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Chemical Constituents of The Essential Oils From The Leaves of *Litsea umbellata* and *Litsea iteodaphne* and Their Mosquito Larvicidal Activity

Do N. Dai [1,2]*, Nguyen D. Hung [1], Nguyen T. Chung [1], Le T. Huong [3], Nguyen H. Hung [4] and Isiaka A. Ogunwande [5]*

[1] Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[2] Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City, Nghe An Province, Vietnam
[3] School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, NghÇ An Province, Vietnam
[4] Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam
[5] Foresight Institute of Research and Translation, Ibadan, Nigeria

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Abstract: The chemical constituents of hydrodistilled essential oils from the leaves of *Litsea umbellata* (Lour.) Merr. and *Litsea iteodaphne* (Ness) Hook. f. growing wild in central Vietnam and mosquito larvicidal activity of these oils against laboratory-reared larvae of *Culex quinquefasciatus* and *Aedes albopictus* were investigated. The major constituents mostly terpenes (percentage abundance, respectively) of the oils were β-pinene (18.8 % and 6.6 %), β-caryophyllene (16.2 % and 21.4 %), α-pinene (10.4 % and 8.7 %) and germacrene D (9.1 % and 15.5 %). The oil of *L. umbellata* displayed larvicidal activity against *Cx. quinquefasciatus* with LC_{50} of 36.19 µg/mL at both 24 h and 48 h, while LC_{50} of 40.09 µg/mL and 27.33 µg/mL at 24 h and 48 h respectively, were exhibited towards *Ae. albopictus*. Also, an LC_{90} value of 54.17 µg/mL was obtained against *Cx. quinquefasciatus* at both 24 h and 48 h, while values of 68.79 and 60.49 µg/mL respectively, were recorded against *Ae. albopictus* at 24 h and 48 h. Moreover, the oil of *L. iteodaphne* exhibited activity against *Cx. quinquefasciatus* with LC_{50} of 37.20 µg/mL (24 h) and 20.21 µg/mL (48 h) while LC_{90} values were 39.27 µg/mL (24 h) and 23.78 µg/mL (48 h). Furthermore, *L. iteodaphne* oil displayed larvicidal activity against *Ae. albopictus* with LC_{50} values of 40.04 µg/mL (24 h) and 16.63 µg/mL (48 h) as well as LC_{90} values of 42.40 µg/mL (24 h) and 23.64 µg/mL (48 h). This is the first report on the chemical composition and larvicidal activity of *L. umbellata* and *L. iteodaphne* essential oils.

Key words: *Litsea umbellata, Litsea iteodaphne,* essential oil composition, terpene, larvicidal activity.

Introduction

Litsea is one of the 35 genera in the Lauraceae family of plants. A total of 400 species are distributed throughout tropical and subtropical Asia, the Pacific, Australia and New Zealand [1]. *Litsea umbellata* (Lour.) Merr is evergreen shrubs or small trees that grow up to 3-9 m tall. The leaves are alternate while the leaf blade is elliptic or oblong-ovate with a dimension of 6-12 × 3-4.2 cm. The globose or subglobose shaped fruits are greenish-

*Corresponding authors (Do N. Dai, Isiaka A. Ogunwande)
E-mail: < daidn23@gmail.com; isiakaogunwande@gmail.com >

whitish which measure up to 0.6-1 cm in diameter. The green color of the young fruits turns to dark purple and black when ripe. Flowering occurs in April and May while fruiting takes place from August to September. Traditionally, the leaves of *L. umbellata* are used to treat boils, microbial infections and also act as insect deterrent [2,3]. *L. iteodaphane* (Ness) Hook. f. is a tree or evergreen shrub which grows up to 3.5 m long. The leaves are linear lanceolates, leathery and about 1-2 cm wide. The flower is unisexual while the fruits are ellipsoids about 9-10 mm long. *L. iteodaphane* flowers mostly in June and July and bear fruits from August to October. The decoction of leaves and fruits of both plants are known to cure inflammation, lower blood pressure, kill insect pests and act as antimalaria [4].

