Tropical Journal of Pharmaceutical Research January 2015; 14 (1): 111-116
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i1.16

Original Research Article

Larvicidal Activity of Essential Oil Derived from *Illicium henryi* Diels (Illiciaceae) Leaf

Xin Chao Liu[1], Qi Yong Liu[2], Ligang Zhou[3] and Zhi Long Liu[1]*
[1]Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, [2]State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, 102206, [3]Department of Plant Pathology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

For correspondence:* ***Email:* *zhilongliu@cau.edu.cn;* ***Tel:*** *+86-10-62732800;* ***Fax:*** *+86-10-62732800*

Received: 26 September 2014 *Revised accepted: 16 December 2014*

Abstract

Purpose: *To determine larvicidal activity of the essential oil derived from Illicium henryi Diels (Illiciaceae) leaf and stem against the larvae of Aedes albopictus Skuse.*
Methods: *The essential oil of I. henryi leaves was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromaotography-mass spectrometry (GC-MS). The activity of the oil was evaluated, using World Health Organization (WHO) procedures, against the fourth instar larvae of A. albopictus for 24 h, and larval mortalities recorded at various essential oil concentrations ranging from 12.5 - 200 µg/mL.*
Results: *A total of 25 components of the essential oil of I. henryi were identified and the principal compounds in the essential oil were safrole (54.09 %), myristicin (22.24 %), and 1, 8-cineole (5.43 %). The essential oil has higher content of (76.48 %) of phenylpropanoids than monoterpenoids (10.79 %) and sesquiternoids (11.72 %). The essential oil exhibited larvicidal activity against A. albopictus with a median lethal concentration (LC_{50}) of 35.43 µg/mL.*
Conclusion: *The findings obtained indicate that the essential oil of I. henryi leaves has potentials for use in the control of A. albopictus larvae and may be useful in the search for newer, safer and more effective natural compounds as larvicides.*

Keywords: *Illicium henryi, Aedes albopictus, Larvicidal activity, Mosquito, Essential oil, Phenylpropanoids, Monoterpenoids, Sesquiternoids*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The Asian tiger mosquito, *Aedes albopictus* Skuse (*Stegomyia albopictus*) (Diptera: Culicidae), is one of two main species of mosquito responsible for dengue fever in China [1]. Currently, light mineral oils and insect-growth regulators and some organophosphates such as temephos, fenthion, and Malathion are used as mosquito larvicides in many countries. However, these mineral oils and organophosphates can also kill other aquatic animals such as fish and crustaceans in the water body [2]. Thus, there is an urgent need to search new materials for controlling mosquitoes in an environmentally safe way, using biodegradable and target-specific insecticides against them. Fortunately, essential oils and their constituents have been suggested as alternative sources for insect control. Many studies have demonstrated that essential oils and their individual components possess promising larvicidal activities against mosquito

vectors [3-8]. During our mass screening program for new agrochemicals from the wild plants, essential oil of *Illicium henryi* Diels leaves (Family: Illiciaceae) was found to possess larvicidal activity against the Asian tiger mosquito, *A. albopictus* Skuse.

Illicium henryi is an evergreen shrub or tree, 3 to 8 m (up to 12 m) tall and distributed in the southwestern part of China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, and Yunnan Province). Its root bark is locally used as a folk medicinal herb for repelling pathogenic wind and assuaging pain in China, and the fruits are poisonous [9]. In the previous phytochemical investigations, various sesquiterpene lactones, lignans, flavonoids, triterpenoids, and prenylated C6-C3 compounds have been isolated and identified from the plant [10-12]. Chemical composition of the essential oil of *I. henryi* fruits has been analyzed previously [13]. However, a literature survey has shown that there is no report on chemical composition and larvicidal activity of the essential oil of *I. henryi* leaves against mosquitoes, thus we decided to investigate chemical composition and larvicidal activity of the essential oil of *I. henryi* leaves against the Asian tiger mosquito.

EXPERIMENTAL

Plant collection and identification

Fresh leaves of *I. henryi* (10 kg) were harvested from Liankang Mountain Nature Reserve Conservation Zone (31.54 ^{o}N and 114.50 ^{o}E, Xinxian, Henan Province, China) in September 2013. The herb was identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU- Illiciaceae-10216) was deposited at the herbarium of Department of Entomology, China Agricultural University.

