Supporting Information

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

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Table S1 : Chemical compositions of leaf essential oils of *Vitex* species collected in north-cental Vietnam

			V. ajugifolia	***V. pinnata***	***V. tifolia* subsp. *litoralis***		***V. tifolia* subsp. *tifolia***	
RI_{calc}	**RI_{db}**	**Compound**	**DND8**	**DND74**	**DND19**	**DND44**	**DND28**	**DND40**
922	923	Ticyclene	---	---	---	---	---	t
925	927	α-Thujene	---	---	0.7	0.5	0.3	0.8
933	932	α-Pinene	0.2	0.5	18.7	15.2	3.1	11.7
949	950	Camphene	---	0.1	0.1	0.1	t	0.1
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
978	978	1-Octen-3-ol	---	---	0.1	0.1	0.2	---
985	986	3-Octanone	---	---	t	---	---	0.1
988	989	Myrcene	---	---	1.0	0.8	0.4	1.1
990	991	2-Pentylfuran	---	0.1	---	---	---	---
997	996	3-Octanol	---	---	0.1	t	t	t
1005	1004	*p*-Mentha-1(7),8-diene	---	---	t	---	---	t
1007	1006	α-Phellandrene	---	---	t	---	---	t
1017	1017	α-Terpinene	---	---	t	0.2	0.1	0.2
1025	1024	*p*-Cymene	0.1	t	2.4	0.7	0.8	2.1
1029	1030	Limonene	0.1	t	1.7	1.3	0.3	1.2
1031	1031	β-Phellandrene	---	---	---	0.5	0.2	0.2
1033	1032	1,8-cineole	---	---	14.5	12.7	8.5	15.7
1035	1034	(*Z*)-β-Ocimene	---	t	t	---	---	t
1046	1046	(*E*)-β-Ocimene	---	0.1	t	t	t	t
1058	1057	γ-Terpinene	---	---	0.2	1.0	0.3	0.5
1070	1069	*cis*-Sabinene hydrate	---	---	0.1	t	t	t
1085	1086	Terpinolene	---	---	0.1	0.3	0.1	0.2
1091	1091	*p*-Cymenene	---	---	t	---	---	---

1100	1099	Linalool	0.1	0.1	0.3	0.1	0.1	0.1
1102	1101	*tans*-Sabinene hydrate	---	---	t	---	t	t
1106	1107	Nonanal	0.1	0.1	t	0.1	t	t
1111	1108	*p*-Menth-2,8-dien-1-ol	---	---	t	---	---	---
1113	1113	(*E*)-4,8-Dimethylnona-1,3,7-tiene	---	t	---	---	---	---
1120	1120	3-Octyl acetate	---	---	0.1	---	---	---
1124	1124	*cis*-*p*-Menth-2-en-1-ol	---	---	0.1	t	0.1	t
1127	1126	α-Campholenal	---	t	t	---	---	---
1141	1141	*tans*-Pinocarveol	---	0.1	0.1	0.1	t	t
1143	1142	*tans*-*p*-Menth-2-en-1-ol	---	---	0.1	t	t	t
1146	1145	*tans*-Verbenol	---	---	0.1	---	---	t
1148	1149	Camphor	---	---	---	---	t	---
1163	1164	Pinocarvone	---	t	0.1	0.2	---	t
1163	1165	Lavandulol	---	---	---	---	t	---
1171	1170	δ-Terpineol	---	---	0.1	0.1	0.1	0.1
1173	1173	Borneol	---	t	---	---	---	---
1181	1180	Terpinen-4-ol	---	---	1.3	0.8	1.8	1.2
1185	1188	Naphthalene	---	---	---	0.3	---	---
1188	1189	*p*-Cymen-8-ol	---	---	0.1	0.1	t	0.1
1193	1192	Methyl salicylate	---	0.1	---	---	---	---
1193	1191	Myrtenol	---	---	---	---	---	t
1194	1195	α-Terpineol	---	---	0.4	0.4	0.8	0.4
1197	1197	Myrtenal	---	0.1	---	---	---	---
1197	1198	*cis*-Piperitol	---	---	t	---	t	---
1200	1201	Safranal	---	t	---	---	---	---
1209	1208	*tans*-Piperitol	---	---	---	---	t	t
1220	1219	β-Cyclocital	---	t	---	---	---	---
1227	1227	Citonellol	---	---	---	---	---	t