Aedes albopictus (Skuse) (Diptera: Culicidae) are important vectors of arboviral infections, including yellow fever, chikungunya virus, dengue virus and Zika virus [5]. It is known as the Asian tiger mosquito. *Culex quinquefasciatus* Say, commonly known as the southern house mosquito, is a medium-sized brown mosquito that exists throughout the tropics. It is a vector of many pathogens of humans, domestic and wild animals. Viruses transmitted by this species include lymphatic filariasis, West Nile virus (WNv), St. Louis encephalitis virus (SLEv), Western equine encephalitis virus (WEEv) and Zika virus [6]. Dengue fever epidemics are frequent and widespread in Vietnam [7] and the outbreaks of chikungunya and Zika infections have been reported lately [8]. The control of the vector is one of the primary approaches to reduce the spread of arboviral infections. Botanical insecticides in general and essential oils, in particular, have emerged as promising, environmentally friendly alternatives to synthetic pesticides for mosquito control [9,10].

As part of our ongoing research on identifying the constituents and biological potentials of essential oils from plant species in Vietnam [11-14], we have obtained essential oils from *L. umbellata* and *L. iteodaphne* and examined their mosquito larvicidal activity for the first time. The authors are aware that there has been no previous investigation on the chemical compositions and biological activities of essential oils from *L. umbellata* and *L. iteodaphne*.

Materials and methods

Plant samples and chemicals

The leaves of *L. umbellata* (1.1 kg) and *L. iteodaphne* (1.3 kg) were collected from Pu Hoat Natural Reserve, Nghe An Province (GPS 19°20′ N 104°50′E), Vietnam. All collections were done in August 2018. The plant samples were identified by Dr. Dai. Voucher specimens DND 702 and DND 705 respectively were deposited at the Botany Museum, Nghe An College of Economics, Vietnam. Plant samples were air-dried for two weeks at ambient temperature, before hydrodistillation. Pure chemicals were distributed products of Sigma-Aldrich (San Louis, MI, USA).

Hydrodistillation of essential oils

A total of 1 kg of each of the pulverized plant samples was used for the separate hydrodistillation experiment. A measured weight of samples was separately and carefully introduced into a 5 L flask. Distilled water was then added until it covered the sample completely. Essential oils were obtained by hydrodistillation method (replicates of 3) which was carried out in an all glass Clevenger-type distillation unit designed according to Vietnamese Pharmacopoeia as described previously [11-14]. The distillation was carried out at normal pressure for 3 h. The volatile oils distilled over water were collected into clean and previously weighed sample bottles by running through the tap in the receiver arm of the apparatus. The essential oils were kept under refrigeration (4°C) until the moment of analysis as described previously [11-14].

Gas chromatography (GC) analysis of the essen-tial oils

Gas chromatography (GC) analysis was performed on an HP 7890A Plus Gas chromatograph (Agilent Technologies, USA) equipped with an FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected (10 % *n*-hexane solution) was

1.0 µL. Inlet pressure was 6.1 kPa. Also, the homologous series of *n*-alkanes (C_6-C_{40}) under identical expeimental conditions of GC above were run for comparative analysis. The quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds.

Gas chromatography-Mass spectrometry (GC/MS) analysis of the oil samples

An Agilent Technologies HP 7890A Plus Chromatograph (Agilent USA) fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD (Agilent, USA) was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as the carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan a mass range of 45-350 amu at a sampling rate of 1.0 scan/s.

The identification of constituents from the GC/MS spectra of *L. umbellata* and *L. iteodaphne* was performed based on a comparison of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. The technique of co-injection with known compounds was also employed where necessary. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition [15,16] and with those in the literature as described previously [11-14].

Mosquito larvae of Culex quinquefasciatus and Aedes albopictus

The larvicidal experiment was conducted at the Center for Entomology and Parasitology Research, Duy Tan University. The adults of *Culex quinquefasciatus* and *Aedes albopictus* used for this study were the laboratory stock of Hoa Khanh Nam Ward, Lien Chieu District, Da Nang city (16°03′ 14.9"N, 108°09′31.2"E). The adults of *Cx. quinquefasciatus* and *Ae. albopictus* were housed in entomological cages built of dimension 40 x 40 x 40 cm. They were fed and sustained on 10 % sucrose solution while feeding on the blood of mice. Furthermore, egg hatching was induced with tap water. The obtained larvae were maintained in plastic trays of dimension 24 × 35 × 5 cm. The larvae were allowed to feed on dog biscuits and yeast powder in a ratio of 3:1. The temperature condition of 25 ± 2°C, the relative humidity of 65-75 % and an equal 12:12 h light: dark cycle were maintained throughout the study.

