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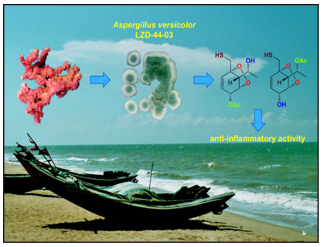

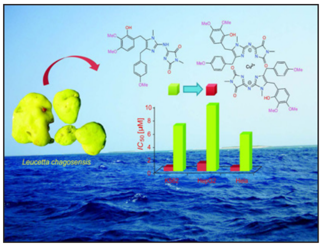

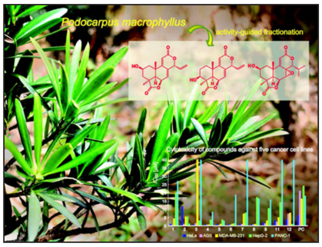

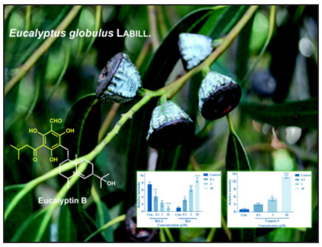

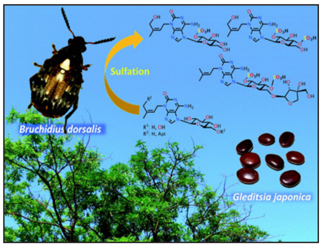

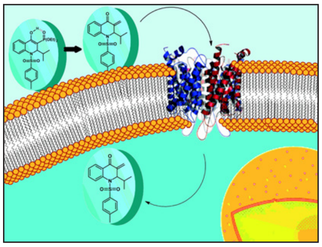

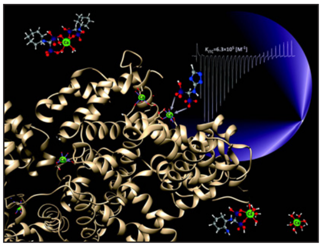

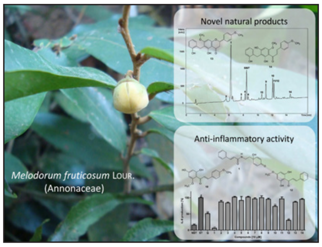

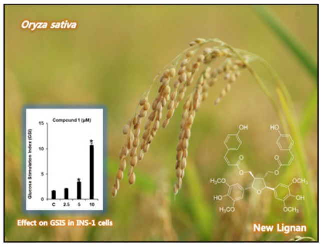

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FULL PAPER

CHEMISTRY & BIODIVERSITY

Lantana camara Essential Oils from Vietnam: Chemical Composition, Molluscicidal, and Mosquito Larvicidal Activity

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Lantana camara is a troublesome invasive plant introduced to many tropical regions, including Southeast Asia. However, the plant does hold promise as a source of essential oils that may be explored for potential use. Fresh water snails such as *Pomacea canaliculata*, *Gyraulus convexiusculus*, and *Tarebia granifera* can be problematic agricultural pests as well as hosts for parasitic worms. *Aedes* and *Culex* mosquitoes are notorious vectors of numerous viral pathogens. Control of these vectors is of utmost importance. In this work, the essential oil compositions, molluscicidal, and mosquito larvicidal activities of four collections of *L. camara* from north-central Vietnam have been investigated. The sesquiterpene-rich *L. camara* essential oils showed wide variation in their compositions, not only compared to essential oils from other geographical locations (at least six possible chemotypes), but also between the four samples from Vietnam. *L. camara* essential oils showed molluscicidal activities comparable to the positive control, tea saponin, as well as other botanical agents. The median lethal concentrations (LC_{50}) against the snails were 23.6–40.2 μg/mL (*P. canaliculata*), 7.9–29.6 μg/mL (*G. convexiusculus*), and 15.0–29.6 μg/mL (*T. granifera*). The essential oils showed good mosquito larvicidal activities with 24-h LC_{50} values of 15.1–29.0 μg/mL, 26.4–53.8 μg/mL, and 20.8–59.3 μg/mL against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*, respectively. The essential oils were more toxic to snails and mosquito larvae than they were to the non-target water bug, *Diplonychus rusticus* (24-h LC_{50} = 103.7–162.5 μg/mL). Sesquiterpene components of the essential oils may be acting as acetylcholinesterase (AChE) inhibitors. These results suggest that the invasive plant, *L. camara*, may be a renewable botanical pesticidal agent.

Keywords: *Pomacea canaliculata*, *Gyraulus convexiusculus*, *Tarebia granifera*, *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*.

Introduction

The native range of *Lantana camara* L. (Verbenaceae) is Central and South America but the plant has been introduced to tropical and subtropical regions around the world, including Africa,[1] Australia, the Mediterranean, India,[2] Malesia, and Southeast Asia,[3] including Vietnam.[4] *Lantana camara* has been identified as one of the top ten invasive species in the world,[2] and in regions where it has invaded it has caused reduction of native species diversity and agricultural productivity.[5]

Lantana camara has been reported to cause intoxication to a variety of animals, especially ruminants, but also dogs, horses, ostriches, kangaroos, and humans.[6] The toxic principles are pentacyclic triterpenoids lantadenes A and B and icterogenin.[7] In spite of its toxicity, the plant has been used as an herbal medicine in numerous cultures. For example, in Jamaica and Trinidad, a decoction of the plant is taken as a remedy for colds and fevers;[8] in Peru, the leaf juice is applied externally to wounds and sores;[9] in Nigeria and Senegal, a leaf infusion is taken to treat coughs and colds;[10] and in Vietnam, the plant is used to treat rheumatism and tuberculosis.[11]

Pomacea canaliculata (Lamarck) (Ampullariidae), the golden apple snail (GAS), is native to South America,[12] but was introduced into Asia in 1980 for aquicultural purposes, and has escaped cultivation and established itself as a troublesome invasive species.[13–15] The snail is polyphagous, feeding on a wide range of aquatic plants,[16] but in many countries of Southeast Asia, including Vietnam, *P. canaliculata* has impacted rice agricultural production.[13–15] In addition to its invasive impact, *P. canaliculata* serves as an intermediate host to the parasitic nematodes *Angiostrongylus cantonensis* (Chen) (Angiostrongylidae), which is the most common cause of eosinophilic meningitis in humans;[17–19] *Angiostrongylus vasorum* (Baillet) (Kamensky) (Angiostrongylidae),[20] which causes canine angiostrongylosis in domestic dogs and wild canids;[21] *Gnathostoma spinigerum* Levinsen (Gnathostomatidae),[22] the cause of human gnathostomiasis.[23] In addition, *P. canaliculata* can serve as an intermediate host for the intestinal trematode *Echinostoma ilocanum* (Garrison) (Echinostomatidae).[24–26]

