Research Article

Mosquito Larvicidal Constituents from the Ethanol Extract of *Inula racemosa* Hook. f. Roots against *Aedes albopictus*

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The aim of this research was to determine larvicidal activity of the ethanol extract of *Inula racemosa* Hook. f. (Compositae) roots against the larvae of the Culicidae mosquito *Aedes albopictus* and to isolate any larvicidal constituents from the extract. Based on bioactivity-guided fractionation, 11,13-dihydroisoalantolactone (1), macrophyllilactone E (2), 5 α -epoxyalantolactone (3), and epoxyisoalantolactone (4) were isolated and identified as the active constituents. Compounds 1 and 2 exhibited strong larvicidal activity against the early fourth-instar larvae of *A. albopictus* with LC₅₀ values of 21.86 μ g/mL and 18.65 μ g/mL, respectively, while the ethanol extract had a LC₅₀ value of 25.23 μ g/mL. Compounds 3 and 4 also possessed larvicidal activity against the Asian tiger mosquitoes with LC₅₀ values of 29.37 μ g/mL and 35.13 μ g/mL, respectively. The results indicated that the ethanol extract of *I. racemosa* and the four isolated constituents have potential for use in the control of *A. albopictus* larvae and could be useful in the search of newer, safer, and more effective natural compounds as larvicides.

1. Introduction

Botanical pesticides are emerging as a potential source for mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable and potentially suitable for controlling mosquitoes. Moreover, herbal sources give a lead for discovering new insecticides [1]. During our mass screening program for new agrochemicals from the wild plants and Chinese medicinal herbs, the ethanol extract of *Inula racemosa* Hook. f. (family: Compositae) roots was found to possess larvicidal activity against the Asian tiger mosquito, *Aedes albopictus*.

I. racemosa is a stout herbaceous alpine perennial, 1.5 m tall, with very large basal leaves and usually terminally borne, yellow flower heads and distributed mainly in Xinjiang, China, and also in Afghanistan, Kashmir, Nepal, and Pakistan [2]. Now it is also cultivated in western areas of China (Sichuan, Shanxi, Yunnan Province, and Tibet Autonomous Region). Roots of *I. racemosa* have been used as a traditional medicinal plant in China, India, and Europe [1]. As a traditional Chinese medicine, roots of *I. racemosa* usually

were used to invigorate the spleen, to regulate the function of the stomach, to relieve the depression of the liver “qi,” to alleviate pain especially between the neck and the shoulders, and to prevent abortion [3]. The characteristic components of the genus *Inula* are sesquiterpenes, such as eudesmanes, germacranes, guaianes, and bis-sesquiterpenes. In the previous reports, various sesquiterpenoids, triterpenoids, and lignans have been isolated and identified in the plant [4–19]. Isoalantolactone derived from *I. racemosa* roots exhibited repellency and insecticidal activity to rice weevil, *Sitophilus oryzae* and ovicidal activity against maize borer, *Chilo partellus* [20, 21]. However, a literature survey has shown that there is no report on larvicidal activity of *I. racemosa* roots against *A. albopictus*. Thus, the present research was therefore undertaken to investigate mosquito larvicidal activity of the ethanol extract of *I. racemosa* roots and to isolate active constituent compounds from the extract.

2. Materials and Methods

2.1. Chinese Medicinal Herb. *I. racemosa* (5 kg, dried roots), purchased from Anguo Chinese Herbs Market, Hebei

Province, China, was ground to a powder. The species was identified by Dr. Liu. Q.R. (College of Life Sciences, Beijing Normal University), and the voucher specimens (CAU-CMH-Zangmuxiang-2013-08-009) were deposited in the museum of the Department of Entomology, China Agricultural University.

2.2. Extraction and Bioassay-Directed Fractionation. The powder of *I. racemosa* roots was extracted with 95% ethanol (20 L) at room temperature over a period of three weeks. The extracts were concentrated using a vacuum rotary evaporator to afford a syrupy gum (215 g). This syrup was partitioned between methanol water and *n*-hexane (3 × 1,000 mL). The *n*-hexane extracts were evaporated off to give a residue (67 g). The aqueous layer was repartitioned with chloroform (3 × 1,000 mL) to provide a residue (77 g) after evaporation of chloroform. Further partitioning with ethyl acetate (3 × 1,000 mL) gave a residue (26 g) after evaporation of the solvent.