Larvicidal test on the oil samples

Larvicidal activity of the essential oils from *L. umbellata* and *L. iteodaphne* were evaluated according to previous methods [11-14]. The essential oil (200 mg) was dissolved in 20 mL of EtOH (1 % stock solution) to obtain an aliquot and was transferred into a 200 mL beaker which contained 20 fourth instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus*. Water was then added to the beaker. Four different concentrations of the essential oils (100, 50, 25 and 12.5 µg/mL) were used for the experiment. EtOH was used as a negative control, while permethrin, a larvicidal drug, was used as a positive control. The mortality of larvae of *Cx. quinquefasciatus* and *Ae. albopictus* was recorded after 24 h and 48 h of exposure to the different concentrations of the essential oil. The experiments were carried out at a temperature of 25 ± 2°C. The larvicidal test was conducted in four replicates.

The mortality rate of *Cx. quinquefasciatus* and *Ae. albopictus* was calculated according to the formula;

$$Mc = Mo\text{-}Mt / 100\text{-}Mt \times 100$$

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality

Statistic analysis

The data obtained were subjected to log-probit analysis [11-14] to obtain LC_{50} values, LC_{90} values and 95 % confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France). Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, ±) of three and four independent measurements, respectively for the chemical constituents and larvicidal test, using Microsoft excel program 2003.

Results and discussion

The percentage yields and color of the essential oils

The average yields of *L. umbellata* and *L. iteodaphne* essential oils (yellow), calculated on a dry weight basis were 1.82 g and 2.13 g, respectively. The yields were obtained with an SD of ± 0.01 for both essential oils and are consistent with data obtained for essential oils of some *Litsea* plants from Vietnam. Previously reported yields from the *Litsea* leaf essential oils growing in Vietnam were *L. helferi* (0.30 %) [11], *L. ferruginea* (0.25 %) [11], *L. verticillata* (0.25 %) [11], *L. viridis* (0.21 %) [12], *L. firma* var. *austroannamensis* (0.28 %) [17] and *L. acutivena* (0.16 %) [18]. However, both *L. glutinosa* (0.95 %) [11] and *L. cubeba* (1.40 %) [11], were obtained in a relatively higher yield.

Nature and percentages of chemical compounds identified in the essential oils

Forty-eight compounds representing 92.8 % of the oil contents were identified in *L. umbellata*. The main classes of compounds present in the oil were monoterpene hydrocarbons (46.9 %) and sesquiterpene hydrocarbons (37.7 %) as seen in Table 1. The constituents occurring in higher amounts in the leaf oil of *L. umbellata* were β-pinene (18.8 %), β-caryophyllene (16.2 %), α-pinene (10 .4 %), germacrene D (9.1 %) and sabinene (5.1 %). In addition, limonene (3.3 %), (*Z*)-β-ocimene (3.3 %) and bicyclogermacrene (3.1 %) were also present in sizeable amount. Also, thirty-eight com-pounds accounting for 97.9 % of the oil contents were identified in *L. iteodaphne*. This comprised of monoterpene hydrocarbons (30.7 %), oxygenated monoterpenes (0.2 %), sesquiterpene hydrocarbons (63.9 %) and oxygenated sesquiterpenes (3.3 %). However, β-caryophyllene (21.4 %), germacrene D (15.5 %), α-pinene (8.7 %), β-pinene (6.6 %), bicyclogermacrene (6.5 %) and camphene (6.4 %) were the main constituents of the oil. Moreover, β-bourbonene (3.6 %), δ-3-carene (3.3 %), α-guaiene (2.8 %), limonene (2.7 %), α-humulene (2.5 %) and δ-cadinene (2.3 %) were also prominent in the oil as presented in Table 1.