Gyraulus convexiusculus (Hutton) (Planorbidae) has a wide geographical distribution from Iraq through eastern Iran, Pakistan, Afghanistan, northern India, to northern Myanmar, China, Korea, Southeast Asia, the Malay Archipelago, and Indonesia.[27–34] This snail is an intermediate host for several trematode parasites[35,36] including *Echinostoma revolutum* (Froelich) Looss (Echinostomatidae), *Australapatemon burti* (Miller) (Strigeidae),[37] *Artyfechinostomum malayanum* (Leiper) (Echinostomatidae)[38] (intestinal flukes), *Sanguinicola armata* (Plehn) (Sanguinicolidae) (blood fluke),[39] and *Cercaria* spp. (Strigeatoidea).[40]

Tarebia granifera (Lamarck) (Thiaridae), the quilted melania, is native to Southeast Asia from China, east to the Philippines, and south through the Indonesian Archipelago,[41] but has become an invasive species in other tropical locations, including Africa[42,43] and the Neotropics.[44,45] This snail species is a host of several parasitic trematodes including *Paragonimus westermani* (Kerbert) (Paragonomidae), the oriental lung fluke, as well as the intestinal flukes *Haplorchis taichui* (Nishigori) (Heterophyidae),[46] *Centrocestus formosanus* (Nishigori) (Heterophyidae), and *Haplorchis pumilio* (Looss) (Heterophyidae).[47]

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) (Culicidae) are recognized vectors of yellow fever virus, dengue virus, chikungunya virus, and Zika virus,[48,49] as well as Mayaro virus,[50,51] Phasi Charoen-like virus,[52] and many other pathogenic viruses.[49,53] *Culex quinquefasciatus* (Say) (Culicidae) is the vector of the parasitic nematode *Wuchereria bancrofti* (Cobbold) Seurat (Onchocercidae), the causative agent of lymphatic filariasis. This mosquito is also known to transmit arboviruses such as St. Louis encephalitis virus, Western equine encephalitis virus, Zika virus and West Nile virus,[54] and has been recognized as a competent vector for Japanese encephalitis virus[55] and Usutu virus.[56]

Exacerbating the problems of arboviral diseases are the accelerating invasion potentials of the disease vectors, which can be attributed to climate change[57,58] and increasing global transportation.[53] Dengue fever, Japanese encephalitis, chikungunya, and Zika virus fever are already widespread in Vietnam.[59,60]

Pest control has historically relied on synthetic insecticide use, but there are increasing concerns regarding environmental contamination, detrimental effects to non-target organisms, and impacts on human health.[61] Problems associated with synthetic pesticide use has raised awareness to the potential of plant-based pesticidal agents in general[62] and essential oils in particular for control of disease vectors.[63,64] In this work we have examined the essential oils of *Lantana camara* collected from several locations in north-central Vietnam, their chemical compositions, and their potential as control agents for *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* larvae, as well as molluscicides against *P. canaliculata*, *G. convexiusculus*, and *T. granifera*.

Results and Discussion

Chemical Composition of the Essential Oils

Hydrodistillation of *L. camara* fresh leaves collected at four different locations obtained variable yield values of 0.043–0.059% (v/w) (*Table 1*). These values are consistent with previously published results from many parts of the world and likely depend on differences in collection time, geographical and environmental differences.[65,66]

The *L. camara* essential oil compositions are listed in *Table 2*. The main components of *L. camara* essential oil from Da Nang were β-caryophyllene (20.1%), sabinene (16.5%), with lesser concentrations of 1,8-cineole (7.1%), α-humulene (6.9%), and bicyclogermacrene (5.6%). The main components of the essential oil from Hai Van were sabinene (17.7%), β-caryophyllene (16.2%), 1,8-cineole (10.6%), with lesser concentrations of α-humulene (6.0%), (*E*)-nerolidol (4.7%), bicyclogermacrene (4.3%), and α-pinene (4.3%). The essential oil composition from Nghe An and Bà Nà were dominated by sesquiterpene hydrocarbons (*Table 2*). The main chemical compositions of essential oil from Nghe An were germacrene D (24.7%), β-caryophyllene (11.1%), *trans*-β-elemene (7.6%), with a lower concentration of α-copaene (4.1%). The main chemical compositions of essential oil from Ba Na were germacrene D (19.0%), β-caryophyllene (14.4%), *trans*-β-elemene (7.9%), with lesser concentrations of α-copaene (5.0%), bicyclogermacrene (4.8%), germacrene B (4.5%), and δ-cadinene (4.1%).

Lantana camara has several subspecies and numerous ornamental cultivars[71,72] and as such, the volatile phytochemistry has shown wide variation.[65] In order to place the volatile phytochemistry of the specimens from Vietnam into perspective, a hierarchical cluster analysis (HCA) was carried out comparing the major (37) essential oil components of 60 essential oil compositions reported in the literature[65,66,73–117] along with the four specimens from Vietnam (*Figure 1*). The cluster analysis reveals five clusters and one outlier. Except for the outlier, a sample from Peru, which was dominated by carvone, β-caryophyllene is a major component of *L. camara* essential oils. Cluster 1 can best be described as a group with lower levels of β-caryophyllene, but high levels of other sesquiterpenoids (e.g., *ar*-curcumene, (*E*)-nerolidol, spathulenol, or caryophyllene oxide). Cluster 2 is a sabinene/β-caryophyllene/1,8-cineole group; cluster 3 is a davanone-rich cluster; cluster 4 is a β-caryophyllene/α-humulene group, and cluster 5 is a germacrene D/β-caryophyllene/bicyclogermacrene cluster. Cluster 5 may be subdivided into a group with bicyclogermacrene > germacrene D, and a group with germacrene D ≫ bicyclogermacrene. Vietnam samples from Da Nang and Hai Van fall into cluster 2 (sabinene/β-caryophyllene/1,8-cineole), with essential oil compositions very similar to samples from Yemen,[65] Iran,[102] and from Benin.[94] In contrast, the Vietnamese samples from Nghe An and Bà Nà are in cluster 5b (germacrene D/β-caryophyllene).

Very recently, Pereira and co-workers have carried out an extensive investigation of 105 essential oil samples of native *L. camara* from Sergipe State, Brazil.[118] β-Caryophyllene dominated the essential oils. A cluster analysis carried out by Pereira et al. showed seven clusters, with clusters largely defined by different concentrations of the sesquiterpenes α-humulene, germacrene D, bicyclogermacrene, and *ar*-curcumene. The Vietnamese Nghe An and Bà Nà samples are chemically similar to the Brazilian samples forming cluster 4 of Pereira et al.[118] Notably, these workers did not observe any davanone-rich, sabinene-rich, or carvone-rich essential oils.

