

Original Research Article

Larvicidal Activity of *Isodon japonicus* var. *glaucoalyx* (Maxim.) H.W.Li Essential Oil to *Aedes aegypti* L. and its Chemical Composition

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Abstract

Purpose: To determine the larvicidal activity of the essential oil derived from *Isodon japonicus* var. *glaucoalyx* (Maxim.) H.W.Li (*Labiatae*) aerial parts at flowering stage against the larvae of *Aedes aegypti* L.

Methods: The essential oil of *I. japonicus* var. *glaucoalyx* aerial parts was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The activity of the essential oil was evaluated, using World Health Organization (WHO) procedures, against the fourth larvae of *A. aegypti* for 24 h, and larval mortality recorded at various essential oil concentrations ranging from 12.5 - 200 µg/mL.

Results: A total of 34 components of the essential oil of *I. japonicus* var. *glaucoalyx* were identified. The principal compounds of the essential oil were thujone (9.65 %), morillo (8.14 %), caryophyllene oxide (7.68 %), β-caryophyllene (7.60 %), α-terpineol (7.22 %), 1,8-cineole (7.09 %), linalool (5.56 %), Z-caryophyllene (5.10 %) and γ-eudesmol (4.71 %). The essential oil exhibited larvicidal activity against *A. aegypti* with a median lethal concentration (LC₅₀) of 40.82 µg/mL.

Conclusion: The findings indicate that the essential oil of *I. japonicus* var. *glaucoalyx* aerial parts has potentials for use in the control of *A. aegypti* larvae and may be useful in the search for newer, safer and more effective natural compounds for use as larvicides.

Keywords: *Isodon japonicus* var. *glaucoalyx*, *Aedes aegypti*, Larvicidal activity, Mosquito, Essential oil

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INTRODUCTION

Mosquitoes play an important role in the transmission of malaria, dengue fever, yellow fever, chikungunya, filariasis, several forms of encephalitis and filariasis as well as several diseases which are today among the greatest health problems in the world. The yellow fever mosquito (*Aedes aegypti* L) and the Asian tiger mosquito (*A. albopictus* Skuse) and are two main

species of mosquito responsible for dengue fever in China.

The use of synthetic insecticides (organophosphates such as dichlorvos, temephos and fenthion) and insect growth regulators (such as diflubenzuron and methoprene) is the most widespread method for control of mosquito larvae [1]. However, heavily repeated use of these synthetic insecticides has

fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organism [2]. Thus, there is urgent need to look for alternative approaches for mosquito control. It is suggested that many essential oils and constituent compounds derived from various essential oils can exert toxic activity against mosquito species [3-6].

During the present author's mass screening program for new agrochemicals from wild plants and Chinese medicinal herbs, the essential oil of *Isodon japonicus* var. *glaucoalyx* (Maxim.) H.W.Li (Family: Labiatae) aerial parts, was found to possess larvicidal activity against the yellow fever mosquito, *A. aegypti*.

I. japonicus var. *glaucoalyx* is widely distributed in northern China (Hebei, Heilongjiang, Jilin, Liaoning, Shandong, and Shanxi Province) and Japan, Korea as well as Russia [7]. It has been used as folk medicine in China for the treatment of hepatitis, gastricism, mastitis, and cough [8]. Phytochemical analysis of *I. japonicus* var. *glaucoalyx* collected from different regions led to the identification of several diterpenoids [9-15]. Glaucoalyxin A (ent-kaurane diterpenoid), the main constituent in *I. japonicus* var. *glaucoalyx*, exhibited inhibiting tumor cell proliferation by inducing apoptosis in human leukemia HL-60 cells through the mitochondria-mediated death pathway [16]. However, a literature survey has shown that there is no report on larvicidal activity of *I. japonicus* var. *glaucoalyx* essential oil against mosquitoes. Hence, the objective of the present study was to investigate the chemical constituents and larvicidal activity of the essential oil of the plant against yellow fever mosquito.

EXPERIMENTAL

Plant collection and identification

Fresh aerial parts at flowering stage of *I. japonicus* var. *glaucoalyx* (15 kg) were harvested in September 2013 from Xiaolongmeng National Forest Park (Mentougou District, Beijing 102300). The herb was identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU-Labiatae-Lanexiangchacai-10012) was deposited at the herbarium of Department of Entomology, China Agricultural University.

Extraction and isolation of essential oil

The samples was air-dried, ground to powder using a grinding mill (Retsch Muhle, Germany), subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and then extracted with n-hexane. The oil was dried over anhydrous Na₂SO₄ and kept in a refrigerator (4 °C) pending subsequent experiments.