The CHCl₃ residue (25 g) was applied to a silica gel column (160–200 mesh, Qingdao Marine Chemical Plant, Shandong Province, China), eluting with petroleum ether containing increasing accounts of ethyl acetate (from 100 : 1 to 0 : 100). Fractions of 500 mL were collected and concentrated at 40°C, and similar fractions according to TLC profiles were combined to yield 14 fractions. Fractions (5–6, 8–11) that possessed mosquito larvicidal toxicity, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC GF254, 300–400 mesh, Qingdao Haiyang Chemical Group Corp., China) with petroleum ether-acetone (10 : 2, v/v) until obtaining the pure compound for determining structures as 11,13-dihydroisantalactone (**1**, 28.5 mg), macrophyllilactone **E** (**2**, 45.6 mg), 5 α -epoxyalantolactone (**3**, 28.1 mg), and epoxyisantalactone (**4**, 14.1 mg).

2.3. NMR Analysis. ¹H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 [300 MHz (¹H)] and AMX500 [500 MHz (¹H)] instruments using deuteriochloroform (CDCl₃) as the solvent with tetramethylsilane (TMS) as the internal standard.

2.4. Isolated Constituent Compounds. 11,13-Dihydroisantalactone (1, Figure 1). Colorless crystal. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 4.78 (1H, brs, H-15a), 4.49 (2H, brs, H-15b, H-8), 2.81 (1H, m, H-11), 2.40 (1H, m, H-7), 2.34 (1H, m, H-3a), 2.17 (1H, dd, J = 15.4, 1.4 Hz, H-9a), 2.00 (1H, m, H-3b), 1.80 (1H, d, J = 12.3 Hz, H-5), 1.59 (1H, m, H-6a), 1.56 (2H, m, H-2), 1.53 (1H, m, H-1a), 1.23 (3H, d, J = 7.2 Hz, 13-CH₃), 1.16 (1H, m, H-6b), 0.81 (3H, s, 14-CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 179.5 (C-12), 149.4 (C-4), 106.4 (C-15), 77.9 (C-8), 46.5 (C-5), 42.2 (C-9), 41.8 (C-1), 41.6 (C-7), 40.3 (C-11), 36.8 (C-3), 22.8 (C-6), 21.3 (C-2), 17.8 (C-14), 9.3 (C-13). The data matched previous report [22].

Macrophyllilactone E (2, Figure 1). Colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 6.37 (1H, s, H-6), 4.79 (1H, dd,

J = 13.0, 6.0 Hz, H-8), 4.41 (2H, s, H-13), 2.77 (1H, m, H-4), 2.15 (1H, dd, J = 13.0, 6.0 Hz, H-9b), 1.92 (1H, m, H-2b), 1.70 (1H, m, 3b), 1.67 (1H, m, H-1b), 1.59 (1H, m, H-1a), 1.55 (1H, m, H-3a), 1.51 (2H, m, H-2a, 9a), 1.29 (3H, d, J = 7.4 Hz, 15-CH₃), 1.27 (3H, s, 14-CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 174.9 (C-12), 163.5 (C-5), 159.3 (C-7), 118.4 (C-11), 112.8 (C-6), 76.5 (C-8), 55.1 (C-13), 43.2 (C-9), 40.6 (C-4), 39.7 (C-1), 38.6 (C-10), 34.1 (C-3), 29.5 (C-14), 20.6 (C-15), 18.0 (C-2). The data matched previous report [23].

5 α -Epoxyalantolactone (3, Figure 1). Colorless crystal. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 6.44 (1H, d, J = 2.8 Hz, H-13a), 5.80 (1H, d, J = 2.8 Hz, H-13b), 4.71 (1H, ddd, J = 8.8 Hz, 4.4 Hz, 1.6 Hz, H-8), 3.70 (1H, ddd, J = 8.8 Hz, 2.5 Hz, 2.5 Hz, H-7), 2.94 (1H, d, J = 2.5 Hz, H-6), 1.92 (1H, dd, J = 15.1 Hz, 4.4 Hz, H-9a), 1.60 (1H, dd, J = 15.1 Hz, 1.4 Hz, H-9b), 1.3–2.0 (7H, m, H-1, 2, 3, 4), 1.15 (3H, s, 14-CH₃), 1.10 (3H, d, J = 7.7 Hz, 15-CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 169.6 (C-12), 136.6 (C-11), 123.7 (C-13), 75.1 (C-8), 67.3 (C-5), 61.1 (C-6), 39.4 (C-1), 37.6 (C-9), 37.3 (C-7), 37.0 (C-4), 32.0 (C-10), 29.1 (C-3), 23.9 (C-14), 18.0 (C-15), 16.4 (C-2). The data matched previous report [23].