1240	1240	Ascaridole	---	---	0.1	0.1	t	0.1
1275	1275	*tans*-Ascaridol glycol	---	---	0.1	---	t	0.1
1284	1285	Bornyl acetate	---	---	0.5	0.7	0.1	0.1
1288	1287	Dihydroedulan IA	---	---	0.1	0.1	0.1	0.1
1292	1291	*cis*-Ascaridol glycol	---	---	t	---	---	---
1293	1284	Lavandulyl acetate	---	---	---	---	0.8	---
1305	1306	*iso*-Ascaridole	---	---	0.1	0.2	0.1	0.1
1312	1313	δ-Terpinyl acetate	---	---	0.5	0.8	0.2	0.2
1329	1328	Bicycloelemene	0.3	1.1	---	---	t	---
1334	1335	δ-Elemene	---	1.5	---	0.2	0.1	---
1346	1346	α-Terpinyl acetate	---	---	12.7	19.0	8.3	8.3
1347	1348	α-Cubebene	0.3	0.3	---	---	---	---
1369	1371	α-Ylangene	2.2	0.3	---	---	---	---
1370	1370	*iso*-Ledene	---	0.1	---	---	---	---
1375	1375	α-Copaene	17.0	0.5	t	0.1	t	0.1
1379	1379	(*E*)-β-Damascenone	0.1	0.1	t	0.1	t	t
1382	1383	*cis*-β-Elemene	0.1	---	---	---	---	t
1383	1382	β-Bourbonene	0.2	0.3	0.1	0.2	0.1	0.1
1387	1387	β-Cubebene	0.1	0.3	---	---	t	t
1388	1391	α-Bourbonene	---	---	---	---	---	---
1389	1390	*tans*-β-Elemene	1.2	0.5	0.1	0.2	0.2	0.2
1392	1389	Sativene	0.2	---	---	---	---	---
1404	1405	(*Z*)-β-Caryophyllene	---	---	---	---	t	t
1405	1405	α-Gurjunene	1.0	0.2	---	t	---	---
1411	1415	β-Maaliene	0.1	0.1	---	---	---	---
1413	1411	Thymohydroquinone dimethyl ether	---	---	---	---	---	0.2
1414	1409	*iso*-Sativene	0.1	---	---	---	---	---
1419	1419	β-Ylangene	---	---	---	t	---	---

1422	1424	(*E*)-β-Caryophyllene	11.7	32.7	0.3	0.5	15.8	14.5
1427	1430	γ-Maaliene	0.2	0.2	---	t	t	---
1430	1430	γ-Elemene	---	3.3	---	---	---	---
1430	1433	β-Copaene	0.3	---	0.1	0.1	t	0.1
1434	1438	α-Maaliene	---	0.1	---	---	---	---
1435	1436	α-Guaiene	3.1	0.2	---	---	---	---
1439	1438	Aromadendrene	2.0	1.5	---	---	---	---
1440	1444	Guaia-6,9-diene	---	---	---	---	t	---
1447	1447	*iso*-Germacrene D	0.2	0.3	---	---	---	t
1446	1446	Myltayl-4(12)-ene	0.2	0.3	---	---	---	---
1448	1447	Geranyl acetone	---	---	---	---	---	t
1451	1452	(*E*)-β-Farnesene	---	---	---	t	t	t
1452	1455	Valerena-4,7(11)-diene	0.2	0.2	---	---	---	---
1455	1454	α-Humulene	9.6	2.0	t	0.1	0.7	0.7
1460	1458	*allo*-Aromadendrene	1.3	0.3	---	---	---	t
1462	1463	*cis*-Muurola-4(14),5-diene	---	0.3	---	---	---	---
1470	1471	4,5-di-*epi*-Aristolochene	0.1	---	---	---	0.1	---
1473	1476	Selina-4,11-diene	0.3	---	---	---	---	---
1475	1475	γ-Muurolene	0.3	1.5	---	t	t	0.1
1479	1476	γ-Gurjunene	0.5	---	---	---	---	---
1480	1480	Germacrene D	0.7	17.1	0.2	0.7	0.4	0.6
1482	1482	Aristolochene	---	---	---	0.1	---	---
1487	1492	*cis*-β-Guaiene	0.1	---	---	---	---	---
1487	1488	δ-Selinene	---	0.4	---	---	---	---
1489	1489	β-Selinene	1.9	0.3	---	0.1	---	---
1489	1491	Eremophilene	---	---	0.1	---	13.4	0.1
1491	1491	Viridiflorene	1.0	1.0	---	---	---	---
1492	1492	*tans*-Muurola-4(14),5-diene	---	---	---	---	---	t

