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Original Research Article

Chemical Composition of *Zanthoxylum avicennae* Essential Oil and its Larvicidal Activity on *Aedes albopictus* Skuse

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Abstract

Purpose: To determine the larvicidal activity of the essential oil derived from *Zanthoxylum avicennae* (Lam.) DC. (Rutaceae) leaves and stems against the larvae of *Aedes albopictus* Skuse.**Methods:** Essential oil of *Z. avicennae* leaves and stems were 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. albopictus* for 24 h and larval mortality recorded at various essential oil concentrations ranging from 12.5 - 200 µg/mL.**Results:** A total of 31 components of the essential oil of *Z. avicennae* were identified. The essential oil had higher content of monoterpenoids (65.70 %) than sesquiterpenoids (33.45 %). The principal compounds of the essential oil were 1,8-cineol (53.05 %), β-elemene (6.13 %), α-caryophyllene (5.96 %), β-caryophyllene (5.09 %) and caryophyllene oxide (4.59 %). The essential oil exhibited larvicidal activity against *A. albopictus* with a median lethal concentration (LC₅₀) value of 48.79 µg/mL.**Conclusion:** The findings obtained indicate that the essential oil of *Z. avicennae* has potentials 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.**Keywords:** *Aedes albopictus*, Essential oil, Larvicidal activity, Mosquito, *Zanthoxylum avicennae*.

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INTRODUCTION

Beyond their itchy, irritating bites, mosquitoes are vectors for some of humanity's most deadly illnesses, such as yellow fever, dengue fever, malaria, several forms of encephalitis and filariasis. The Asian tiger mosquito (*Aedes albopictus* Skuse) and the yellow fever mosquito (*A. aegypti* L.) are two main species of mosquito

responsible for dengue fever and malaria in China.

All mosquitoes need water to breed. The control of mosquito larvae worldwide depends currently on continued application of synthetic insecticides and insect growth regulators [1]. However, repeated use of these synthetic insecticides has caused 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 new strategies for mosquito control.

From this point of view, botanical pesticides, including essential oils, are promising since they are effective, environmentally friendly, easily biodegradable, and often inexpensive [2]. It is suggested that many essential oils and constituent compounds derived from various essential oils can exert toxic activity against mosquito species [3-5]. During our mass screening program for new agrochemicals from wild plants and Chinese medicinal herbs, the essential oil of *Zanthoxylum avicennae* (Lam.) DC. (Family: Rutaceae) leaves and stems, was found to possess larvicidal activity against the Asian tiger mosquito, *A. albopictus*.

Zanthoxylum avicennae is an evergreen shrub distributed in India, Indonesia, Malaysia, Philippines, Thailand, Vietnam, southern China (Fujian, Guangdong, Guangxi, Hainan, and Yunnan Province) and Taiwan [6]. It is used as a folk medicine for treatment of rheumatism, abdominal pain, jaundice, chronic hepatitis, and common cold in China [7]. The chemical constituents of this medicinal herb have been studied. Alkaloids, coumarins, terpenoids, flavonoids, and neolignans, and their derivatives were isolated from this plant in previous studies [8-13]. Chemical composition of the essential oil of *Z. avicennae* has also been determined [14-16]. However, a literature survey has shown that there is no report on larvicidal activity of *Z. avicennae* essential oil against mosquitoes, thus we decided to investigate the chemical constituents and larvicidal activity of the essential oil against the Asian tiger mosquito.

EXPERIMENTAL

Plant collection and identification

Fresh aerial parts (leaves and stems) of *Z. avicennae* (5 kg) were harvested from Qujing City (25.51° N latitude and 103.79° E longitude, Yunnan Province, China) in October 2012. The herb was identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU- Rutaceae-Yingbubo-10002) was deposited at the herbarium of Department of Entomology, China Agricultural University. Extraction and isolation of essential oil.

The samples were air-dried for two weeks, 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. The retention indices were 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 National Institute of Standards and Technology 05 (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

The 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 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 150 groups of ten specimens in 100 ml tubes with 50 mL of mineral water and a small amount of cat food (3:1, dried bread and poulder of pork liver). 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>) with L16:D9, 26-27°C, 78-80 % relative humidity. Mortality was recorded after 24 h of exposure to essential oil 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 PriProbit Program V1.6.3 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 *Z. avicennae* essential oil was 0.07 % (v/w) while its density was determined to be

0.85 g/mL. A total of 31 components of the essential oil of *Z. avicennae* were identified (Table 1). The principal compounds in *Z. avicennae* essential oil were 1,8-cineol (53.05 %), β-elemene (6.13 %), α-caryophyllene (5.96 %), β-caryophyllene (5.09 %) and caryophyllene oxide (4.59 %). Monoterpenoids represented 15 of the 31 compounds, corresponding to 65.70 % of the whole essential oil while 14 of the 31 constituents were sesquiterpenoids corresponding to 33.45 % of the essential oil of *Z. avicennae*.

The essential oil possessed strong larvicidal activity against the 4th instar larvae of *A. albopictus* with a LC₅₀ value of 48.79 µg/mL (Table 2).

