SHORT REPORT

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Investigation of Pesticidal Activities of Essential Oils Obtained from *Vitex* Species

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Abstract: Essential oils from renewable plant sources are important considerations for environmentally benign botanical pesticides. In this work, the leaf essential oils of four species of *Vitex* (Lamiaceae) have been collected from north-central Vietnam, analyzed by gas chromatographic techniques, and screened for mosquito larvicidal activity and molluscicidal activity. *Vitex ajugifolia* and *V. pinnata* essential oils were dominated by sesquiterpenoids (97.8% and 95.8%, respectively). In contrast, however, the essential oils of *V. trifolia* subsp. *litoralis* showed monoterpenoids to be dominant. The essential oils of *V. trifolia* subsp. *trifolia* also showed abundant monoterpenoids (38.4% and 68.0%), but also included (*E*)-β-caryophyllene (15.8 and 14.5%). *Vitex pinnata* essential oil showed excellent larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*. Both *V. trifolia litoralis* and *V. trifolia trifolia* demonstrated notable molluscicidal activities against *Gyraulus convexiusculus* and *Pomacea canaliculata*. The research results suggest that essential oils of *Vitex* species might have potential to be used as natural pesticides.

Keywords: Mosquito larvicidal; molluscicidal; chemical composition; botanical pesticide.

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Pesticidal activity of *Vitex* leaf essential oils

1. Plant Source

There are currently 223 recognized species of *Vitex* L., which are found throughout the tropics and subtropics of both hemispheres [1]. The genus was previously placed in the Verbenaceae, but is now placed in the Lamiaceae [2]. Several members of the genus are important ethnobotanically, including *Vitex agnus-castus* L., *Vitex negundo* L., *Vitex trifolia* subsp. *trifolia* L., and *Vitex trifolia* subsp. *litoralis* Steenis (syn. *Vitex rotundifolia* L.f.) [3–5].

In this work, the leaf essential oils of four taxa of *Vitex* (*Vitex ajugiflora* Dop, *Vitex pinnata* L., *Vitex trifolia* subsp. *litoralis* Steenis, and *Vitex trifolia* subsp. *trifolia* L.) growing wild in north-central Vietnam were obtained by hydrodistillation, analyzed by gas chromatographic techniques, and were screened for mosquito larvicidal and molluscicidal activities.

2. Previous Studies

Botanical pesticides have emerged as potential alternatives to synthetic pesticides for mosquito management [6] and control of snail parasite vectors [7]. The leaf essential oil composition of *V. quinata* from Vietnam has been reported [8]; leaf essential oil compositions of *V. trifolia* subsp. *litoralis* from South Korea [9], Taiwan [10], and south Vietnam [11] have been reported; and leaf essential oils of *V. trifolia* subsp. *trifolia* from Bangkok, Thailand [12], Imphal, Manipur, India [13], and Denpasar, Bali, Indonesia [14], have also been reported. As far as we are aware, this is the first report of the essential oil composition of *V. ajugifolia* and the first to examine the mosquito larvicidal, molluscicidal, and non-target insecticidal activities of these Vietnamese *Vitex* essential oils.

3. Present Study

Essential oils from hydrodistillation of fresh *Vitex* leaves were obtained in yields of around 0.1% (Table 1).

Table 1. Collection details of *Vitex* species from north-central Vietnam

***Vitex* Species**	**Collection Site**	**Voucher Numbers**	**Mass Plant Material (kg)**	**Essential Oil Yield (% *v/w*)**
V. ajugifolia	Son Tra Peninsula, Da Nang province 16°08′36″ N, 108°14′10″ E, 127 m elevation	DND8	4.0	0.09
V. pinnata	Chu Mom Ray National Park 14°25′33.5″ N, 107°43′15.6″ E, 672 m elevation	DND74	4.0	0.14
V. trifolia subsp. *litoralis*	Hoa Vang district – Da Nang City 16°04′44″ N, 108°14′48″ E, 4 m elevation	DND19	4.0	0.12
V. trifolia subsp. *litoralis*	Son Tra Peninsula, Da Nang province 16°06′04″ N, 108°17′00″ E, 4 m elevation	DND44	4.0	0.10
V. trifolia subsp. *trifolia*	Hoa Vang district – Da Nang City 16°02′57″ N, 108°09′34″ E, 7 m elevation	DND28	4.0	0.11
V. trifolia subsp. *trifolia*	Hoa Vang district – Da Nang City 16°02′57″ N, 108°09′34″ E, 7 m elevation	DND40	4.0	0.12

The *Vitex* leaf essential oils were analyzed by gas chromatography-mass spectrometry as described previously [15]. Identification of the essential oil components was carried out by comparison of their retention indices and mass spectral fragmentation patterns with those found in the databases. The major components of the essential oils are summarized in Table 2.