Analysis of the essential oil

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 % dimethylpolysiloxane, 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 °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 °C for 1 min and ramped at 10 °C min⁻¹ to 180 °C where it was held for 1 min, and then ramped at 20 °C min⁻¹ to 280 °C and held there for 15 min. The injector temperature was maintained at 270 °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. Retention index was determined in relation to a homologous series of n-alkanes (C₈ – C₂₄) 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 [17]. 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. aegypti* 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 dehydrated eggs were placed on a plastic tray containing tap water to hatch and yeast pellets served as food for the emerging larvae. The eggs batches, collected daily, were kept wet for 24 h and then placed in distilled water in the laboratory at 24-26 °C and natural summer photoperiod for hatching. The newly emerged larvae were then isolated in groups of ten specimens in 100 ml tubes with mineral water and a small amount of dog or cat food. Larvae were daily controlled until they reached the fourth instar stage, when they were utilized for bioassay (within 12 h).

Larvicidal bioassay

Range-finding studies were run to determine the appropriate testing concentrations. Concentrations of 200, 100, 50, 25, and 12.5 µg/mL of essential oil were tested. The larval mortality bioassay was carried out according to the test method for larval susceptibility proposed by the World Health Organization (WHO) [18]. 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 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 (Ningbo Jiangnan Instrument Factory, Ningbo 315012, China. <http://www.nb-jn.com>) (L16:D9, 26-27°C, 78-80 % relative humidity). Mortality was recorded after 24 h of exposure and the larvae were starved of food over this period.

Statistical analysis

Percent mortality was corrected for control mortality using Abbott's formula [19]. Results from all replicates for the pure compounds/oil were subjected to probit analysis using Probit Program V1.6.3 (<http://ars.usda.gov/Services/docs.htm?docid=11284>) to determine LC₅₀ values and their 95 % confidence intervals [20]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The yield of essential oil from *I. japonicus* var. *glaucocalyx* aerial parts at flowering stage was 0.14 % (v/w) while its density was determined to be 0.86 g/mL. A total of 34 components of the essential oil of *I. japonicus* var. *glaucocalyx* were identified (Table 1). The principal compounds of the essential oil were thujone (9.65 %), morillool (8.14 %), caryophyllene oxide (7.68 %), β-caryophyllene (7.60 %), α-terpineol (7.22 %), 1,8-cineole (7.09 %), linalool (5.56 %), Z-caryophyllene (5.10 %) and γ-eudesmol (4.71 %). Monoterpenoids represented 13 of the 34 compounds, corresponding to 41.27 % of the whole essential oil while 18 of the 34 constituents were sesquiterpenoids corresponding to 43.16 % of the essential oil of *I. japonicus* var. *glaucocalyx* aerial parts.

The essential oil possessed strong larvicidal activity against the 4th instar larvae of *A. aegypti* with a LC₅₀ value of 40.82 µg/mL (Table 2).

DISCUSSION

The main constituents of *I. japonicus* var. *glaucocalyx* essential oil were thujone, morillool, caryophyllene oxide, β-caryophyllene, α-terpineol, 1,8-cineole, linalool, Z-caryophyllene and γ-eudesmol. Its chemical composition was quite different from that reported in the previous studies [21,22]. For example, the major compounds in the essential oil of *I. japonicus* var. *glaucocalyx* aerial parts collected from Gansu province, China were 2-ethoxy propane (15.49 %), 2-methyl hexane (8.06 %) and methyl salicylate (3.58 %) [21]. However, the essential oil of *I. japonicus* var. *glaucocalyx* aerial parts harvested from Shandong province, China contained 50 constituent compounds and the main constituents were germane A (17.92 %), β-caryophyllene (14.73 %), (Z)-β-ocimene (14.34 %), β-pinene (8.14 %), 1,8-cineole (7.78 %) and α-pinene (4.33 %) [22]. The above results suggest that there were some variations in chemical composition of essential oil of *I. japonicus* var. *glaucocalyx* collected from different sites and at different collect times. Studies on plant cultivation and essential oil standardization are needed because chemical composition of essential oil varies greatly with plant population.

The essential oil of *I. japonicus* var. *glaucocalyx* possessed strong larvicidal activity against the 4th instar larvae of *A. aegypti*. The commercial insecticide, chlorpyrifos showed larvicidal activity

Table 1: Main compounds of the essential oil of *Isodon japonicus* var. *glaucocalyx* aerial parts