Table 1. Plant collection and hydrodistillation details of *Lantana camara* from Vietnam.

Collection site	Voucher Number	Collection month/year	Yield (%, v/w)
Da Nang City 16°02′03″N; 108°03′44″E	DND 30	June 4, 2019	0.059
Nghe An province 15°50′11″N; 108°11′28″E	DND 34	July 12, 2019	0.051
Hai Van Pass 16°09′12″N; 108°08′01″E	DND 38	June 9, 2019	0.048
Bà Nà Hill 19°20′10″N; 105°25′56″E	DND 48	June 6, 2019	0.043

Table 2. Chemical compositions of the leaf essential oils of *Lantana camara*.[a]

RI_{calc}	RI_{db}	Compound	DND30 Da Nang	DND34 Nghe An	DND38 Hai Van	DND48 Bà Nà
872	872	2-Methylbutyl acetate	tr	–	tr	–
922	923	Tricyclene	tr	tr	tr	tr
925	927	α-Thujene	0.5	0.1	0.7	tr
932	933	α-Pinene	3.7	0.6	4.3	0.5
947	948	α-Fenchene	tr	–	tr	–
949	953	Camphene	1.4	0.2	1.8	0.1
952	953	Thuja-2,4(10)-diene	tr	tr	tr	–
972	972	Sabinene	16.5	0.6	17.7	0.5
978	978	β-Pinene	2.9	0.4	3.3	0.3
982	986	6-Methylhept-5-en-2-one	–	–	tr	–
982	986	3-Octanone	–	–	tr	–
989	991	Myrcene	1.5	0.2	1.9	0.1
997	999	3-Octanol	tr	–	0.1	–
1007	1007	α-Phellandrene	0.2	0.1	0.4	0.1
1009	1009	δ-3-Carene	2.3	tr	2.6	tr
1015	1015	2-Methylbutyl isobutyrate	tr	tr	tr	–
1017	1018	α-Terpinene	0.1	0.1	0.3	tr
1017	1022	*m*-Cymene	–	–	tr	–
1025	1025	*p*-Cymene	0.9	1.1	0.9	1.0
1029	1030	Limonene	1.7	0.9	2.3	0.8
1031	1031	β-Phellandrene	0.2	0.1	–	tr
1032	1032	1,8-Cineole	7.1	0.4	10.6	0.4
1035	1034	(*Z*)-β-Ocimene	0.8	0.2	1.0	0.1
1044	1045	(*E*)-β-Ocimene	0.8	0.5	1.3	0.2
1058	1058	γ-Terpinene	0.3	0.9	0.6	0.2
1070	1069	*cis*-Sabinene hydrate	0.1	–	0.1	–
1081	1082	*p*-Mentha-2,4(8)-diene	tr	–	tr	–
1085	1086	Terpinolene	0.2	0.1	0.4	0.1
1100	1101	Linalool	0.4	0.2	0.3	0.2
1102	1101	*trans*-Sabinene hydrate	tr	–	tr	–
1103	1103	2-Methylbutyl 2-methylbutyrate	0.2	0.1	0.2	tr
1103	1104	Nonanal	–	tr	–	–
1111	1113	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	–	0.1	tr	tr
1124	1124	*cis*-*p*-Menth-2-en-1-ol	tr	–	tr	–
1128	1127	*allo*-Ocimene	tr	–	tr	–
1140	1140	Geijerene	–	–	–	tr
1146	1146	Verbenol	tr	–	–	–
1148	1149	Camphor	0.6	tr	0.9	0.1
1168	1367	Cyclosativene	0.1	0.1	–	0.2
1169	1170	δ-Terpineol	tr	–	0.1	–
1173	1173	Borneol	0.2	tr	0.3	tr
1179	1180	Terpinen-4-ol	0.7	tr	1.0	tr
1188	1189	*p*-Cymen-8-ol	tr	tr	tr	tr
1189	1192	Methyl salicylate	–	0.2	tr	0.2
1196	1195	α-Terpineol	0.2	0.1	0.4	tr
1209	1207	(3*E*)-Octenyl acetate	0.1	–	–	–
1323	1328	Silphiperfol-5-ene	–	tr	–	–
1331	1330	Bicycloelemene	0.5	0.4	0.6	0.5
1335	1335	δ-Elemene	0.1	1.0	0.1	1.0
1342	1349	7-*epi*-Silphiperfol-5-ene	–	0.2	–	tr
1347	1349	α-Cubebene	tr	0.2	tr	0.2
1364	1372	2,2-Dimethyl-1-decanol	–	tr	–	0.1
1375	1375	α-Copaene	0.8	4.1	0.6	5.0
1382	1383	*cis*-β-Elemene	0.1	0.5	0.1	0.4
1382	1382	β-Bourbonene	tr	0.5	tr	0.6
1387	1387	β-Cubebene	0.5	0.6	0.4	0.6

Table 2. (cont.)