The *Vitex* leaf essential oils were screened for mosquito larvicidal activity against *Aedes aegypti* (L.) (Culicidae), *Aedes albopictus* (Skuse), *Culex quinquefasciatus* (Say) as previously described [16] (Table 3). The essential oils were also screened and for insecticidal activity against the non-target water bug, *Diplonychus rusticus* as previously reported [17]. Lethality data were subjected to log-probit analysis to obtain LC_{50} values, LC_{90} values and 95% confidence limits using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA). All four *Vitex* essential oils tested (*V. ajugifolia*, *V. pinnata*, *V. trifolia*

subsp. *litoralis*, and *V. trifolia* subsp. *trifolia*) demonstrated appreciable mosquito larvicidal activity against the three mosquito species with 48-h LC_{50} values less than 100 μg/mL [18].

Table 2. Major components of leaf essential oils of *Vitex* species collected in north-central Vietnam

			V. ajugifolia	***V. pinnata***	***V. trifolia* subsp. *litoralis***		***V. trifolia* subsp. *trifolia***	
RI_{calc}	**RI_{db}**	**Compound**	**DND8**	**DND74**	**DND19**	**DND44**	**DND28**	**DND40**
933	932	α-Pinene	0.2	0.5	18.7	15.2	3.1	11.7
973	972	Sabinene	–	–	15.2	12.2	10.6	19.4
977	978	β-Pinene	–	0.2	4.9	4.1	1.3	3.7
1033	1032	1,8-cineole	–	–	14.5	12.7	8.5	15.7
1346	1346	α-Terpinyl acetate	–	–	12.7	19.0	8.3	8.3
1375	1375	α-Copaene	17.0	0.5	t	0.1	t	0.1
1422	1424	(*E*)-β-Caryophyllene	11.7	32.7	0.3	0.5	15.8	14.5
1455	1454	α-Humulene	9.6	2.0	t	0.1	0.7	0.7
1480	1480	Germacrene D	0.7	17.1	0.2	0.7	0.4	0.6
1497	1497	Bicyclogermacrene	3.8	11.1	–	0.2	–	–
1577	1576	Spathulenol	8.7	2.0	0.2	0.3	–	–
1583	1587	Caryophyllene oxide	4.3	1.2	0.1	0.1	3.8	1.9

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

The leaf essential oil of *V. pinnata*, rich in the germacrane sesquiterpenes germacrene D and bicyclogermacrene, was especially toxic to *Ae. aegypti* and *Cx. quinquefasciatus* larvae with 24-h LC_{50} values of 7.11 and 9.19 μg/mL, respectively. Notably, *V. pinnata* essential oil was less toxic to *Ae. albopictus* larvae (24-h LC_{50} = 45.1 μg/mL). Germacrene D has shown notable larvicidal activity against both *Ae. aegypti* (LC_{50} = 18.8 μg/mL) and *Cx. quinquefasciatus* (LC_{50} = 21.3 μg/mL) [19]. Furthermore, essential oils rich in bicyclogermacrene have also shown good larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* [20,21].

Table 3. Mosquito larvicidal activity of *Vitex* leaf essential oils from north-central Vietnam[a]