The authors are not aware of any report on the volatile constituents and biological activities of essential oils from *L. umbellata* and *L. iteodaphne*, hence the present data represent the first of its kind in these regards. A comparative analysis of the present data on the chemical constituents of *L. umbellata* and *L. iteodaphne* essential oils with known oil constituents of *Litsea* plants from Vietnam revealed both quantitative and qualitative variations. For example, the leaf oil of *L. helferi* [11] contained high amounts of limonene (17.5 %), β-caryophyllene (14.2 %), bicyclogermacrene (13.1 %) and bicycloelemene (12.4 %) while sabinene (34.5 %), α-pinene (10.1 %), γ-terpinene (7.8 %) and limonene (6.9 %) were present in higher quantities in *L. ferruginea*. The significant constituents of *L. verticillata* were linalool (23.4 %), α-pinene (26.1 %) and β-pinene (11.7 %). (*E*)-β-Ocimene (57.4 %) was the most abundant compound of the essential oil of *L. glutinosa* [11]. The main constituents identified in the leaf, stem, fruits and roots oils of *L. cubeba* were (*Z*)-citral (32.9 %-66.1 %), sabinene (1.4 %-14.2 %), limonene (7.0 %-13.6 %) and linalool (1.9 %-9.5 %), respectively [11]. The main compounds in the leaf oil of *L.viridis* were bicyclogermarcrene (25.5 %), decanal (14.4 %), α-pinene (11.1 %) and β-pinene (8.3 %) [12] while *L. firma* var. *austroannamensis* contained nerol (14.4 %), (*E*)-β-ocimene (10.2 %) and *cis*-geraniol (10.2 %) [17]. The main compounds of *L. acutivena* from Vietnam were α-phellandrene (30.4 %) and α-pinene (10.2 %) [18]. The essential oils from *Litsea* plants growing in Vietnam are rich sources of terpene compounds.

The information showed that the compositions of the oils of *L. ferruginea, L. verticillata, L. cubeba and L. glutinosa* [11]*; L L. firma* Hook. f. var. *austroannamensis* [17] and *L. acutivena* [18] from Vietnam were dominated by monoterpene compounds. Also, the essential oils of *L. cubeba* from China [19] and several other parts of the worlds [11,20] contain monoterpene compounds. Monoterpene compounds were also the main chemical class in the oils of *L. akoensis* [21] and *L. pungens* [22]. The leaf oils of *L. helferi* from Vietnam [11], *L. acutivena* from Taiwan [23], *L. glutinosa* from Vietnam [24], *L. nakaii* from Taiwan [25], *L. kostermansii* from Taiwan [26], *L. acuminata* from Taiwan [27], *L. linii* [28] from Taiwan and *L. mushaensis* from Taiwan [28] had high contents of sesquiterpene

Table 1. Chemical composition of essential oils of *L. umbellata* and *L. iteodaphne*

