedes*

mosquito larvae were reared in plastic containers and were fed on Koi fish food, the water in the plastic containers was renewed daily with tap water overnight. The eggs and first and early second instar larvae of *Culex quinquefasciatus* were collected in wild environments such as tires and ditches. The larvae were fed on the ripe fruit of *Ficus racemosa* until reaching the third and early fourth instar.

The essential oils were screened for larvicidal activity against *Ae. albopictus* and *Cx. quinquefasciatus* as described previously[32]: 4 replicates, 20 larvae third and early fourth instar each, with essential oil concentrations of 100, 50, 25, 12.5, 6, and 3 μg/mL, permethrin positive control, larvicidal effects assessed after 24 and 48 h, lethality data analyzed by log-probit[49] analysis to obtain LC_{50}, LC_{90}, and 95% confidence limits using Minitab® 19 (Minitab, LLC).

Insecticidal Assay

Adults of *Diplonychus rusticus* (water bug) were collected from the wild, maintained in glass tanks (60 cm long × 50 cm wide) with a water depth of 20 cm under the same conditions for other organisms. *Eichhornia crassipes* plants were introduced into the tanks to help the water bug maintain its behaviors.

The essential oils were screened for insecticidal activity against the non-target water bug, *Diplonychus rusticus* as previously described[50]: Quadruplicate assays, 20 adult insects each, essential oil concentrations of 200, 150, 100, 75, 50, and 25 μg/mL, insect lethality assessed after 24 and 48 h, log-probit analysis.

Molluscicidal Assays

The *Premna* and *Callicarpa* essential oils were screened for molluscicidal activity against *Gyraulus convexiusculus*, *Pomacea canaliculata*, and *Tarebia granifera* as previously described[34]: Quadruplicate assays, 20 snails each, essential oil concentrations of 100, 50, 25, 12.6, and 6 μg/mL, tea saponin positive control, 24-h treatment, followed by 24-h recovery in fresh water, lethality data analyzed by log-probit.

Conclusions

Based on the pesticidal activity data presented in this report, we conclude that *C. rubella* essential oil could be an effective mosquito control agent for *Cx. quinquefasciatus* with limited environmental impact. In addition, the essential oils *C. rubella, P. chevalieri, P. mekongensis*, and *P. tomentosa* should be considered for control of the agricultural pest *P. canaliculata*. Additional research is needed to determine appropriate formulations to extend the lifetimes of the essential oils in the field as well as field trials to determine efficacy in the field as well as any detrimental environmental effects. These studies are currently being carried out in the laboratory of Nguyen Huy Hung. Additional studies are also necessary to examine more essential oil components for biological activity, both individually and in mixtures to assess synergistic activities.

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Supplemental material

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