RI$_{\text{calc}}$	RI$_{\text{db}}$	Compound	DND30 Da Nang	DND34 Nghe An	DND38 Hai Van	DND48 Bà Nà
1390	1390	*trans*-β-Elemene	2.0	7.6	1.3	7.9
1400	1405	Sesquithujene	–	–	0.1	–
1402	1405	(*Z*)-Caryophyllene	–	–	tr	–
1406	1406	α-Gurjunene	tr	0.2	tr	0.2
1414	1414	α-Cedrene	–	–	0.1	–
1421	1424	(*E*)-Caryophyllene	20.1	11.1	16.2	14.4
1424	1428	β-Duprezianene	–	–	0.1	–
1429	1432	γ-Elemene	–	3.2	–	3.3
1432	1432	*trans*-α-Bergamotene	tr	0.4	tr	0.4
1432	1433	β-Copaene	1.1	–	0.8	–
1435	1438	α-Guaiene	–	1.7	–	2.8
1438	1438	Aromadendrene	tr	tr	tr	–
1438	1439	(*Z*)-β-Farnesene	–	–	tr	–
1439	1440	6,9-Guiadiene	–	tr	–	–
1443	1447	*iso*-Germacrene D	–	0.2	–	0.2
1445	1447	Geranyl acetone	–	0.1	–	–
1447	1448	*cis*-Muurola-3,5-diene	–	0.1	–	–
1449	1451	*trans*-Muurola-3,5-diene	–	0.2	–	–
1452	1452	(*E*)-β-Farnesene	–	2.1	0.3	1.2
1455	1454	α-Humulene	6.9	2.3	6.0	3.2
1457	1457	*allo*-Aromadendrene	0.3	1.8	0.2	1.9
1474	1478	γ-Muurolene	0.4	0.5	0.2	0.7
1475	1482	γ-Curcumene	–	–	0.4	–
1482	1480	Germacrene D	2.3	24.7	2.2	19.0
1483	1476	Selina-4,11-diene	–	0.4	–	0.4
1488	1487	β-Selinene	0.1	0.7	0.1	0.4
1490	1490	γ-Amorphene	–	0.3	0.1	0.4
1492	1496	α-Zingiberene	–	–	0.8	–
1496	1497	Bicyclogermacrene	5.6	3.7	4.3	4.8
1497	1497	α-Muurolene	0.5	0.8	0.3	1.0
1501	1505	α-Bulnesene	–	0.8	–	1.3
1502	1502	2-Davana ether	0.1	–	–	–
1502	1504	(*E*,*E*)-α-Farnesene	–	0.5	–	0.5
1506	1508	β-Bisabolene	–	0.9	0.1	1.0
1507	1511	β-Curcumene	–	–	0.2	–
1507	1511	Germacrene A	0.1	–	–	–
1513	1512	γ-Cadinene	0.1	0.3	tr	0.4
1515	1519	Cubebol	0.4	0.3	0.2	0.3
1518	1518	δ-Cadinene	0.3	3.8	0.3	4.1
1520	1527	*trans*-Calamenene	–	–	–	0.1
1522	1521	4-Davana ether	0.1	–	–	–
1535	1538	α-Cadinene	–	–	–	0.2
1539	1540	(*E*)-α-Bisabolene	–	0.4	–	0.4
1546	1546	α-Elemol	0.1	0.2	tr	0.2
1550	1556	Davanone C	0.6	–	0.4	–
1557	1557	Davanone B	0.6	–	0.3	–
1558	1557	Germacrene B	0.4	3.4	0.2	4.5
1561	1562	(*E*)-Nerolidol	2.5	–	4.7	–
1563	1562	Davanone D	0.3	–	–	–
1571	1568	Dendrolasin	–	0.6	–	0.2
1578	1576	Spathulenol	2.4	1.5	1.6	1.9
1583	1587	Caryophyllene oxide	1.5	0.6	1.0	1.1
1587	1594	Viridiflorol	–	0.2	–	–
1594	1593	Guaiol	0.1	0.3	–	0.1
1595	1592	Globulol	0.1	–	–	–
1599	1601	Humulene epoxide I	0.1	–	–	–

Table 2. (cont.)

RI_{calc}	RI_{db}	Compound	DND30 Da Nang	DND34 Nghe An	DND38 Hai Van	DND48 Bà Nà
1603	1600	α-Oplopenone	0.1	–	–	–
1611	1613	Humulene epoxide II	0.3	–	0.3	0.3
1613	1614	1,10-di-*epi*-Cubenol	–	–	–	0.1
1629	1629	*iso*-Spathulenol	0.1	0.8	0.1	0.8
1630	1630	Caryophylla-4(12),8(13)-dien-5α-ol	–	–	–	0.3
1633	1635	(Z,Z)-Geranyl linalool	2.0	–	1.3	–
1637	1644	*allo*-Aromadendrene epoxide	–	0.5	0.1	0.2
1640	1639	*cis*-Guaia-3,9-dien-11-ol	0.1	–	–	–
1640	1640	τ-Cadinol	0.2	0.2	0.1	0.3
1642	1645	τ-Muurolol	–	0.4	–	0.4
1644	1651	α-Muurolol (=δ-Cadinol)	–	0.3	–	0.3
1654	1655	α-Cadinol	–	1.3	0.1	1.1
1655	1655	α-Eudesmol	0.4	–	–	–
1656	1660	Selin-11-en-4α-ol	–	0.2	–	0.1
1662	1662	9-Methoxycalamenene	–	–	–	0.2
1681	1683	Germacra-4(15),5,10(14)-trien-1α-ol	–	0.2	–	–
1692	1695	Shyobunol	–	1.0	–	0.6
1705	1705	14-Hydroxy-4,5-dihydrocaryophyllene	–	0.2	–	–
1712	1715	Pentadecanal	–	0.2	–	–
1718	1719	1-Phenylhepta-1,3,5-triyne	–	0.3	–	–
1806	1807	Eudesm-11-en-4α,6α-diol	–	0.3	–	–
1859	1860	Platambin	–	0.2	–	0.6
2018	2018	(*E*,*E*)-Geranyl linalool	–	0.1	–	0.1
2105	2109	(*E*)-Phytol	–	2.2	–	0.9
		Monoterpene hydrocarbons	34.0	6.0	39.1	4.0
		Oxygenated monoterpenoids	9.4	0.7	13.6	0.7
		Sesquiterpene hydrocarbons	42.4	79.3	35.9	83.1
		Oxygenated sesquiterpenoids	10.1	9.3	8.8	9.2
		Diterpenoids	2.0	2.3	1.3	1.0
		Others	0.3	0.9	0.2	0.3
		Total identified	98.1	98.6	98.9	98.3

[a] $RI_{calc.}$ = Retention indices determined with reference to a homologous series of *n*-alkanes on a ZB-5 ms column. RI_{db} = Retention indices from the databases.[67–70] tr = trace (< 0.05 %). – = not detected.

Molluscicidal Activity

The *L. camara* essential oils were screened for molluscicidal activity against *Pomacea canaliculata*, *Gyraulus convexiusculus*, and *Tarebia granifera* (*Table 3*). The *L. camara* essential oils showed molluscicidal activities comparable to tea saponin, which was used as the positive control.[119]

Pereira and co-authors have reviewed the molluscicidal activities of several essential oils against schistosomiasis transmitting snails (*Biomphalaria* spp. and *Bulinus* spp.).[64] Most of the essential oils in the review were monoterpenoid-rich essential oils, however. There is a paucity of information in the literature regarding the molluscicidal effects of essential oils on *Pomacea canaliculata*, *Gyraulus convexiusculus*, or *Tarebia granifera*. There have been reports published on effects of plant extracts and individual phytochemicals on *P. canaliculata*, however.[120] The fatty-acid rich petroleum ether extract of *Chimonanthus nitens* flowers showed a 48-h LC_{50} of 160 μg/mL.[121] A butanol extract of the unripe fruit of *Ilex paraguariensis* was active against *P. canaliculata* with 24-h LC_{50} of 24.75 μg/mL.[122] The methanol leaf extract of *Ipomoea batatas* had a 48-h LC_{50} of 1000 μg/mL.[123] The cardiac glycosides fraction from *Nerium indicum* leaves had a 48-h LC_{50} of 20.12 μg/mL.[124] The methanol extract of *Ambrosia artemisiifolia* showed a 24-h LC_{50} of 194 μg/mL, with the sesquiterpene lactones psilostachyin (LC_{50} = 15.9 μg/mL) and psilostachyhin B (LC_{50} = 27.0 μg/mL) as the active constituents.[125] The methanol extract of leaves and twigs of *Aglaia duperreana*