Extraction of the essential oil

The leaves (10 kg) were air-dried, ground to powder using a grinding mill (Retsch Muhle, Haan, Germany). Each portion of the powder (600 g) was soaked in water at a ratio of 1:3 (w/v) for 3 h, prior to hydrodistillation using a round bottom flask (3000 mL) over a period of 6 h. The volatile essential oil was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separating funnel using n-hexane and the n-hexane layer was then dried over anhydrous Na_2SO_4. The solvent was evaporated at 40 ^{o}C using a Buchi Rotavapor R-124 vacuum rotary evaporator. The oil was kept in a refrigerator at 4 ^{o}C pending subsequent experiments.

Analysis of the essential oils

Capillary gas chromatography was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector and fused silica capillary column HP-5 (5 % diphenyl and 95 % dimethylpolysyloxane, 30 m × 0.25 mm, 0.25 µm film thickness), at a flow rate of 1 mL min^{-1}. Temperature was programmed from 60 to 280 °C (at a rate of 2 °C min−1); injector and detector temperatures were 270 and 300 ^{o}C, respectively. The components of the essential oil were separated and identified by gas chromatography–mass spectrometry (GC-MS) using Agilent 6890N gas chromatography coupled to Agilent 5973N mass selective detector. The system was equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 µm). GC settings were as follows: the initial oven temperature was held at 60 ^{o}C for 1 min and ramped at 10 ^{o}C min^{-1} to 180 ^{o}C where it was held for 1 min, and then ramped at 20 ^{o}C min^{-1} to 280 ^{o}C and held there for 15 min. The injector temperature was maintained at 270 ^{o}C. The samples (1 µL, diluted to 100:1 with acetone) were injected, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 ml min^{-1}. Spectra were obtained over the scan range 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those published in the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8-C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [14]. Relative contents of the oil components were calculated based on GC peak areas without applying correction factors.

Insect cultures and rearing conditions

Mosquito eggs of *A. albopictus* utilized in bioassays were obtained from a laboratory colony maintained in the Department of Vector Biology and Control, Institute for Infectious Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The original eggs of *A. albopictus* were collected from Nanjing, Jiangsu province, China in 1997. Adults were maintained in a cage (60 × 30 × 30 cm) at

28 – 30 °C and 75 – 85 % RH. The females were fed with blood every alternate day whereas the males were fed with 10 % glucose solution soaked on cotton pad, which were hung in the middle of the cage. A beaker with strips of moistened filter paper was kept for oviposition. The eggs laid on paper strips were kept wet for 24 h and then dehydrated at room temperature. The dehydrated eggs were put into plastic tray containing tap water in our laboratory at 26 – 28 °C and natural summer photoperiod for hatching and yeast pellets served as food for the emerging larvae. The newly emerged larvae were then isolated in groups of ten specimens in 100 ml tubes with tap water and a small amount of cat food. Larvae were daily controlled until they reached the fourth instar, when they were utilized for bioassays (within 12 h).

Larvicidal bioassay

Range-finding studies were run to determine the appropriate testing concentrations. The essential oil was first diluted to 10 % (v/v) using acetone as a solvent and final concentrations of 200, 100, 50, 25, and 12.5 µg/mL of the essential oil were tested. The larval mortality bioassay was carried out according to the test method for larval susceptibility recommended by the World Health Organization (WHO) [15]. Twenty larvae were placed in glass beaker with 250 ml of aqueous suspension of tested material at various concentrations, and an emulsifier dimethyl sulfoxide (DMSO) was added in the final test solution (0.05 %). Five replicates per concentration were run simultaneously and with each experiment, a set of controls using 0.05 % DMSO and acetone and untreated sets of larvae in tap water, were also run for comparison. For comparison, commercial chlorpyrifos (purchased from National Center of Pesticide Standards, Tiexi District, Shenyang 110021 China) was used as positive control. The toxicity of chlorpyrifos was determined at concentrations of 5, 2.5, 1.25, 0.6, and 0.3 µg/mL. The assay was carried out in a growth chamber (L16:D9, 26 - 27 ^{o}C, 78 - 80 % relative humidity). Mortality was recorded after 24 h of exposure.