Epoxyisantalactone (4, Figure 1). Colorless crystal. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 6.07 (1H, d, J = 1.0 Hz, H-13a), 5.53 (1H, d, J = 1.0 Hz, H-13b), 4.45 (1H, dt, J = 4.8, 1.4 Hz, H-8), 2.88 (1H, m, H-7), 2.64 (1H, dd, J = 4.3, 1.8 Hz, H-15a), 2.52 (1H, d, J = 4.4 Hz, H-15b), 2.16 (1H, dd, J = 15.7, 1.3 Hz, H-9a), 1.55 (1H, m, H-6a), 1.48 (1H, dd, J = 15.7, 4.5 Hz, H-9b), 0.95 (3H, s, 14-CH₃), 0.9 (1H, m, H-6b), 0.8–1.9 (6H, m, H-1, H-2, H-3). ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 170.5 (C-12), 141.8 (C-11), 120.5 (C-13), 76.6 (C-8), 58.5 (C-4), 50.7 (C-15), 44.1 (C-5), 41.8 (C-1), 41.3 (C-9), 40.3 (C-7), 35.3 (C-3), 34.2 (C-10), 23.1 (C-6), 20.3 (C-2), 18.6 (C-14). The data matched previous report [23].

2.5. 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 dehydrated eggs were put into plastic tray containing tap water to hatch and yeast pellets served as food for the emerging larvae. The egg batches, daily collected, were kept wet for 24 h and then placed in mineral water in laboratory at 26–28°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, when they were utilized for bioassays (within 12 h).

2.6. Bioassays. Range-finding studies were run to determine the appropriate testing concentrations. Concentrations of 150.0, 75.0, 37.5, 18.5, and 9.0 μ g/mL of the crude extract/compounds were tested. The larval mortality bioassays were carried out according to the test method of larval susceptibility as suggested by the World Health Organization

FIGURE 1: Constituents isolated from *Inula racemosa* roots.

[24]. Twenty larvae were placed in glass beaker with 250 mL of aqueous suspension of tested material at various concentrations, and an emulsifier (DMSO) was added in the final test solution (less than 0.05%). Five replicates were run simultaneously per concentration 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, China)) was used as a positive control. The toxicity of chlorpyrifos was determined at 5, 2.5, 1.25, 0.6, and 0.3 $\mu\text{g/mL}$. The assays were placed in a growth chamber (L16:D9, 26–27°C, 78–80% relative humidity). Mortality was recorded after 24 h of exposure and the larvae were starved within this period.

2.7. Data Analysis. The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates in larvicidal toxicity were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LD_{50} and LD_{90} values [25].

3. Results and Discussion

The ethanol extract of *I. racemosa* roots possessed larvicidal activity against the Asian tiger mosquitoes with a LC_{50} value of 25.23 $\mu\text{g/mL}$ (Table 1). The isolated constituent compounds, 11,13-dihydroisoalantolactone (1) and macrophyllilactone E (2), exhibited strong larvicidal activity against the early fourth-instar larvae of *A. albopictus* with LC_{50} values of 21.86 $\mu\text{g/mL}$ and 18.65 $\mu\text{g/mL}$, respectively (Figure 2). 5α-Epoxyalantolactone (3) and epoxyisoalantolactone (4) also possessed larvicidal activity against the Asian tiger mosquitoes with LC_{50} values of 29.37 $\mu\text{g/mL}$ and 35.13 $\mu\text{g/mL}$, respectively. On the basis of LC_{50} and LC_{95} values, only

compound 2 possessed significantly stronger (no overlap in 95% fiducial limits) toxicity to the larval Asian tiger mosquitoes than ethanol extract and compound 4 exhibited significant lesser toxicity than the crude extract (Table 1). Moreover, some minor constituents in ethanol extract of *I. racemosa* roots may play a role in larvicidal activity against *A. albopictus*. For example, isoalantolactone was demonstrated to exhibit larvicidal activity against *A. aegypti* with a LC_{50} value of 10.0 $\mu\text{g/mL}$ [26]. Thus, further studies are needed to isolate and determine the insecticidal activity of those minor constituents against *A. albopictus* and to measure whether synergy exists among the constituents derived from *I. racemosa* roots. The commercial insecticide, chlorpyrifos, showed larvicidal activity against the mosquitoes with a LC_{50} value of 1.86 $\mu\text{g/mL}$; thus, the ethanol extract of *I. racemosa* roots was about 14 times less toxic to *A. albopictus* larvae compared with chlorpyrifos. However, compared with the other extracts/essential oils in the literature, the ethanol extract of *I. racemosa* roots exhibited stronger larvicidal activity against *A. albopictus* larvae, for example, ethanol extract from *Evodia rutaecarpa* (LC_{50} = 43.21 $\mu\text{g/mL}$) [27], *Cryptomeria japonica* (LC_{50} = 93.8 $\mu\text{g/mL}$) [28], and *Knema attenuata* (LC_{50} = 141 ppm) [29], essential oil of *Clinopodium gracile* aerial parts (LC_{50} = 42.56 $\mu\text{g/mL}$) [30], *Zanthoxylum avicennae* leaves (LC_{50} = 48.79 $\mu\text{g/mL}$) [31], *Toddalia asiatica* roots (LC_{50} = 69.09 $\mu\text{g/mL}$) [32], and *Eucalyptus urophylla* (LC_{50} = 95.5 $\mu\text{g/mL}$) [33], and essential oils of *Achillea millefolium* (LC_{50} = 211.3 $\mu\text{g/mL}$), *Helichrysum italicum* (LC_{50} = 178.1 $\mu\text{g/mL}$), and *Foeniculum vulgare* (LC_{50} = 142.9 $\mu\text{g/mL}$) [34]. Moreover, compared with chlorpyrifos, the isolated constituents exhibited 10–19 times less toxic to *A. albopictus* larvae (Table 1).