1493	1490	γ-Amorphene	---	0.3	---	---	---	---
1496	1501	α-Selinene	---	---	---	---	0.1	0.1
1497	1497	Bicyclogermacrene	3.8	11.1	---	0.2	---	---
1499	1500	α-Muurolene	0.6	0.6	t	t	0.1	0.1
1500	1502	ε-Amorphene	---	0.1	---	---	---	---
1501	1505	α-Bulnesene	3.1	---	---	---	---	---
1502	1503	(*E*,*E*)-α-Farnesene	0.2	1.3	---	0.1	---	t
1503	1507	Eremophila-1(10),8,11-tiene	---	---	---	---	0.1	---
1506	1508	β-Bisabolene	---	---	---	t	---	---
1511	1510	1,11-Oxidocalamenene	---	---	---	0.1	0.1	---
1513	1512	γ-Cadinene	0.4	0.9	0.1	0.1	0.1	0.2
1516	1516	Sobrerol 8-acetate	---	---	t	---	---	---
1518	1518	δ-Cadinene	1.5	1.8	0.1	0.2	0.3	0.4
1521	1519	*tans*-Calamenene	1.2	---	---	---	---	t
1533	1533	*tans*-Cadina-1,4-diene	---	0.1	---	---	---	---
1537	1540	Selina-4(15),7(11)-diene	0.2	---	---	---	---	---
1537	1538	α-Cadinene	---	0.4	---	---	---	t
1541	1541	α-Calacorene	0.8	0.2	---	---	t	---
1552	1551	(*Z*)-Caryophyllene oxide	---	---	---	---	---	0.2
1559	1560	Germacrene B	---	3.4	---	---	0.1	---
1560	1560	(*E*)-Nerolidol	---	---	t	0.1	0.1	t
1561	1560	β-Calacorene	0.3	0.2	---	---	t	t
1570	1568	Palustol	0.6	0.1	---	---	---	---
1570	1571	Maaliol	---	0.1	---	---	---	---
1570	1571	(3*Z*)-Hexenyl benzoate	---	---	---	---	0.2	---
1573	1571	Dendrolasin	---	0.9	---	---	---	---
1576	1575	Germacra-1(10),5-dien-4β-ol	---	---	---	---	t	0.1
1577	1576	Spathulenol	8.7	2.0	0.2	0.3	---	---

1582	1582	*epi*-Globulol	---	---	---	0.1	---	---
1583	1587	Caryophyllene oxide	4.3	1.2	0.1	0.1	3.8	1.9
1584	1583	*allo*-Spathulenol	---	---	---	0.1	0.1	t
1587	1590	Globulol	2.1	0.9	---	---	---	---
1588	1591	β-Copaen-4α-ol	1.6	---	---	---	---	---
1594	1594	Viridiflorol	1.5	0.6	---	---	---	---
1596	1593	Guaiol	0.9	---	---	---	---	---
1597	1596	Cubeban-11-ol	---	0.2	---	---	---	---
1599	1593	Humulene epoxide I	0.3	---	---	---	---	---
1602	1598	Dehydroxy-isocalamendiol	0.2	---	---	---	---	---
1602	1600	α-Oplopenone	---	---	---	---	t	0.1
1605	1605	Ledol	1.0	---	---	---	---	---
1607	1609	Rosifoliol	0.4	0.1	---	---	---	---
1610	1611	Humulene epoxide II	1.9	---	---	---	0.2	0.1
1623	1623	Humulane-1,6-dien-3-ol	---	0.1	---	---	---	---
1626	1624	Muurola-4,10(14)-dien-1β-ol	0.4	---	---	---	---	---
1629	1629	*iso*-Spathulenol	1.6	0.5	---	0.1	0.3	---
1628	1628	1-*epi*-Cubenol	0.5	---	---	---	---	t
1631	1631	Eremoligenol	0.2	---	---	---	---	---
1633	1630	Caryophylla-4(12),8(13)-dien-5α-ol	---	0.3	---	---	0.2	t
1634	1634	*cis*-Cadin-4-en-7-ol	0.2	---	---	---	---	---
1636	1636	Caryophylla-4(12),8(13)-dien-5β-ol	0.2	0.5	---	---	0.3	0.1
1641	1644	*allo*-Aromadendrene epoxide	---	0.1	---	---	---	---
1641	1640	τ-Cadinol	---	0.2	0.1	t	0.1	0.1
1643	1643	Cubenol	0.3	---	---	---	---	---
1644	1644	τ-Murrolol	0.4	0.2	t	0.1	0.1	0.1
1647	1651	α-Muurolol	---	---	---	---	---	t
1655	1655	α-Eudesmol	1.8	---	---	---	---	---