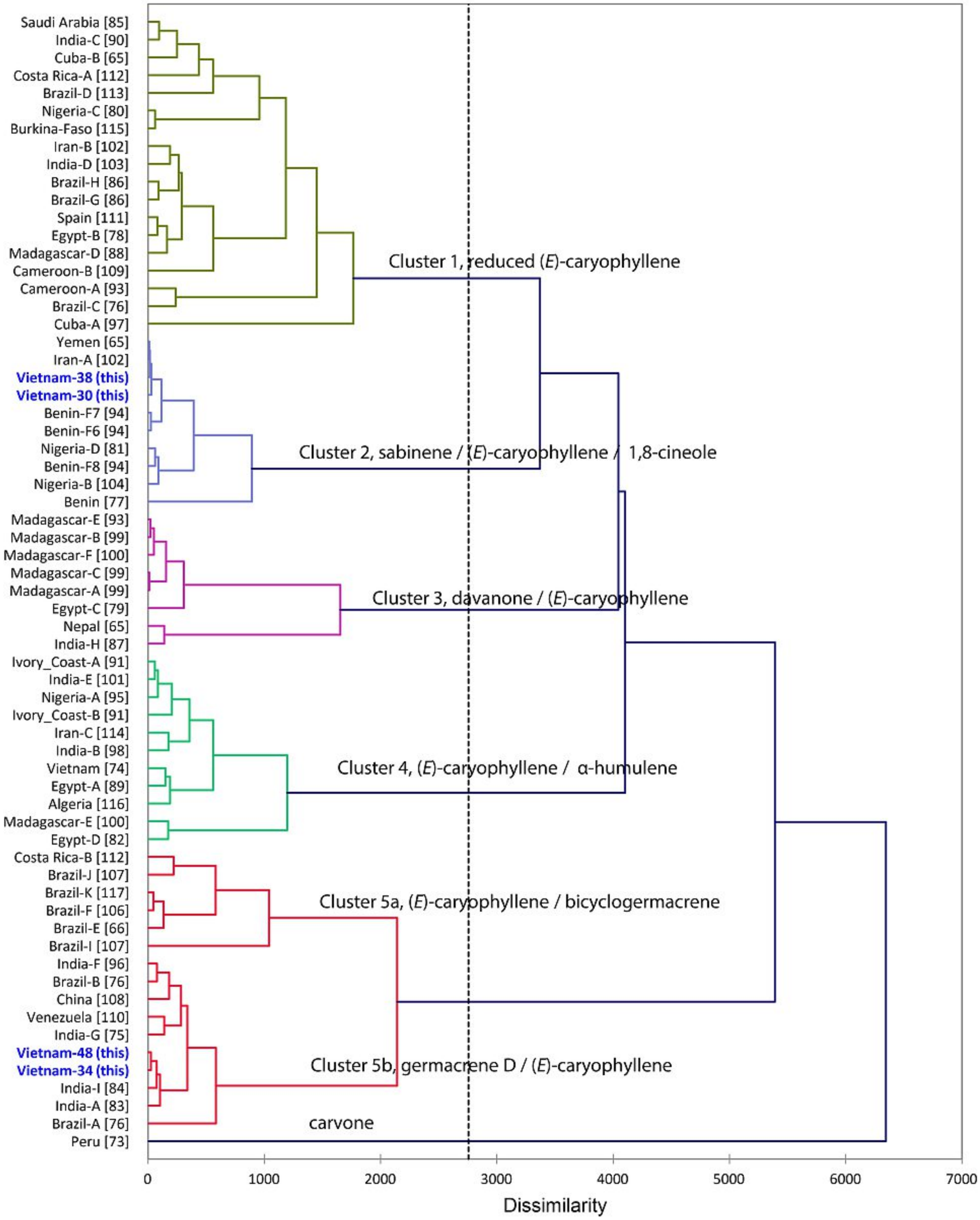

Figure 1. Dendrogram obtained from the agglomerative hierarchical cluster analysis of *Lantana camara* leaf essential oil compositions.

Table 3. Molluscicidal activities of *Lantana camara* leaf essential oils from Vietnam.[a]

Sample	LC$_{50}$ (μg/mL)	LC$_{90}$ (μg/mL)	χ^2	p
	Pomacea canaliculata			
DND30 (Da Nang City)	36.06 (33.13–39.33)	43.98 (40.58–48.40)	16.96	0.001
DND34 (Nghe An province)	37.82 (34.33–41.92)	44.21 (40.31–49.61)	5.971	0.113
DND38 (Hai Van Pass)	40.23 (37.46–43.13)	56.53 (52.70–61.63)	12.80	0.005
DND48 (Bà Nà Hill)	23.63 (22.16–25.33)	31.37 (29.03–35.03)	0.2252	0.973
Positive control (tea saponin)	24.78 (23.26–26.72)	32.62 (29.98–37.10)	0.1301	0.988
β-Caryophyllene	16.67 (15.48–18.08)	21.75 (20.06–24.14)	2.258	0.521
α-Humulene	18.99 (17.74–20.27)	24.93 (23.40–26.93)	0.2817	0.963
Caryophyllene oxide	17.77 (16.56–19.07)	22.92 (21.39–24.96)	0.1732	0.982
	Gyraulus convexiusculus			
DND30 (Da Nang City)	7.912 (6.927–8.991	22.29 (18.70–27.91)	15.51	0.004
DND34 (Nghe An province)	29.55 (27.16–32.29)	44.41 (40.65–49.51)	4.020	0.259
DND38 (Hai Van Pass)	9.579 (8.020–11.233)	22.16 (19.53–26.04)	1.866	0.760
DND48 (Bà Nà Hill)	13.25 (12.03–14.78)	25.59 (22.24–31.08)	14.14	0.003
Positive control (tea saponin)	37.28 (33.55–41.73)	65.86 (58.87–75.82)	7.223	0.065
β-Caryophyllene	26.62 (22.85–31.03)	103.8 (81.0–145.5)	16.29	0.001
α-Humulene	18.94 (16.81–21.27)	46.64 (39.71–57.41)	8.140	0.043
Caryophyllene oxide	13.68 (12.64–14.98)	19.10 (17.35–21.89)	3.602	0.308
	Tarebia granifera			
DND30 (Da Nang City)	29.62 (26.62–33.02)	53.00 (47.64–60.53)	17.72	0.001
DND34 (Nghe An province)	23.80 (19.88–27.80)	57.44 (50.22–68.33)	12.26	0.007
DND38 (Hai Van Pass)	15.00 (13.23–16.95)	41.41 (34.91–51.30)	9.265	0.055
DND48 (Bà Nà Hill)	25.48 (22.86–28.38)	56.05 (48.29–67.96)	4.169	0.244
Positive control (tea saponin)	17.16 (15.74–18.69)	26.14 (23.98–29.15)	3.156	0.368
β-Caryophyllene	26.47 (23.96–29.31)	44.93 (40.69–50.75)	9.035	0.029
α-Humulene	18.60 (16.24–21.21)	39.567 (35.17–45.71)	7.263	0.123
Caryophyllene oxide	37.24 (34.63–40.09)	52.43 (48.70–57.38)	0.9116	0.823