In the previous reports, several sesquiterpene lactones exhibited larvicidal activity against mosquitoes (*A. aegypti*,

FIGURE 2: Dose-response curves of fourth-instar larvae of *Aedes albopictus* treated with the ethanol extract and isolated constituents from *Inula racemosa* roots.

A. albopictus, and *A. atropalpus*) [26, 35–39]. For example, alantolactone and isoalantolactone showed LC_{50} values of 2.7 $\mu\text{g/mL}$ and 11.9 $\mu\text{g/mL}$ for *A. albopictus* [36]. Moreover, dehydrocostus lactone and costunolide exhibited strong larvicidal activity against *A. albopictus* with LC_{50} values of 2.34 and 3.26 $\mu\text{g/mL}$, respectively [37]. Among the four isolated constituent compounds, 11,13-dihydroisoalantolactone was demonstrated to possess larvicidal activity against *A. albopictus* [36]. The other three constituents were the first time to evaluate larvicidal activity against mosquitoes. Considering that the currently used larvicides are synthetic insecticides, larvicidal activity of the crude ethanol and the four isolated compounds are quite promising and they show potential for

use in control of *A. albopictus* larvae and could be useful in the search of newer, safer, and more effective natural compounds as larvicides. For the practical use of the ethanol extract of *I. racemosa* roots and its constituents as novel larvicides or insecticides to proceed, further research is needed to establish their human safety and environmental safety. In traditional Chinese medicine, *I. racemosa* roots have been used for their peptic, relieving phlegm, detumescence, anti-inflammatory, and vermifuge properties [3]. However, no experimental data about the safety of ethanol extract of this medicinal herb and the four other isolated constituents is available so far. Therefore, any attempt to develop an agrochemical must be carefully evaluated for harmful effects. Additionally,

TABLE 1: Larvicidal activity of the ethanol extract of *Inula racemosa* roots and the isolated constituents against fourth-instar larvae of *Aedes albopictus*.

Treatment	LC ₅₀ (μg/mL) (95% FL)	LC ₉₅ (μg/mL) (95% FL)	Slope ± SD	Chi-square value
Ethanol extract	25.23 (22.71–27.41)	95.48 (84.43–105.08)	2.52 ± 0.25	9.73*
11,13-Dihydroisoalantolactone (1)	21.86 (19.58–24.21)	83.45 (75.87–93.21)	2.49 ± 0.24	9.45*
Macrophyllilactone E (2)	18.65 (15.59–20.41)	68.23 (61.92–75.12)	2.68 ± 0.27	8.894*
5α-Epoxyalantolactone (3)	29.37 (26.79–32.23)	111.43 (100.34–123.04)	2.56 ± 0.26	11.12*
Epoxyisoalantolactone (4)	35.13 (31.54–38.63)	129.43 (127.55–142.32)	2.23 ± 0.23	9.45*
Chlorpyrifos	1.86 (1.71–2.05)	6.65 (6.21–7.48)	0.87 ± 0.01	3.13*

*Significant at $P < 0.05$ level.

their larvicide modes of action need to be established and formulations for improving larvicidal potency and stability, thereby reducing costs, need to be developed. Moreover, field evaluation and further investigations on the effects of the extract and the four isolated constituent compounds on nontarget organisms are necessary.

4. Conclusion

The study indicates that the ethanol extract of *I. racemosa* roots and its isolated constituent compounds have potential for use in the control of *A. albopictus* larvae and could be useful in search of newer, safer, and more effective natural compounds as larvicides.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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