DISCUSSION

The main constituents of *Z. avicennae* essential oil were 1,8-cineol, β-elemene, α-caryophyllene, β-caryophyllene and caryophyllene oxide. Its chemical composition was quite different from that reported in the previous studies [14-16]. For example, the major compounds in the essential oil of *Z. avicennae* fruits collected from Hainan province, China were sylvestrene (50.0%), α-pinene (16.0%), and octanal (8.7%) [14]. The essential oil of *Z. avicennae* leaves harvested from Hainan province, China contained 72 constituent compounds and the main constituents were linalool (24.36%), β-elemene (12.03%), (E)-2-hexen-1-ol (11.73%), and caryophyllene oxide (10.84%) [15]. However, the essential oil of *Z. avicennae* leaves collected from Nghean province, Vietnam contained 53 constituent compounds and the major constituents were β-caryophyllene (17.01%), α-caryophyllene (10.38%), α-pinene (10.07%) and β-phellandrene (9.42%), and γ-terpinene (4.53%) [16]. The above results suggest that there were some variations in chemical composition of essential oil of *Z. avicennae* collected from different sites and from different parts of the plants. 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 *Z. avicennae* possessed strong larvicidal activity against the 4th instar larvae of *A. albopictus*. The commercial insecticide, chlorpyrifos showed larvicidal activity against the mosquitoes with a LC₅₀ value of 1.86 µg/mL, thus the essential oil of *C. gracile* was 26 times less toxic to *A. albopictus* larvae compared with chlorpyrifos. However, compared with the other essential oils/extracts in the literature, the

Table 1: Main compounds of the essential oil of *Zanthoxylum avicennae* leaves and stems.

Peak no.	Compound	Retention index	(%)
Monoterpenoids			
1	α -Pinene	939	0.25
2	β -Pinene	981	1.56
3	β -Myrcene	991	0.01
4	(+)-4-Carene	1016	0.25
5	β -Phellandrene	1028	0.02
6	1,8-Cineol	1031	53.05
7	Linalool	1094	3.28
8	Fenchol	1117	0.36
9	Camphor	1145	2.08
10	Isoborneol	1160	0.21
11	Borneol	1168	0.45
12	4-Terpineol	1177	0.75
13	α -Terpineol	1188	3.20
14	Fenchyl acetate	1225	0.19
15	Bornyl acetate	1285	0.04
Sesquiterpenoids			
16	α -Cubebene	1350	0.35
17	Copaene	1375	1.78
18	β -Elemene	1389	6.13
19	Longifolene	1412	0.62
20	β -Caryophyllene	1420	5.09
21	α -Caryophyllene	1454	5.96
22	α -Selinene	1494	0.28
23	Calamenene	1520	0.57
24	δ -Cadinene	1523	0.73
25	α -Calacorene	1524	0.70
26	(-)-Spathulenol	1578	2.29
27	Caryophyllene oxide	1583	4.59
28	Humulene oxide II	1608	3.42
29	β -Eudesmol	1648	0.94
Others			
30	Isobutyl 2-methylbutyrate	1015	0.24
31	Phytol	2119	0.28
	<i>Total identified</i>		99.67
	Monoterpenoids		65.70
	Sesquiterpenoids		33.45
	Others		0.52

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

Treatment	LC ₅₀ (μ g/mL) (95% CL)	LC ₉₅ (μ g/mL) (95% CL)	Slope \pm SD	Chi-square value (χ^2)
Z. avicennae	48.79	141.22	0.65 \pm 0.06	9.12*
Mean Range	(39.52-45.76)	(129.77-152.43)		
<i>Chlorpyrifos</i>	1.86	6.65	0.87 \pm 0.01	3.13*
Mean Range	(1.71-2.05)	(6.21-7.48)		

* Significant at $p < 0.05$

essential oil of *Z. avicennae* exhibited the same level of or stronger larvicidal activity against *A. albopictus* larvae, e.g., essential oil of *E. urophylla* (LC₅₀ = 95.5 μ g/mL) [21]; essential oil of *Cinnamomum osmophloeum* of cinnamaldehyde type (LC₅₀ = 40.8 μ g/mL) [22]; leaf essential oil of *Cryptomeria japonica* (LC₅₀ = 51.2 μ g/mL) [23]; leaf and twig essential oils from

Clausena excavata (LC₅₀ = 41.1 μ g/mL) [24]; essential oils of *Salvia elegans* and *S. splendens* (LC₅₀ = 46.4 ppm and LC₅₀ = 59.2 ppm, respectively) [25]; and ethanolic extractives of *Borassus flabellifer* (LC₅₀ = 60 μ g/mL) [26]. However, the essential oil of *Z. avicennae* possessed weaker larvicidal activity against *A. albopictus* larvae than essential oil of *Eucalyptus*

camaldulensis ($LC_{50} = 31.0 \mu\text{g/mL}$) [21] and hexane extract of *Acorus calamus* ($LC_{50} = 21.26 \text{ ppm}$) [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 LC_{50} value of $34 \mu\text{g/mL}$ [28]. Caryophyllene oxide exhibited strong larvicidal activity against *A. albopictus* larvae with a 24 h LC_{50} value of $65.6 \mu\text{g/mL}$ while another main constituent, 1,8-cineol, exhibited weaker larvicidal activity ($LC_{50} > 100 \mu\text{g/mL}$) [22]. 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 two of main constituents, β -elemene and α -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 *Z. avicennae* 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.

For the actual use of *Z. avicennae* essential oil and its constituents as novel larvicides or insecticides to be realized, further research is needed to establish their human and environmental safety. In traditional Chinese medicine, the plant is used to treat abdominal pain, chronic hepatitis, and common cold [7] and appears to be safe for human consumption. However, no experimental data on its toxicity to human is available. 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 *Z. avicennae* demonstrates some activity against *Aedes albopictus* mosquito larvae but needs to be further evaluated for safety in human and to enhance its activity.

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