[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.

showed molluscicidal activity against *P. canaliculata* with a 72-h LC$_{50}$ of 33.4 μg/mL.[126] The active compounds isolated were naringenin trimethyl ether, 4′,5,7-trimethoxydihydroflavonol, and eudesmin (72-h LC$_{50}$ = 3.9, 10.6, and 15.8 μg/mL, respectively). Three triterpenoids from the methanol bark extract of *Eucalyptus exserta*, 2α-hydroxyurs-12-en-28-oic acid-3β-*trans*-isoferulate, 2α,3α,24-trihydroxyolean-18-en-28-oic acid, and 2α-hydroxyursolic acid showed activity against *P. canaliculata* with 72-h LC$_{50}$ values of 35.5, 42.6, and 27.3 μg/mL, respectively.[127] Thus, *L. camara* essential oils show molluscicidal activities comparable to other botanical agents.

Mosquito Larvicidal Activity

The four *L. camara* essential oils from Vietnam were screened for mosquito larvicidal activity against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*. The 24-h and 48-h larvicidal results are summarized in *Table 4*.

Several essential oils rich in sesquiterpene hydrocarbons have shown remarkable mosquito larvicidal activities.[63,128] For example, *Hymenaea courbaril* fruit peel essential oil (31.9 % germacrene D and 27.1 % β-caryophyllene) has shown excellent larvicidal activity against *Ae. aegypti* with a LC$_{50}$ of 28.4 μg/mL.[129] However, the larvicidal activities of β-caryophyllene and α-humulene are relatively weak compared to the *L. camara* essential oils, suggesting that these components are not individually responsible for the observed activities. In addition, there does not seem to be a correlation between larvicidal activities against the mosquito species and sesquiterpene hydrocarbon concentrations in the *Lantana* essential oils. The Nghe An and Bà Nà samples had much higher sesquiterpene hydrocarbon and germacrene D concentrations than the Da Nang and Hai Van samples. Synergistic and/or

Table 4. Larvicidal activities of *Lantana camara* leaf essential oils from Vietnam.[a]

Sample	24 h LC_{50} (μg/mL)	LC_{90} (μg/mL)	χ^2	*p*
	Aedes aegypti			
DND30 (Da Nang City)	18.87 (16.76–21.25)	34.68 (30.81–40.50)	10.08	0.018
DND34 (Nghe An province)	28.96 (25.05–33.27)	62.93 (55.32–74.18)	13.88	0.003
DND38 (Hai Van Pass)	23.98 (22.41–25.89)	33.60 (29.74–40.24)	0.002204	0.999
DND48 (Bà Nà Hill)	15.07 (13.13–17.08)	29.27 (25.93–34.41)	7.748	0.052
Positive control (permethrin)	0.00638 (0.00548–0.00744)	0.0232 (0.0182–0.0318)	8.868	0.031
β-Caryophyllene	61.15 (55.75–66.95)	120.4 (103.9–145.2)	6.939	0.139
α-Humulene	44.43 (40.03–49.48)	91.85 (79.01–112.10)	6.070	0.194
Caryophyllene oxide	127.9 (120.1–136.2)	188.4 (176.5–203.6)	22.67	0.000
	Aedes albopictus			
DND30 (Da Nang City)	53.75 (49.27–59.11)	81.83 (74.44–91.94)	4.858	0.183
DND34 (Nghe An province)	48.35 (44.46–52.64	77.58 (69.30–90.64)	1.296	0.730
DND38 (Hai Van Pass)	34.83 (32.10–37.93)	52.91 (48.22–59.29)	3.401	0.334
DND48 (Bà Nà Hill)	26.36 (24.04–28.88)	46.35 (41.11–54.38)	2.919	0.404
Positive control (permethrin)	0.00237 (0.00211–0.00265)	0.00424 (0.00379–0.00492)	4.644	0.031
β-Caryophyllene	56.87 (51.05–63.88)	120.8 (101.8–153.4)	2.514	0.473
α-Humulene	43.86 (39.94–48.15)	78.59 (69.38–92.77)	1.273	0.866
Caryophyllene oxide	20.61 (19.28–22.00)	27.56 (25.79–29.99)	0.3637	0.948
	Culex quinquefasciatus			
DND30 (Da Nang City)	20.77 (18.09–21.73)	64.43 (53.18–82.53)	6.299	0.098
DND34 (Nghe An province)	59.32 (54.28–65.39)	88.70 (80.34–100.88)	14.17	0.001
DND38 (Hai Van Pass)	48.18 (43.68–53.54)	79.08 (71.11–90.52)	5.251	0.072
DND48 (Bà Nà Hill)	55.34 (51.12–60.97)	76.67 (69.30–87.90)	1.585	0.453
Positive control (permethrin)	0.0165 (0.0149–0.0181)	0.0305 (0.0267–0.0367)	5.235	0.073
β-Caryophyllene	161.1 (152.5–170.6)	234.0 (214.0–267.6)	4.434	0.350
α-Humulene	108.3 (101.4–115.5)	158.2 (148.5–170.50	0.9970	0.910
Caryophyllene oxide	98.52 (90.70–108.68)	144.5 (129.6–165.7)	1.601	0.809
	48 h			
	Aedes aegypti			
DND30 (Da Nang City)	14.20 (12.36–16.05)	27.24 (24.25–31.81)	11.23	0.011
DND34 (Nghe An province)	12.52 (10.93–14.21)	3 (36.67–49.89)	14.17	0.001
DND38 (Hai Van Pass)	18.64 (16.07–21.04)	32.24 (29.56–36.87)	0.5030	0.778
DND48 (Bà Nà Hill)	12.76 (10.77–14.64)	26.44 (23.38–31.20)	8.152	0.043
β-Caryophyllene	53.08 (47.72–59.03)	120.4 (103.9–145.2)	16.74	0.002
α-Humulene	37.89 (33.97–42.38)	83.95 (71.70–103.13)	7.840	0.098
Caryophyllene oxide	110.7 (103.4–118.6)	169.6 (158.4–183.6)	14.82	0.005
	Aedes albopictus			
DND30 (Da Nang City)	51.27 (46.87–56.56)	80.20 (72.75–90.42)	9.473	0.024
DND34 (Nghe An province)	46.16 (42.47–50.19)	73.39 (65.72–85.46)	0.1810	0.981
DND38 (Hai Van Pass)	33.30 (30.46–36.56)	52.64 (48.35–58.39)	12.81	0.005
DND48 (Bà Nà Hill)	24.93 (22.72–27.32)	44.12 (39.07–51.84)	1.041	0.791
β-Caryophyllene	49.48 (44.77–54.86)	96.93 (83.80–118.03)	1.973	0.578
α-Humulene	38.72 (35.52–42.19)	63.40 (56.66–73.80)	0.6204	0.961
Caryophyllene oxide	18.29 (17.06–19.58)	24.18 (22.62–26.23)	0.3756	0.945
	Culex quinquefasciatus			
DND30 (Da Nang City)	12.52 (10.93–14.21)	32.46 (27.14–41.45	15.70	0.001
DND34 (Nghe An province)	38.46 (35.09–42.38)	61.03 (55.16–69.66)	0.2152	0.898
DND38 (Hai Van Pass)	43.92 (39.65–48.98)	73.97 (66.23–85.34)	3.148	0.207
DND48 (Bà Nà Hill)	50.98 (46.69–56.28)	76.42 (69.17–87.42)	1.189	0.552