No.	RT (min)	Compound [a]	RI (Cal.)	RI (Lit.)	Concentration *L. umbellata* [c]	Concentration *L. iteodaphne* [c]
1	10.01	α-Thujene	929	921	0.2	-
2	10.29	α-Pinene [b]	938	932	10.4	8.7
3	10.79	Camphene	954	946	0.6	6.4
4	11.50	Sabinene	977	970	5.1	0.1
5	11.69	β-Pinene [b]	983	980	18.8	6.6
6	11.90	Myrcene	990	988	1.3	1.5
7	12.50	α-Phellandrene	1009	1004	0.3	0.2
8	12.70	δ-3-Carene	1015	1014	0.1	3.3
9	13.18	o-Cymene	1028	1022	0.5	0.2
10	13.32	Limonene	1033	1030	3.3	2.7
11	13.37	β-Phellandrene	1034	1032	0.4	-
12	13.44	1,8-Cineole	1036	1034	0.4	-
13	13.45	(*Z*)-β-Ocimene	1037	1034	3.3	-
14	13.84	(*E*)-β-Ocimene	1048	1044	1.8	0.2
15	14.33	γ-Terpinene	1062	1056	0.2	-
16	15.39	Terpinolene	1093	1089	0.2	0.3
17	15.69	Linalool	1102	1100	0.6	0.2
18	16.49	Dehydro Sabina ketone [b]	1124	1125	0.4	-
19	17.54	Citronellal	1154	1158	1.0	-
20	18.66	Terpinen-4-ol	1186	1187	0.3	-
21	20.17	Citronellol	1229	1232	0.4	-
22	20.71	Nerol	1245	1245	0.2	-
23	21.69	Geranial	1273	1271	0.2	-
24	24.14	δ-Elemene	1346	1345	0.3	-
25	24.55	α-Cubebene	1358	1356	-	0.4
26	25.52	α-Copaene	1387	11387	1.4	1.8
27	25.77	1,5-di-*epi*-β-Bourbonene [b]	1396	1392	-	1.0
28	25.87	β-Bourbonene	1401	1403	0.2	3.6
29	25.98	*cis*-β-Elemene	1402	1408	1.0	-
30	26.67	α-Gurjunene	1410	1412	0.1	-
31	27.06	β-Caryophyllene [b]	1420	1417	16.2	21.4
32	27.29	γ-Elemene	14.43	1443	0.7	-
33	27.49	α-Guaiene	1449	1450	-	2.8
34	27.78	α-Humulene	1456	1458	1.6	2.5
35	28.13	(*Z*)-β-Farnesene	1458	1458	0.2	-
36	28.36	9-*epi*-(*E*)-Caryophyllene [b]	1470	1467	0.2	-
37	28.43	*trans*-Cadina-1(6),4-diene [b]	1482	1480	-	1.2
38	28.66	Aromadendrene	1485	1484	-	0.3
39	28.73	γ-Muurolene	1489	1487	0.1	1.2
40	28.66	α-Amorphene	1493	1493	-	0.3
41	28.98	Germacrene D	1497	1495	9.1	15.5
42	29.15	β-Selinene	1502	1500	0.4	0.9
43	29.33	γ-Amorphene	1508	1502	-	0.3

table 1. (continued).

No.	RT (min)	Compound [a]	RI (Cal.)	RI (Lit.)	Concentration *L. umbellata* [c]	Concentration *L. iteodaphne* [c]
44	29.39	(*E,E*)-α-Farnesene	1510	1505	0.9	-
45	29.44	Bicyclogermacrene	1512	1510	3.1	6.5
46	29.56	β-Bisabolene	1516	1516	-	0.3
47	19.64	α-Bulnesene	1519	1520	-	0.9
48	29.93	γ-Cadinene	1528	1525	0.1	0.4
49	30.12	δ-Cadinene	1534	1531	0.7	2.4
50	31.13	(*E*)-Nerolidol	1568	1563	1.6	-
51	31..34	Germacrene B	1575	1570	1.4	-
52	31.97	Spatahulenol	1596	1591	0.9	0.8
53	32.16	Caryophyllene oxide	1603	1602	1.3	1.2
54	33.37	Alismol	1620	1622	0.2	-
55	33.66	Isospathulenol	1656	1646	-	0.2
56	34.13	α-Cadinol	1672	1670	0.1	0.4
57	36.44	Zerumbone	1756	1754	0.5	0.7
58	45.30	Phytol	2123	2119	0.4	-
		Total			92.8	97.9
Monoterpene hydrocarbons (Sr. No. 1-10, 13-16)					46.9	30.7
Oxygenated monoterpenes (Sr. No. 12, 17-23)					3.1	0.2
Sesquiterpene hydrocarbons (Sr. No. 24-49, 51)					37.7	63.9
Oxygenated sesquiterpenes (Sr. No. 50, 52-57)					4.7	3.3
Diterpenes (Sr. No. 58)					0.5	-

[a] Elution order on HP-5MS column, identification by RI, and MS except where stated
[b] Further identification by co-injection with known compounds
[c] Standard deviation (SD±) were insignificant and excluded from the Table to avoid congestion
R (Cal.) Retention indices on HP-5MS column
R (Lit.) Literature retention indices (NIST, 2018)
RT retention times s on HP-5MS column
- Not identified

compounds. The mixture of monoterpene and sesquiterpene compounds were found in *L.viridis* [12]. On the other hand, the mixture of sesquiterpene compounds and fatty acids were the main components in *L. coreana* from Taiwan [27]. From the present study, the oils of *L. umbellata* and *L. iteodaphne* were dominated by monoterpene and sesquiterpene compounds. However, the identity of the individual monoterpene and sesquiterpene components of the species differed. Moreover, the main components of the individual species varied from one another. It can be postulated that both intra and interspecies variation could be observed in the essential oils of *Litsea* plants.