Essential oil (voucher number)	**LC_{50} (μg/mL)**	**LC_{90} (μg/mL)**	**χ^2**	***p***
		Aedes aegypti, 24-hour		
Vitex ajugifolia (DND8)	30.63 (27.75-33.84)	59.21 (51.69-70.74)	5.173	0.160
Vitex pinnata (DND74)	7.110 (6.469-7.876)	11.68 (10.59-13.12)	4.509	0.720
Vitex trifolia subsp. *litoralis* (DND44)	23.82 (21.90-26.24)	35.22 (31.83-40.38)	4.278	0.233
Vitex trifolia subsp. *trifolia* (DND28)	24.90 (23.17-27.16)	35.04 (31.76-40.18)	0.5119	0.916
Positive control (permethrin)	0.0064 (0.0055-0.0074)	0.023 (0.018-0.032)	8.868	0.031
		Aedes aegypti, 48-hour		
Vitex ajugifolia (DND8)	28.13 (25.44-31.12)	55.83 (48.67-66.81)	1.313	0.726
Vitex pinnata (DND74)	4.530 (3.982-5.186)	9.586 (8.447-11.204)	17.59	0.014
Vitex trifolia subsp. *litoralis* (DND44)	21.46 (19.60-23.72)	33.69 (30.33-38.73)	10.96	0.012
Vitex trifolia subsp. *trifolia* (DND28)	24.71 (22.84-27.12)	34.05 (21.00-39.02)	1.627	0.653
		Aedes albopictus, 24-hour		
Vitex ajugifolia (DND8)	25.58 (22.83-28.68)	61.29 (52.21-75.27)	4.661	0.324
Vitex pinnata (DND74)	46.34 (43.41-49.73)	62.40 (57.59-69.46)	0.4340	0.935
Vitex trifolia subsp. *litoralis* (DND44)	29.63 (27.33-32.47)	41.55 (37.87-46.86)	8.045	0.045
Vitex trifolia subsp. *trifolia* (DND28)	37.92 (35.06-40.98)	52.18 (48.34-57.37)	5.341	0.148
Positive control (permethrin)	0.0024 (0.0021-0.0027)	0.0042 (0.0038-0.0042)	4.644	0.031

Pesticidal activity of *Vitex* leaf essential oils

Table 3 continued..

		Aedes albopictus, 48-hour		
Vitex ajugifolia (DND8)	18.45 (16.57-20.55)	40.02 (34.57-48.29)	1.427	0.840
Vitex pinnata (DND74)	45.55 (42.53-49.08)	62.17 (57.56-69.13)	0.9803	0.806
Vitex trifolia subsp. *litoralis* (DND44)	28.70 (26.46-31.52)	40.44 (36.76-45.86)	5.321	0.150
Vitex trifolia subsp. *trifolia* (DND28)	31.81 (29.01-35.06)	49.32 (44.73-55.78)	5.236	0.155
		Culex quinquefasciatus, 24-hour		
Vitex ajugifolia (DND8)	100.3 (93.0-109.9)	126.8 (108.0-159.5)	1.217	0.749
Vitex pinnata (DND74)	9.191 (8.055-10.463)	28.78 (24.05-35.92)	8.342	0.138
Vitex trifolia subsp. *litoralis* (DND44)	38.51 (35.17-42.14)	66.41 (58.99-77.76)	1.794	0.616
Vitex trifolia subsp. *trifolia* (DND28)	140.4 (130.2-151.7)	201.3 (186.5-220.6)	1.285	0.733
Positive control (permethrin)	0.017 (0.015-0.018)	0.031 (0.027-0.037)	5.235	0.073
		Culex quinquefasciatus, 48-hour		
Vitex ajugifolia (DND8)	36.65 (31.89-42.46)	122.1 (96.6-167.7)	7.483	0.058
Vitex pinnata (DND74)	4.234 (3.518-4.911)	12.01 (10.08-15.26)	5.826	0.120
Vitex trifolia subsp. *litoralis* (DND44)	21.74 (19.64-21.07)	43.85 (38.17-52.53)	2.802	0.423
Vitex trifolia subsp. *trifolia* (DND28)	39.04 (34.91-43.80)	90.25 (76.52-112.07)	0.4251	0.935
		Diplonychus rusticus, 24-hour		
Vitex ajugifolia (DND8)	39.46 (36.79-42.30)	54.23 (50.52-59.27)	1.158	0.561
Vitex pinnata (DND74)	142.2 (136.5-148.2)	175.1 (167.2-185.8)	3.147	0.550
Vitex trifolia subsp. *litoralis* (DND44)	55.82 (49.36-64.02)	140.1 (113.2-189.7)	9.979	0.007
Vitex trifolia subsp. *trifolia* (DND28)	91.64 (88.14-95.50)	115.1 (108.5-125.6)	0.3605	0.948
		Diplonychus rusticus, 48-hour		
Vitex ajugifolia (DND8)	30.58 (28.29-34.07)	39.72 (35.78-46.85)	1.070	0.586
Vitex pinnata (DND74)	92.70 (89.68-95.96)	109.3 (104.8-116.1)	0.0429	1.000
Vitex trifolia subsp. *litoralis* (DND44)	39.51 (35.32-44.42)	92.33 (77.65-116.38)	10.36	0.006
Vitex trifolia subsp. *trifolia* (DND28)	86.28 (83.19-89.48)	105.5 (100.3-113.5)	0.04034	0.998

[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.