1656	1655	α-Cadinol	---	0.6	0.2	0.2	2.3	0.3
1658	1658	Selin-11-en-4α-ol (= Kongol)	0.5	---	---	---	---	---
1659	1664	(*E*,*Z*)-Geranyl linalool	---	---	0.1	---	---	---
1665	1662	9-Methoxycalamenene	0.2	---	---	---	---	---
1666	1666	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	---	0.2	---	---	0.1	t
1684	1676	8-Hydroxyisobornyl isobutanoate	---	---	---	---	---	t
1788	---	Unidentified diterpenoid[a]	---	---	1.4	1.7	---	---
1798	1797	Solavetivone	---	---	---	---	0.1	---
1836	1836	Neophytadiene	---	---	---	---	---	---
1841	1841	Phytone	---	0.1	---	0.1	---	---
1881	1882	Cubitene	---	---	1.8	1.9	2.0	1.3
1886	1886	Sclareol oxide	---	---	---	---	t	t
1906	1907	Isopimara-9(11),15-diene	---	---	0.1	0.4	0.1	0.1
1939	---	Unidentified diterpenoid[b]	---	---	---	---	5.3	---
1943	1948	β-*iso*-Methylionone	---	---	1.3	1.2	1.0	1.0
1943	1943	Beyerene isomer	---	---	0.9	1.0	1.3	0.8
1958	---	Unidentified diterpenoid[c]	---	---	1.3	1.3	---	---
1962	---	Unidentified diterpenoid[d]	---	---	4.5	4.5	1.9	2.1
1980	---	Unidentified diterpenoid[e]	---	---	2.3	2.9	2.9	1.4
1993	1994	Manoyl oxide	---	---	0.3	0.3	0.1	0.1
2018	---	Unidentified diterpenoid[f]	---	---	0.2	1.2	---	---
2049	2049	Abietatiene	---	---	3.7	2.0	2.9	0.5
2066	2062	Manool	---	---	0.2	---	0.2	0.1
2071	2079	Kolavelool	---	---	---	0.2	---	---
2107	2106	Phytol	0.7	1.6	1.4	1.4	---	---
		Monoterpene hydrocarbons	0.4	0.8	44.9	36.8	17.6	41.4
		Oxygenated monoterpenoids	0.1	0.4	31.1	35.2	20.8	26.6

Sesquiterpene hydrocarbons	68.2	87.1	1.1	2.8	31.5	17.1
Oxygenated sesquiterpenoids	29.6	8.7	0.5	1.0	7.7	3.0
Diterpenoids	0.7	1.6	8.4	7.2	6.6	2.9
Others	0.2	0.5	1.7	2.0	1.3	1.2
Total identified	99.2	99.1	87.7	85.1	85.6	92.2

RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Retention index from the databases (Adams, 2007; FFNSC 3; NIST17). t = tace (< 0.05%). --- = not detected.

[a] MS(EI): 286(8%), 268(4%), 253(7%), 205(14%), 203(17%), 187(20%), 151(100%), 136(51%), 119(33%), 105(25%), 91(23%), 81(33%), 55(24%), 43(29%), 41(21%).

[b] MS(EI): 272(5%), 257(22%), 190(23%), 175(23%), 163(19%), 149(60%), 134(100%), 133(59%), 121(58%), 119(72%), 109(64%), 107(75%), 105(53%), 95(68%), 93(66%), 91(54%), 81(55%), 79(48%), 69(76%), 55(60%), 43(24%), 41(84%).

[c] MS(EI): 272(5%), 257(17%), 204(8%), 189(28%), 175(12%), 161(15%), 147(12%), 133(25%), 119(27%), 107(30%), 105(24%), 93(27%), 91(32%), 81(26%), 80(100%), 79(25%), 69(21%), 55(19%), 41(23%).

[d] MS(EI): 290(1%), 275(45%), 257(44%), 192(49%), 177(46%), 163(14%), 161(13%), 149(25%), 137(39%), 135(25%), 123(47%), 121(39%), 109(73%), 95(82%), 81(89%), 69(58%), 67(45%), 55(64%), 43(100%), 41(50%).

[e] MS(EI): 272(1%), 257(4%), 245(2%), 191(100%), 189(23%), 136(34%), 121(47%), 119(52%), 107(34%), 95(30%), 93(30%), 81(31%), 80(89%), 71(24%), 69(25%), 55(28%), 43(28%), 41(27%).

[f] MS(EI): 272(30%), 257(20%), 148(92%), 133(38%), 106(100%), 105(83%), 93(38%), 91(40%), 81(19%), 69(30%), 55(21%), 43(35%), 41(32%).