Mortality test

The mosquito larvicidal activity of *L. umbellata* leaf essential oil was summarized in Fig. 1. From the calculated marginal effects, the essential oil of *L. umbellata* exhibited 83.7 % (50 µg/mL) and 100 % (100 µg/mL) mortality towards the larvae of *Cx. quinquefasciatus* at 24 h (Fig. 1). The same effects were observed at 48 h test period. On the other hand, *L. umbellata* leaf essential oil displayed mortality of 79 % and 86 % against *Ae. albopictus* at 24 h and 48 h respectively when the concentration was 50 µg/mL. Also, no noticeable difference was observed in the percentage mortality at 24 h (97.5 %) and 48 h (98.0 %) at a

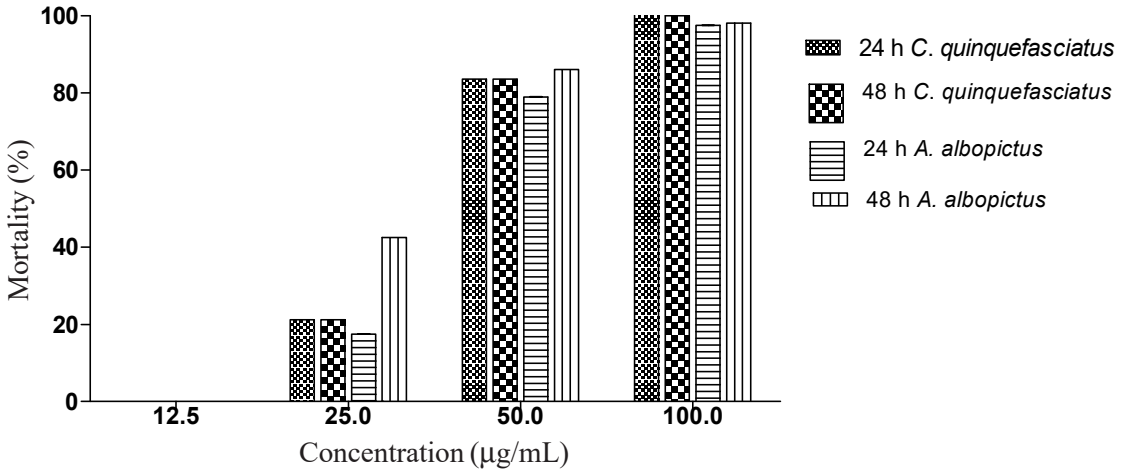

Fig. 1. Percentage mortality of *L. umbellata* towards *C. quinquefasciatus* and *A. albopictus*. Each value is mean ± SEM (n = 4)

tested concentration of 100 µg/mL (Fig. 1). Likewise, the essential oil of *L. iteodaphne* displayed a mortality of 100 % against larvae of *Ae. albopictus* and *Cx. quinquefasciatus* (Fig. 2) at concentration of 100 µg/mL, after 24 h and 48 h. Also, *L. iteodaphne* also showed a mortality of 65 % (concentration of 50 µg/mL) against *Cx. quinquefasciatus* at 48 h test period. There was no mortality in the EtOH controls. From the present data, it could be seen that the percentage of mortality was dependent on the concentration of the tested oil samples. Thus, higher inhibition of mosquito larvae was observed in the 100 µg/L compared to other concentrations. As a result, very low concentrations of the tested extracts resulted in low mortality rates. These findings showed that the concentrations of test substances affected the degree of toxicity, mortality speed and mortality rates. The essential oils of *L. cubeba* were reported previously to displayed 77 % mortality to larvae of *Ae. aegypti* mosquito vector [28].