Table 4. (cont.)

Sample	24 h LC_{50} (μg/mL)	LC_{90} (μg/mL)	χ^2	*p*
β-Caryophyllene	139.1 (130.0–149.0)	213.1 (197.8–233.5)	11.87	0.018
α-Humulene	87.81 (81.14–94.89)	140.0 (130.0–152.7)	9.798	0.044
Caryophyllene oxide	95.19 (86.68–106.26)	141.0 (127.6–160.8)	4.006	0.405

[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 24 h and 48 h of treatment.

Table 5. Insecticidal activity of *Lantana camara* leaf essential oils against *Diplonychus rusticus.*[a]

Sample	LC_{50} 24 h	LC_{90}	χ^2	*p*
DND30 (Da Nang City)	103.7 (98.7–109.2)	136.2 (128.3–147.1)	6.599	0.086
DND34 (Nghe An province)	162.5 (156.3–168.5)	190.1 (183.2–198.6)	27.82	0.000
DND38 (Hai Van Pass)	106.9 (101.7–112.5)	143.3 (135.3–153.9)	13.29	0.004
DND48 (Bà Nà Hill)	106.7 (101.2–112.6)	147.0 (138.4–158.2)	12.23	0.007
	48 h			
DND30 (Da Nang City)	102.9 (98.0–108.4)	135.8 (127.9–146.8)	6.917	0.075
DND34 (Nghe An province)	106.4 (102.0–112.0)	128.4 (121.0–140.2)	1.427	0.699
DND38 (Hai Van Pass)	96.52 (92.34–101.23)	122.7 (116.0–132.3)	12.32	0.006
DND48 (Bà Nà Hill)	83.61 (79.62–87.66)	114.1 (106.9–124.5)	4.956	0.175

[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 24 h and 48 h of treatment.

antagonistic effects between sesquiterpenes and other components likely plays an important role in the larvicidal effects.

Non-Target Species

In order to provide some information regarding the environmental impact of *L. camara* essential oils on non-target species, they were tested for insecticidal activity against the water bug, *Diplonychus rusticus* Fabricius (Belostomatidae) (*Table 5*). The toxicity of *L. camara* essential oils to snails and to mosquito larvae were overall higher than they were to the water bug, suggesting some degree of selectivity toward the target species.

Acetylcholinesterase (AChE) Inhibition

Acetylcholinesterase (AChE) is a known target for insecticides. In order to determine if AChE inhibition contributes to the larvicidal activity, the *L. camara* essential oils were screened for activity against electric eel (*Electrophorus electricus*) AChE (*Table 6*). The *Lantana* essential oils showed relatively weak AChE inhibitory activities, much less than the sesquiterpenes β-caryophyllene, α-humulene, or caryophyllene oxide. Dos Santos and co-workers had examined the AChE inhibitory activity of *L. camara* from Brazil (also rich in β-caryophyllene and germacrene D).[130] Unfortunately, these workers did not determine the IC_{50} values. In comparison, sesquiterpene-rich essential oils of *Piper capitarianum* from Brazil showed much greater AChE inhibitory activities (IC_{50} 14.3–18.1 μg/mL).[131] Sesquiterpene-poor *Crithmum maritimum* essential oils

Table 6. Acetylcholinesterase inhibitory activities of *Lantana camara* essential oils from north-central Vietnam.[a]

Sample	IC_{50} (μg/mL)
DND30 (Da Nang City)	327.79 ± 10.16
DND34 (Nghe An province)	226.42 ± 8.42
DND38 (Hai Van Pass)	240.53 ± 21.51
DND48 (Bà Nà Hill)	240.23 ± 9.19
Positive control (galantamine)	1.78 ± 0.13
β-Caryophyllene	89.10 ± 6.10
α-Humulene	160.48 ± 13.48
Caryophyllene oxide	102.88 ± 7.84

[a] Data are presented as IC_{50} values ± standard deviations obtained graphically from four independent experiments carried out in triplicate.

showed very weak AChE inhibition (IC_{50} 3500–7400 μg/mL).[132] Thus, sesquiterpene components may be acting as AChE inhibitors contributing to the mosquito larvicidal activities of *Lantana* essential oils.

Conclusions

There are several points that can be concluded from this study. There is much variation in the essential oil compositions of *L. camara*, even from the same region in Vietnam. Although it is a troublesome invasive plant, *L. camara* is a renewable source of essential oil that shows promise as a vector control agent. The molluscicidal and larvicidal activities of *L. camara* essential oil can likely be attributed to sesquiterpene hydrocarbons such as (*E*)-caryophyllene and α-humulene. Acetylcholinesterase may be a protein target of the essential oils.