Statistical analysis

Percent mortality was corrected for control mortality using Abbott's formula [19]. Results from all replicates for the oil were subjected to probit analysis using PriProbit Program V1.6.3 (http://ars.usda.gov/Services/docs.htm?docid=11284) to determine LC_{50} values and their 95 % confidence intervals [17]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The yield of *I. henryi* essential oil was 0.23 % (v/w) while its density was determined to be 0.96 g/mL. A total of 25 components from the essential oil of *I. henryi* leaves were identified, accounting for 98.90 % of the total essential oil. The principal constituents of the essential oil of *I. henryi* leaves were safrole (54.09 %), myristicin (22.24 %), and 1, 8-cineole (5.43 %) (Table 1).

The essential oil possessed strong larvicidal activity against the 4th instar larvae of *A. albopictus* with a LC_{50} value of 35.43 µg/mL (Table 2).

DISCUSSION

The major constituents of the essential oil of *I. henryi* leaves were safrole, myristicin, and 1, 8-cineole (Table 1). The essential oil has higher content of phenylpropanoids (76.48 %) than monoterpenoids (10.79 %) and sesquiternoids (11.72 %). This is the first time to report the composition of the essential oil derived from *I. henryi* leaves. However, the essential oil of *I. henryi* fruits contained limonene (12.4 - 32.8 %), caryophyllene (1.5 - 8.5 %), safrole (0.4 - 2.7 %) and 1, 8-cineole (1.2 - 2.1 %) [13].

The essential oil of *I. henryi* leaves demonstrated larvicidal activity against the fourth instar larvae of *A. albopictus*. The commercial insecticide, chlorpyrifos showed larvicidal activity against the mosquitoes with a LC_{50} value of 1.86 µg/ml thus the essential oil of *I. henryi* leaves was only 19 times less toxic to *A. albopictus* larvae compared with chlorpyrifos. However, compared with the other essential oils using the same bioassay in the literature, essential oil of *I. henryi* leaves exhibited the same level or stronger larvicidal activity against *A. albopictus* larvae, e.g. essential oils of *Foeniculum vulgare* fruits (LC_{50} = 142.9 µg/ml) [18], *Eucalyptus urophylla* leaves (LC_{50} = 95.5 µg/ml) [19], *Allium macrostemon* bulbs (LC_{50} = 72.86 µg/ml) [6], *Toddalia asiatica* roots (LC_{50} = 69.09 µg/ml) [5], *Zanthoxylum avicennae* leaves (LC_{50} = 48.79 µg/ml) [7], aerial parts of *Salvia elegans* and *S. splendens* (LC_{50} = 46.4 ppm and LC_{50} = 59.2 ppm, respectively) [20] and *Clinopodium gracile* aerial parts (42.56 µg/ml) [4].

In the previous studies, one of the major constituent, safrole was found to possess larvicidal activity against two mosquitoes, *A. aegypti* and *A. albopictus* [21,22].

Table 1: Main compounds of the essential oil of *Illicium henryi* leaves

Peak no.	Compound	Retention index	(%)
	Monoterpenoids		***10.70***
1	α-Pinene	939	0.57
2	Camphene	954	0.11
3	β-Pinene	974	0.20
4	Sabinene	975	0.16
5	β-Myrcene	991	0.09
6	β-Phellandrene	1030	0.12
7	1,8-Cineole	1032	5.43
8	Linalool	1094	1.12
9	Borneol	1174	0.85
10	4-Terpineol	1177	0.96
11	α-Terpineol	1189	1.09
	Sesquiterpenoids		***11.72***
12	Copaene	1375	0.21
13	β-Elemene	1389	0.14
14	Caryophyllene	1420	0.88
15	α-Santalene	1422	0.34
16	β-Gurjunene	1434	0.11
17	Germacrene D	1485	0.18
18	Calamenene	1520	1.24
19	δ-Cadinene	1523	2.65
20	α-Calacorene	1540	0.76
21	Spathulenol	1578	1.87
22	α-Cadinol	1654	3.34
	Phenylpropanoids		***76.48***
23	***Safrole***	**1287**	**54.09**
24	Eugenol	1356	0.15
25	**Myristicin**	**1517**	**22.24**
	Total identified		98.90