Peak no.	Compound	Retention index	(%)
1	α -Pinene	939	1.29
2	Camphene	954	0.17
3	β -Pinene	974	1.82
4	Morillool	979	8.14
5	3-Octanone	984	3.06
6	β-Myrcene	991	0.23
7	ρ-Cymene	1025	0.14
8	Limonene	1029	3.78
9	1,8-Cineole	1034	7.09
10	(Z)-β-Ocimen	1037	2.28
11	γ -Terpinene	1059	0.32
12	Linalool	1097	5.56
13	Thujone	1105	9.65
14	4-Terpineol	1177	1.72
15	α-Terpineol	1188	7.22
16	Eugenol	1356	2.62
17	<i>Copaene</i>	1375	0.48
18	<i>β-Elemene</i>	1389	0.13
19	Z-Caryophyllene	1409	5.10
20	β-Caryophyllene	1420	7.60
21	β -Gurjunene	1434	0.27
22	α -Caryophyllene	1454	2.86
23	γ -Selinene	1475	0.11
24	Germacrene D	1484	0.69
25	β -Ionone	1487	2.62
26	<i>α-Muurolene</i>	1498	2.06
27	Germacrene A	1505	0.16
28	Dihydroactinidiolide	1522	2.14
29	δ -Cadinene	1524	0.12
30	Spathulenol	1578	3.32
31	Caryophyllene oxide	1583	7.68
32	γ -Eudesmol	1621	4.71
33	α -Cadinol	1648	2.68
34	Phytol	2119	0.43
	Total identified		98.25
	Monoterpenoids		41.27
	Sesquiterpenoids		43.16
	Others		13.82

*RI = retention index

Table 2: Larvicidal activity of *Isodon japonicus* var. *glaucocalyx* essential oil against fourth-instar larvae of *Aedes aegypti*

Treatment	LC ₅₀ (μ g/mL) (95% CL)	LC ₉₅ (μ g/mL) (95% CL)	Slope \pm SD	Chi-square value (χ^2)
<i>I. japonicus</i> var. <i>glaucocalyx</i>	40.82 (36.86-44.43)	134.17 (121.34-147.65)	4.65 \pm 0.43	11.32*
<i>Chlorpyrifos</i>	1.53 (1.36-1.75)	5.34 (4.78-5.88)	0.93 \pm 0.04	4.24*

* Significant at $p < 0.05$ level

against the mosquitoes with a LC₅₀ value of 1.53 μ g/mL, thus the essential oil of *I. japonicus* var. *glaucocalyx* was 27 times less toxic to *A. aegypti* larvae compared with chlorpyrifos. However, compared with the other essential oils/extracts in the literature, the essential oil of *Z. avicennae* exhibited the same level of or stronger larvicidal activity against *I. japonicus* var. *glaucocalyx* larvae, e.g., essential oil of *Eucalyptus urophylla*

(LC₅₀ = 95.5 μ g/mL) [23]; essential oils from four *Guarea* species (*G. humaitensis* branches, LC₅₀ = 48.6 μ g/mL; *G. scabra* leaves, LC₅₀ = 98.6 μ g/mL; *G. silvatica* leaves, LC₅₀ = 117.9 μ g/mL and *G. convergens* branches 145.1 μ g/mL) [24]; leaf essential oil of *Cryptomeria japonica* (LC₅₀ = 56.8 μ g/mL) [25] and leaf and twig essential oils from *Clausena excavata* (LC₅₀ = 40.1 μ g/mL) [26]. However, the essential oil of *I. japonicus*

var. *glaucocalyx* possessed weaker larvicidal activity against *A. albopictus* larvae than essential oil of *Eucalyptus camaldulensis* (LC₅₀ = 31.0 µg/mL) [23] and hexane extract of *Acorus calamus* (LC₅₀ = 4.44 ppm) [27].

In previous reports, one of the main constituent compounds of the essential oil, β-caryophyllene was demonstrated to possess larvicidal activity against *A. aegypti* larvae with a 48 h LC₅₀ value of 34 µg/mL [28]. Caryophyllene oxide exhibited strong larvicidal activity against *A. albopictus* larvae with a 24 h LC₅₀ value of 65.6 µg/mL while another main constituents, 1,8-cineol, linalool and α-terpineol exhibited weaker larvicidal activity (LC₅₀ > 100 µg/mL) [23]. Although 1,8-cineole did not exhibit any significant mosquito larvicidal activity, it was moderately effective as a feeding repellent and highly effective as an ovipositional repellent against adult yellow fever mosquito (*A. aegypti*) [29]. However, another three of main constituents, morillool and thujone and Z-caryophyllene have not been evaluated for larvicidal activity against mosquitoes so far. The isolation and identification of the bioactive compounds in the essential oil of *I. japonicus* var. *glaucocalyx* 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. aegypti* larvae and could be useful in the search for newer, safer and more effective natural compounds as larvicides.

For the actual use of *I. japonicus* var. *glaucocalyx* essential oil and its constituents as novel larvicides or insecticides to be realized, further research is needed to establish their human safety and environmental safety. In traditional Chinese medicine, the plants are used to treat hepatitis, gastricism, mastitis, and cough [8] and appear to be safe for human consumption. However, no experimental data on its toxicity to human is available, to the best of our knowledge. Additionally, their larvicide modes of action have to be established, and formulations for improving larvicidal potency and stability need to be developed. Furthermore, field evaluation and further investigation of the effects of the essential oil on non-target organisms are necessary.

CONCLUSION

The essential oil of *I. japonicus* var. *glaucocalyx* demonstrates some activity against *Aedes aegypti* mosquito larva but needs to be further

evaluated for safety in humans and to enhance its activity.

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