Experimental Section

Plant Material

Fresh leaves of *L. camara* were harvested from shrubs wild-growing in north-central Vietnam. Leaves from several individual plants were collected from each site and pooled. The plants were identified by Dr. Do Ngoc Dai, and voucher specimens (*Table 1*) have been deposited in the Pedagogical Institute of Science, Vinh University. Plant fresh materials (4.0 kg from each collection site) were shredded and hydrodistilled for 4 h using a Clevenger-type apparatus.

Gas Chromatographic-Mass Spectral (*GC/MS*) *Analysis*

Each of the essential oils of *L. camara* was analyzed by GC/MS as previously described:[133] Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD, USA), electron impact mode (electron energy = 70 eV, scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s), ZB-5 ms GC column (Phenomenex®, Torrance, CA, USA), GC oven temperature program was 50 °C initial temperature, increased 2 °C/min to 260 °C. Essential oils (0.1 μL of 5 % w/v in CH_2Cl_2) were injected split (30 : 1) mode. Retention indices were calculated by reference to a homologous series of *n*-alkanes. Compound identification was carried out by comparison of their mass spectral fragmentation patterns and retention indices with those recorded in the databases.[67–70]

Molluscicidal Assay

Wild *G. convexiusculus* were collected in Hoa Vang district, Da Nang city (16°00′25′′ N, 108°06′53′′ E). Snails that were 2.5–5.0 mm in diameter were used for the testing molluscicidal activity. Snails were identified by Dr. Nguyen Huy Hung.

Wild *T. granifera* were collected on Thu Bon river, Quang Nam province (15°50′00′′ N, 108°11′26′′ E) and were identified by Dr. Nguyen Huy Hung. The snails with lengths of 10–14 mm were used for molluscicidal activity screening.

Eggs of *P. canaliculata* were collected from a rice field at Hoa Vang District, Da Nang City (16°01′02.4′′ N, 108°06′34.8′′ E). Eggs were incubated in the laboratory under laboratory conditions with temperature 25 ± 2 °C and relative humidity 70 ± 5%. The newly hatched snails were reared in an incubator at 26 ± 2 °C with a 12 : 12 (light/dark) photoperiod and fed on fresh leaves of *Ipomoea aquatica* Forssk. for 7 days. Seven-day-old juvenile snails that had a shell length of between 1.0 and 3.0 mm were used for further experiments. Snails were identified by Dr. Nguyen Huy Hung.

Molluscicidal activity of essential oils and pure compounds were evaluated according to the protocol of Ding and co-workers[125] with slight modifications. For each assay, 150 mL of water containing 20 snails were placed into 250-mL beakers and aliquots of the essential oils dissolved in EtOH (1 % stock solution) were then added. A set of controls using EtOH only (negative control) and tea saponin (positive control) were included for comparison. Each test was conducted in quadruplicate with five concentrations (100, 50, 25, 12.5 and 6 μg/mL). After 24 h, the treated snails were placed in a separate container with 150 mL of distilled water to recover. Snails that did not recover after an additional 24 h in distilled water were scored as dead. During the experiment, the laboratory temperature was maintained at 26 ± 2 °C with a 12 : 12 h (light/dark) photoperiod.

Mosquito Larvicidal Assay

Aedes aegypti larvae were raised from eggs (Institute of Biotechnology, Vietnam Academy of Science and Technology) while *Aedes albopictus* and *Culex quinquefasciatus* adults were collected from the wild, and larvae reared as previously described.[134,135] Mosquitoes were identified by Dr. Nguyen Huy Hung. Larval developmental stages were maintained at 25 ± 2 °C, 65–75 % relative humidity and a 12:12 h light/dark cycle in the Laboratory of the Faculty of Environmental and Chemical Engineering of Duy Tan University, Da Nang, Vietnam.

Larvicidal activities of the essential oils and pure compounds against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were carried out as previously described:[133,134] Quadruplicate assays, 20 fourth-instar mosquito larvae, 25 ± 2 °C, six essential oil concentrations (200, 100, 50, 25, 12.5 and 6 μg/mL), permethrin positive control, mortality was recorded after 24 h and after 48 h of exposure.

Diplonychus rusticus Insecticidal Assay

Adults of *D. rusticus* (identified by Dr. Nguyen Huy Hung) were collected in the field and maintained as previously described.[136] The insects (20 *D. rusticus* adults) were screened against *L. camara* essential oils at concentrations of 200, 150, 100, 75, 50, and 25 μg/mL (four replicates each), and mortality recorded after 24 h and 48 h exposure.

Acetylcholinesterase (*AChE*) *Inhibition Assay*

The acetylcholinesterase enzyme inhibitory activity was performed according to the method described by Ellman et al.[137] The test samples (essential oils and pure compounds) were dissolved in 100 % dimethyl sulfoxide (DMSO) solvent, then, diluted to different concentrations with H_2O (deionized distilled water). Test wells containing a mixture of 140 μL of phosphate buffer solution (pH: 8), 20 μL of test samples at concentrations (500, 100, 20, and 4 μg/mL) and 20 μL of the enzyme AChE (0.25 IU/mL) then, were mixed and incubated at 25 °C for 15 min. Next, 10 μL dithiobisnitrobenzoic acid (DTNB, 2.5 mM) and 10 μL acetylthiocholine iodide (ACTI, 2.5 mM) were added to the test wells respectively and continued incubation for 10 min at 25 °C. The solution was then measured for absorbance at 405 nm (standard is 412 nm). Galantamine was used as a positive control. The negative control well did not contain the test sample. Each test was carried out in triplicate.

Data Analysis

Agglomerative hierarchical cluster (AHC) analysis was carried out using XLSTAT Premium, version 2018.1.1 (Addinsoft, Paris, France). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method. Lethality data were subjected to log-probit analysis[138] to obtain LC_{50} values, LC_{90} values and 95 % confidence limits using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA).

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Author Contribution Statement

Plant samples, plant identification and essential oil extraction were collected by Nguyen Huy Hung (N.H.H.), Do Ngoc Dai (D.N.D.), Le Thi Huong (L.T.H.), Thieu Anh Tai (T.A.T.), Dinh Quang Hung (D.Q.H.). Collection, identification, and rearing test snails were performed by Nguyen Huy Hung (N.H.H.), Bui Thi Chinh (B.T.C.), Dinh Quang Hung (D.Q.H.), Thieu Anh Tai (T.A.T.). Testing the biological activities were performed by Nguyen Huy Hung (N.H.H.), Dinh Quang Hung (D.Q.H.), Thieu Anh Tai (T.A.T.). GC/MS and data analysis were performed by Prabodh Satyal (P.S.) and William N. Setzer (W.N.S.). Preparing manuscript was performed by William N. Setzer (W.N.S.).

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