Table 2: Larvicidal activity of *Illicium henryi* essential oil against fourth-instar larvae of *Aedes albopictus*

Treatment	LC_{50} (μg/mL) (95% CL)	LC_{95} (μg/mL) (95% CL)	Slope ± SD	Chi-square value (χ^2)
I. henryi	35.43	143.12	7.97 ± 0.68	11.12
Mean range	(31.78-38.23)	(134.63-162.87)		
Chlorpyrifos	1.86	6.65	0.87 ± 0.01	3.13
Mean range	(1.71-2.05)	(6.21-7.48)		

However, myristicin was shown to have contact and fumigant toxicity against several insect/mites and to exhibit strong synergistic activity because it has been demonstrated to possess strong inhibitory effects on many P450s, such as houseflies (*Musca domestica*) [23], hairy caterpillars (*Spilarctia obliqua*) [24], armyworms (*Pseudaletia unipuncta*) [25], navel orangeworm (*Amyelois transitella*) [26], and house dust mites (*Dermatophagoides farinae, D. pteronyssinus*) and mould mites (*Tyrophagus putrescentiae*) [27]. The isolation and identification of the bioactive compounds in the essential oil are of utmost importance to determine if their potential application in controlling mosquito pests can be fully exploited. Considering that the currently used larvicides are synthetic insecticides, larvicidal activity of the crude essential oil is quite promising and it shows its potential for use in the control of *A. albopictus* larvae and could be useful in the search for newer, safer and more effective natural compounds as larvicides.

A literature survey has shown that there is no experimental data on toxicity of the essential oil of *I. henryi* leaves to human is available, to the best of our knowledge. Thus, to develop a practical application for the essential oil as novel insecticides, further research into the safety of the essential oil to humans is needed. The isolation and identification of the bioactive constituent compounds in the essential oil of *I. henryi* leaves are of utmost importance so that their potential application in controlling mosquitoes can be fully exploited. Additional studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost. Moreover, field evaluation and further investigations on the

effects of the essential oil on non-target organisms are necessary.

CONCLUSION

The essential oil of *I. henryi* leaves demonstrates some activity against *Aedes albopictus* mosquito larva but needs to be further evaluated for safety in humans and to develop formulations to improve the efficacy and stability as well as reducing cost.

ACKNOWLEDGEMENT

This work was funded by Hi-Tech Research and Development of China (grant no. 2011AA10A202). The authors are grateful to Dr Liu QR from College of Life Sciences, Beijing Normal University, Beijing, China for the identification of the investigated plant. The authors also thank Yang H and Zhang SQ for help with collection of the plant.