Larvicidal activity

From Table 2, *L. umbellata* essential oil exhibited toxicity towards *Cx. quinquefasciatus* with LC_{50} of 36.19 µg/mL at 24 h and 48 h. However, the oil displayed larvicidal activity against *Ae. albopictus* with LC_{50} of 40.09 µg/mL and 27.33 µg/mL at 24 h and 48 h respectively. The LC_{90} value of 54.17 µg/mL (24 h/28 h) was recorded against *Cx. quinquefasciatus,* while the LC_{90} values of 68.79 µg/mL and 60.49 µg/mL respectively were obtained at 24 h and 48 h against *Ae. albopictus* (Table 2). The essential oil of *L. iteodaphne* exhibited significant larvicidal action against *Cx. quinquefasciatus* with LC_{50} of 37.20 µg/mL (24 h) and 20.21 µg/mL (48 h) as well as LC_{90} of 39.27 µg/mL (24 h) and 23.78 µg/mL (48 h). Moreover, larvicidal activity was observed against *Ae. albopictus* with LC_{50} of 40.04 µg/mL (24 h) and 16.63 µg/mL (48 h), while exhibiting LC_{90} values of 42.30 µg/mL (24 h) and 23.64 µg/mL (48 h). Permethrin, the standard drug used as control displayed larvicidal activity against *Cx. quinquefasciatus* and *Ae. albopictus* with LC_{50} values in the range of 2.19 - 3.43 µg/mL.

The essential oils of *L. umbellata* and *L. iteodaphne* in this present study will be categorized according to previous procedures [29,30] where the substances with LC_{50} > 100 µg/mL were considered not active, substances with LC_{50} between 100 - 50 µg/mL were considered active and those with LC_{50} < 50 µg/mL were considered highly active. Overall results in this study showed that the essential oils of *L. umbellata* and *L. iteodaphne* were considered highly active against *Ae. albopictus* and *Cx. quinquefasciatus*. The larvicidal activities of the studied essential oils are comparable to data obtained from other *Litsea* essential oils screened for mosquito larvicidal activity. For example, the essential oil of *L. petiolata* leaf was considered as an effective larvicide against *Ae. aegypti* with LC_{50} of 28.32 mg/L [31]. The leaf oil of *L. elliptica* exhibited potent

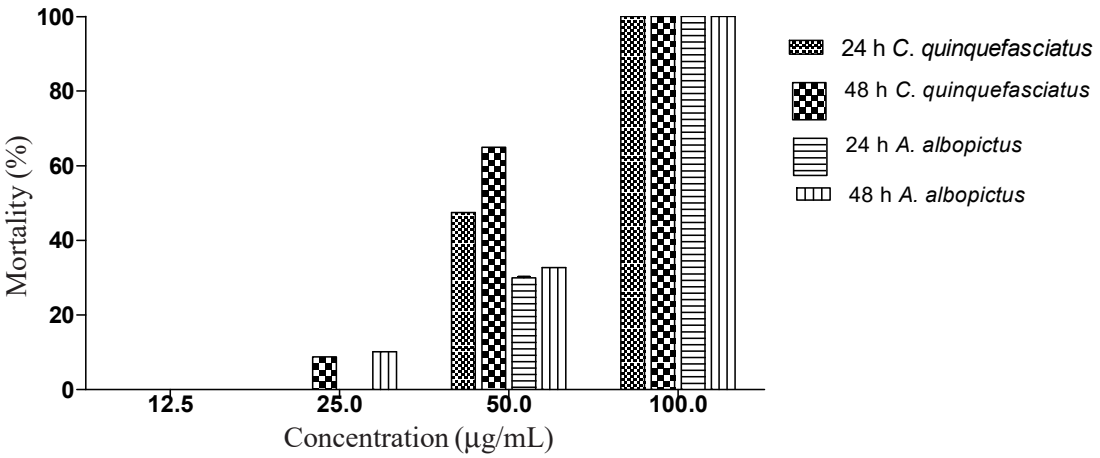

Fig. 2. Percentage mortality of *L. iteodaphne* towards *C. quinquefasciatus* and *A. albopictus* Each value is mean ± SEM (n = 4)

Table 2. Larvicidal activity of *L. umbellata* and *L. iteodaphne* essential oils