Gas Chromatographic-Mass Spectal Analysis

Each of the *Vitex* essential oils was analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ulta (Shimadzu Scientific Instuments, Columbia, MD, USA) gas chromatograph equipped with a 30 m × 0.25 mm i.d. with a 0.25 μm stationary film thickness ZB-5ms fused silica capillary column (Phenomenex, Torrance, CA, USA) coupled with a mass selective detector operated in the electon impact (EI) mode (electon energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. The injector temperature was 250 °C and the ion source temperature was 200 °C. The GC oven temperature program was programmed for 50 °C initial temperature, temperature increased at a rate of 2 °C/min to 260 °C. A 5% *w*/*v* solution of the sample in CH_2Cl_2 was prepared and 0.1 μL was injected with a splitting mode of 30:1. The essential oil components were identified by comparing mass specta fragmentation patterns and linear retention indices (RI), based on a series of homologous C_8-C_{26} *n*-alkanes, with those reported in databases (Adams, 2007; FFNSC 3; NIST17).

Mosquito Larvicidal Assays

Larvae of *Ae. aegypti* were obtained from a mosquito colony maintained at Laboratory of Parasitology and Entomology of Duy Tan University, Da Nang, Vietnam. Wild larvae of *Ae. albopictus* were collected from Hoa Khanh Nam distict (16° 3′ 14.9″ N, 108° 9′ 31.2″ E). The *Ae. aegypti* and *Ae. albopictus* larvae were fed on Koi fish food. Wild *Cx. quinquefasciatus* 1st and 2nd instar larvae were collected from car tires containing the fruit of *Ficus racemosa* L. and were continued to be fed on fruit of *F. racemosa* in the laboratory until the third - early fourth instar. The *Vitex* essential oils were screened for mosquito larvicidal activity against the three mosquito species using the protocol reported by Dai et al. For each assay, 20 larvae (fourth instar) and 150 mL water were placed in 250-mL beakers. Aliquots of the *Vitex* essential oils (from 1% stock solutions in ethanol) were added to give final concentations of (100, 50, 25, 12.5 and 6 μg/mL). A set of contols using ethanol only (negative contol) and permethrin (positive contol) were also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutitional supplement was added. Experiments were carried out at 25 ± 2 °C. Each assay was carried out with four replicates.

Molluscicidal Assays

Wild *G. convexiusculus* were collected in Hoa Vang distict, Da Nang city (16° 00′ 25″ N, 108° 06′ 53″ E). Snails with diameters of 2.5–5.0 mm were used for molluscicidal activity screening. Wild *T. granifera* were collected from Thu Bon river, Quang Nam province (15° 50′ 00″ N, 108° 11′ 26″ E). Snails with lengths of 10–14 mm were used for molluscicidal screening. Eggs of *P. canaliculata* were collected from a rice field at Hoa Vang Distict, Da Nang City (16° 01′ 02.4″ N, 108° 06′ 34.8″ E). Eggs were incubated in the laboratory with temperature of 25 ± 2°C and relative humidity of 70 ± 5%. The newly hatched snails were kept 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. The 7-day-old juvenile snails with shell lengths of 1.0–3.0 mm were used for screening. Snails were identified by Dr. Nguyen Huy Hung (Duy Tan University).

Molluscicidal activity of *Vitex* essential oils against *G. convexiusculus*, *P. canaliculata*, and *T. granifera*, was assessed using the following protocol: For each assay, 20 snails were placed into 250-mL beakers along with 150 mL of water. Aliquots of the essential oils dissolved in EtOH (1% stock solution) were then added to give final concentations of 100, 50, 25, 12.5 and 6 μg/mL. A set of contols using EtOH only (negative contol) and tea saponin (positive contol) were included for comparison. Each test was carried out in quadruplicate with five concentations. The snails were kept in the test solutions for 24 h, after which they were placed in a separate container with 150 mL of distilled water to recover. Snails that did not recover after this 48-h period were determined to be dead. During the molluscicidal assay, the laboratory was maintained at 26 ± 2 °C with a 12:12 h (light:dark) photoperiod.

Diplonychus rusticus Insecticidal Assay

Adult water bugs (*D. rusticus*), identified by Dr. Nguyen Huy Hung, Duy Tan University, were collected in the field and maintained in glass aquaria (60 cm long × 50 cm wide) containing water at 25 °C and a depth of 20 cm. The insects (20 *D. rusticus* adults) were screened against the *Vitex* essential oils at concentations of 200, 150, 100, 75, 50, and 25 µg/mL (four replicates each), and mortality recorded after 24 h and again after 48 h exposure. Ethanol was used as a negative contol.