REFERENCES

1. *Liu ZL, Liu QZ, Du SS, Deng ZW. Larvicidal activity of alkaloids and limonoids derived from Evodia rutaecarpa unripe fruits against Aedes albopictus (Diptera: Culicidae). Parasitol Res 2012; 111: 991-996.*
2. *Isman MB. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006; 51: 45-66.*
3. *Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZW. Essential oil composition and larvicidal activity of Saussurea lappa roots against the mosquito Aedes albopictus (Diptera: Culicidae). Parasitol Res 2012; 110: 2125-2130.*
4. *Chen XB, Liu XC, Zhou L, Liu ZL. Essential oil composition and larvicidal activity of Clinopodium gracile (Benth.) Matsum (Labiatae) aerial parts against the Aedes albopictus mosquito. Trop J Pharm Res 2013; 12: 799-804.*
5. *Liu XC, Dong HW, Zhou L, Du SS, Liu ZL. Essential oil composition and larvicidal activity of Toddalia asiatica roots against the mosquito Aedes albopictus (Diptera: Culicidae). Parasitol Res 2013; 112: 1197-1203.*
6. *Liu XC, Liu Q, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of Allium macrostemon Bunge and its selected major constituent compounds against Aedes albopictus (Diptera: Culicidae). Parasit. Vector 2014; 7: 184.*
7. *Liu XC, Liu Q, Zhou L, Liu QR, Liu ZL. Chemical composition of Zanthoxylum avicennae essential oil and its larvicidal activity on Aedes albopictus Skuse. Trop J Pharm Res 2014; 13: 399-404.*
8. *He Q, Liu XC, Sun RQ, Deng ZW, Du SS, Liu ZL. Mosquito larvicidal constituents from the ethanol extract of Inula racemosa Hook.f. Roots against Aedes albopictus. J Chem 2014; doi:10.1155/2014/738796*
9. *Editorial Board of Flora of China of China Academy of Sciences. Flora Reipublicae Popularis Sinicae, Vol. 30, no. 1, Science Press, Beijing, China; 1996; pp 226-227. [cited 2014 July 12]. Available from: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200008457*
10. *Xiang WJ, Ma L, Hu LH. Neolignans and flavonoids from the root bark of Illicium henryi. Fitoterapia 2010; 81: 1228-1231.*
11. *Liu JF, Wang YF, Bi YP, Li HJ, Jia L, Bi YF, Zhang YB. Unusual nor-sesquiterpene lactone from the fruits of Illicium henryi. Tetrahedron Lett 103; 54: 4834-4836.*
12. *Zhuang PY, Ma SG, Zhang GJ, Wang XJ, Zhang Y, Yu SS, Liu YB, Qu J, Li Y, Hou Q. New prenylated C6-C3 compounds from the roots of Illicium henryi. Phytochem Lett 2013; 6: 444-448.*
13. *Howes MJR, Kite GC, Simmonds MSJ. Distinguishing Chinese star anise from Japanese star anise using thermal desorption-gas chromatography-mass spectrometry. J Agric Food Chem 2009; 57: 5783-5789.*
14. *Adams RP. Identification of Essential Oil Components by Gas Chromatograph /Mass Spectrometry. 4th edn. Allured Publishing Corporation, Carol Stream, USA, 2007.*
15. *World Health Organization. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticide. WHO/VBC/81.80. 1981.*
16. *Finney DJ. Probit analysis. 3rd edn. Cambridge University, London, 1971.*
17. *Sakuma M. Probit analysis of preference data. Appl Entomol Zool 1998; 33: 339-347.*
18. *Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito Aedes albopictus (Diptera: Culicidae). Parasitol Res 2010; 107: 1455-1461.*
19. *Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. Bioresour Technol 2009; 100: 452-456.*
20. *Mathew J, Thoppil JE. Chemical composition and mosquito larvicidal activities of Salvia essential oils. Pharm Biol (London) 2011; 49: 456-463.*
21. *Jantan I, Yalvema MF, Ahmad NW, Jamal JA. Insecticidal activities of the leaf oils of 8 Cinnamomum species against Aedes aegypti and Aedes albopictus. Pharm Biol (Philadelphia, USA) 2005; 43: 526-532.*
22. *Cheng SS, Chang HT, Lin CY, Chen PS, Huang CG, Chen WJ, Chang ST. Insecticidal activities of leaf and twig essential oils from Clausena excavata against*

Aedes aegypti and Aedes albopictus larvae. Pest Manag Sci 2009; 65: 339-343.

23. *Fuhremann TW, Lichtenstein EP. Insecticide toxicity and degradation in houseflies as affected by naturally occurring food plant components. J Agric Food Chem 1979; 27: 87-91.*
24. *Srivastava S, Gupta MM, Prajapati V, Tripathi AK, Kumar S. Insecticidal activity of myristicin from Piper mullesua. Pharm Biol (Lisse, Netherlands) 2001; 39: 226-229.*
25. *Passreiter CM, Akhtar Y, Isman MB. Insecticidal activity of the essential oil of Ligusticum mutellina roots. Z Naturforsch 2005; 60(C): 411-414.*
26. *Niu G, Pollock HS, Lawrance A, Siegel JP, Berenbaum MR. Effects of a naturally occurring and a synthetic synergist on toxicity of three insecticides and a phytochemical to navel orangeworm (Lepidoptera: Pyralidae). J Econ Entomol 2012; 105: 410-417.*
27. *Song HY, Yang JY, Suh JW, Lee HS. Acaricidal activities of apiol and its derivatives from petroselinum sativum seeds against Dermatophagoides pteronyssinus, Dermatophagoides farinae, and Tyrophagus putrescentiae. J Agric Food Chem 2011; 59: 7759-7764.*