Larvae	*L. umbellata* (µg/mL)[a]	*L. iteodaphne* (µg/mL) [a]
Cx. quinquefasciatus		
LC_{50} (24 h)	36.19	37.20
LC_{90} (24 h)	54.17	39.27
Cx. quinquefasciatus		
LC_{50} (48 h)	36.19	20.21
LC_{90} (48 h)	54.17	23.78
Ae. albopictus		
LC_{50} (24 h)	40.09	40.04
LC_{90} (24 h)	68.79	42.40
Ae. albopictus		
LC_{50} (48 h)	27.33	16.63
LC_{90} (48 h)	60.49	23.64

[a](n=4)

larvicidal action against *Anophe-les maculataes* with LC_{50} of 13.61 µg/mL*, Ae. aegypti* with LC_{50} of 16.01 µg/mL and *Cx. quinquejasciatus* with LC_{50} of 14.63 µg/Ml [32]. The larvicidal activity of essential oils from *L. cubeba* and *L. salicifolia* has been reported against *An. arabiensis*, *An. gambiae* and *Cx. Quinqefasciatus* [33,34]. Similarly, *L. cubeba* oil showed the highest repellency against *Ae. albopictus* among the tested oil samples [35]. Several essential oils of *Litsea* have demonstrated good repellent efficacy against different mosquito species as compared to synthetic chemical DEET [36,37].

This study demonstrated that the essential oils of *L. umbellata* and *L. iteodaphne* exhibited good mortality and larvicidal activity towards the larva of *Ae. albopictus* and *Cx. quinquefasciatus* with median lethal concentration comparable to many other essential oils from Vietnam screened for mosquito larvicidal activity [12-14]. The mosquito larvicidal activities (LC_{50}) of essential oils against *Cx. quinquefasciatus* have generally ranged between 25.6 µg/mL and 225 µg/ML [38,39]. Thus, *Cx. quinquefasciatus* larvicidal activity of the *L. umbellata* (LC_{50} = 36.19 µg/mL) and *L. iteodaphne* (LC_{50} = 20.21-37.20 µg/mL) were good compared to other essential oils.

The observed mosquito larvicidal activity of the

essential oils may be due to individual or/and synergistic actions of the major compounds namely α-pinene, β-pinene, β-caryophyllene and germacrene D present in the oil sample. These compounds have previously shown to demonstrate larvicidal activity against the tested mosquito vectors [12-14]. Their presence in the studied essential oils may be of valuable contribution to the observed activities of the essential oils. This will enable an assessment of the potential environmental impact of using the *Litsea* essential oils as a larvicidal control agent. Literature information indicated that various researches have recorded varying degrees of activity for the tested compounds against the different mosquito vectors. For example, β-caryophyllene, α-pinene and β-pinene showed activity against *Aedes* species with LC_{50} values of 26.0 ppm, 49.5-65.7 ppm and 35.9-56.50 ppm respectively [40]. Likewise, β-pinene exhibited larvicidal activity against *Cx. quinquefasciatus* with LC_{50} values of 32.23 µg/mL [41] and 19.6 µg/mL [42]. Germacrene D was shown to be toxic towards *Cx. quinquefa-sciatus* with LC_{50} value of f 14.01 µg/mL [41] and *Ae. aegypti* with LC_{50} value of 14.01 µg/ML [41] but not *Ae. albopictus*. α-Pinene was shown to exhibit toxicity towards *Ae. albopictus* with LC_{50} in the range 34.0-74.0 µg/mL [43], with β-caryophyllene having the values of 39.52-44.77 µg/mL [43]. It seems there is a scarcity of information on the larvicidal activity of pure compounds against *Ae. albopictus* in the literature.

Conclusions

The current study enriched the information for medicinal uses and phytochemical compounds in the essential oils of the leaves of *L. umbellata* and *L. iteodaphne*. Moreover, the presence of the major compounds namely α-pinene, β-pinene, β-caryophyllene and germacrene D in the essential oil was detected and reported in this study. The obtained essential oils fraction from *L. umbellata* and *L. iteodaphne* leaves and their major compounds displayed larvicidal activity against *Cx. quinquefasciatus* and *Ae. albopictus* which can be used for the prevention of these insects and damage they can cause to human beings. Our results are a contribution to a better valorization and medicinal uses of these medicinal plants.

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