The *Vitex* leaf essential oils were screened for molluscicidal activity against three fresh-water snail species, *Gyraulus convexiusculus* (Hutton) (Planorbidae), *Pomacea canaliculata* (Lamarck) (Ampullariidae), and *Tarebia granifera* (Lamarck) (Thiaridae) as previously described [17] (Table 4). According to the World Health Organization (WHO), a crude plant extract or essential oil is considered "active" if it has a $LC_{90} \leq 20$ µg/mL after 24 h of exposure [22]. Thus, *V. ajugifolia*, *V. trifolia* subsp. *litoralis*, and *V. trifolia* subsp. *trifolia* showed remarkable molluscicidal activity against *P. canaliculata* with LC_{90} values of 11.3, 13.5, and 10.4 µg/mL, respectively. *Vitex trifolia* subsp. *litoralis* essential oil also showed notable activity against *G. convexisculus* (LC_{90} = 18.6 µg/mL). *Tarebia granifera* snails were less susceptible to the *Vitex* essential oils with LC_{90} values ranging from 49.6 µg/mL (*V. trifolia* subsp. *trifolia*) to 81.2 µg/mL (*V. trifolia* subsp. *litoralis*).

The essential oil compositions reported here are new for *V. ajugifolia*, and help to clarify the leaf essential oil compositions of *V. pinnata*, *V. trifolia* subsp. *litoralis*, and *V. trifolia* subsp. *trifolia* from north-central Vietnam. *Vitex pinnata* and *V. trifolia* subsp. *trifolia* showed notable pesticidal activities with reduced non-target lethality, and may be useful sources of renewable botanical pesticides. Additional research is needed to examine methods to increase essential oil yields, assess the potential for cultivation, and explore methods for retaining effective essential oil concentrations in the field (e.g., microencapsulation or nanoemulsions).

Table 4. Molluscicidal activity of *Vitex* leaf essential oils from north-central Vietnam[a]

Essential oil (voucher number)	LC_{50} (µg/mL)	LC_{90} (µg/mL)	χ^2	*p*
		Gyraulus convexiusculus		
Vitex ajugifolia (DND8)	20.85 (19.23-22.58)	31.89 (28.80-36.68)	1.863	0.761
Vitex pinnata (DND74)	9.269 (8.106-10.586)	30.20 (25.04-38.08)	6.594	0.243
Vitex trifolia subsp. *litoralis* (DND44)	8.238 (7.382-9.207)	18.56 (15.94-22.52)	6.726	0.347
Vitex trifolia subsp. *trifolia* (DND28)	9.462 (8.373-10.705)	25.542 (21.62-32.07)	8.464	0.076
Positive control (tea saponin)	37.28 (33.55-41.73)	65.86 (58.87-75.82)	7.223	0.065
		Pomacea canaliculata		
Vitex ajugifolia (DND8)	5.903 (5.348-6.521)	11.25 (9.85-13.37)	5.986	0.541
Vitex pinnata (DND74)	43.85 (40.44-47.48)	67.05 (60.49-77.34)	0.7287	0.866
Vitex trifolia subsp. *litoralis* (DND44)	6.835 (6.181-7.572)	13.46 (11.72-16.11)	5.219	0.516
Vitex trifolia subsp. *trifolia* (DND28)	6.501 (5.976-7.099)	10.36 (9.18-12.38)	0.32150	0.999
Positive control (tea saponin)	24.78 (23.26-26.72)	32.62 (29.98-37.10)	0.1301	0.988
		Tarebia granifera		
Vitex ajugifolia (DND8)	23.59 (21.02-26.46)	57.50 (48.98-70.56)	6.786	0.148
Vitex pinnata (DND74)	25.48 (22.86-28.38)	56.05 (48.29-67.96)	4.169	0.244
Vitex trifolia subsp. *litoralis* (DND44)	53.66 (49.27-58.86)	81.20 (74.06-90.91)	3.437	0.329
Vitex trifolia subsp. *trifolia* (DND28)	33.88 (31.31-36.77)	49.64 (45.77-54.76)	3.894	0.273
Positive control (tea saponin)	17.16 (15.74-18.69)	26.14 (23.98-29.15)	3.156	0.368

[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.

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Supporting Information

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