Larvicidal activities of sesquiterpenes from *Inula helenium* (Compositae) against *Aedes albopictus* (Diptera: Culicidae) and *Paratanytarsus grimmii* (Diptera: Chironomidae)

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Abstract

The larvicidal activities of three sesquiterpenes, alantolactone, isoalantolactone and dihydroisoalantolactone, isolated from the roots of *Inula helenium* (Compositae) against 3rd and 4th instars of *Aedes albopictus* (Diptera: Culicidae) and *Paratanytarsus grimmii* (Diptera: Chironomidae), were examined. The two sesquiterpenes, alantolactone and isoalantolactone, showed LC₅₀ values of 2.7 µg/ml and 11.9 µg/ml for *A. albopictus*, and 5.1 µg/ml and 4.1 µg/ml for *P. grimmii* within 48 h, respectively. Alantolactone was significantly more toxic than isoalantolactone against *A. albopictus*; however, dihydroisoalantolactone did not entirely show lethal effects against the larvae of both species at a concentration of 1,000 µg/ml.

Key words: *Aedes albopictus*; *Paratanytarsus grimmii*; *Inula helenium*; larvicidal activity; sesquiterpenes

INTRODUCTION

A medicinal plant, *Inula helenium* (Compositae), which is native to Europe and north Asia, is cultivated all over the world. The roots of *I. helenium* have been used as diaphoretic, diuretic and expectorant agents in Europe, a fragrance agent for home remedies in Japan (Okuda, 1986), in the treatment of tuberculotic enterorrhea, chronic enterogastritis and bronchitis, and as a preservative of crude drugs in China (Jiangsu New Medical College, 1986). The roots of this plant have been used as a substitute for Chinese medicinal herbs to relieve pain associated with abdominal distention and indigestion with anorexia, nausea, and vomiting. The root has also shown strong anthelmintic, antibacterial activities (Cantrell et al., 1999), and some sesquiterpene lactones isolated from this root showed strong antiproliferative activities against three tumor cell lines (Konishi et al., 2002).

Thousands of sesquiterpene lactones have been isolated from plant sources, and many have been shown to possess various biological activities, including antitumoral (Lee et al., 2001), antimicrobial (Fischer et al., 1998), insect growth inhibitory (Arnason et al., 1985; Srivastava and Proksch, 1990), insect feeding deterrent (Mullin et al., 1991) and insect larvicidal (Neves et al., 1999; Tsukamoto et al., 2005) activity. *Aedes albopictus* (Diptera: Culicidae) occurs throughout the Oriental region from the tropics of Southeast Asia, the Pacific and Indian Ocean islands, north through China and Japan and west to Madagascar. *Ae. albopictus* is associated with the transmission of dengue and potentially with West Nile viruses. The insecticidal activities of synthetic insecticides (Gill, 1977; Ponlawat et al., 2005), polypeptides of *Bacillus thuringiensis* subsp. *je-gathesan* (Kawalek et al., 1995) and seed kernels extracts of *Carapa guianensis* (Meliaceae) (Silva et al., 2004) against *Ae. albopictus* have been reported, as well as the effects of sesquiterpene lactones against the growth and development of *Ae. atropalpus* (Arnason et al., 1985). However no in-
vestigations have reported the insecticidal activities of sesquiterpene lactones against larvae of *Ae. albopictus*.

Chironomid midges (Diptera: Chironomidae) include economically and medically important species that are a serious nuisance and produce allergies on outbreak (Cranston, 1995); however, there is little information about the larvicidal activities of plant extracts against *Paratanytarsus grimmii*.

In the present study, we report the larvicidal activities of isolated compounds from the root of *I. helenium* against dengue vector mosquito, *Ae. albopictus* and the common midge, *P. grimmii*.

**MATERIALS AND METHODS**

The dry roots of *Inula helenium* were purchased from Mitsuboshi Seiyaku Co. Ltd. (Nara, Japan). A voucher specimen (KPU-001952) was deposited in the herbarium of the Department of Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University, Japan. The cut roots (80 g) were extracted with methanol (MeOH, Nacalai Tesque, Inc., Kyoto, Japan) at room temperature for 2 weeks. The MeOH extract was filtrated and then the solvent evaporated under reduced pressure to obtain a viscous mass (12 g).

This MeOH extract was suspended in water and partitioned with hexane, chloroform, ethyl acetate and 1-butanol, respectively, to give each organic fraction. The chloroform extract (0.21 g) and hexane extract (7.57 g) fractions showed significant activity (≥80% mortality) within 48 h against both larvae of *Ae. albopictus* and *P. grimmii* at 1,000 μg/ml. But other extracts did not show high larvicidal activity (≤40% mortality); therefore, we used the hexane extract for the isolation of larvicidal compounds.

The hexane fraction (7.57 g) was fractionated by Si-gel column chromatography and reverse phase HPLC to afford alantolactone (830 mg), isosalantolactone (486 mg) and dihydroisoalantolactone (56 mg), respectively (Fig. 1) (Konishi et al., 2002). The chemical structures of these compounds were determined by spectroscopic data and physicochemical properties, and comparison with data from the literature (Marshall and Cohen, 1964; Kaur and Kalsi, 1985; Milmanis, 1990).

Both α-santonin (Tokyo Chemical Industry Co. Ltd.) and sesquiterpene lactones obtained by the above-mentioned procedure were used for bioassay. A juvenile hormone analog, methoprene (IC$_{50}$) (Buei et al., 1975) was referenced.

Larvae of *Paratanytarsus grimmii* (Diptera: Chironomidae) and *Aedes albopictus* (Diptera: Culicidae) were used as test organisms. The chironomid colony was initiated with individuals collected in 1992 from the Yamazaki river in Nagoya city, Japan. The mosquito colony “Toyama” was collected in 1992 in Sugitani village, Toyama, Japan. The larvicidal activity of the lactones was determined by the immersion method (Kondo et al., 2004).

The test compound (each 10 mg) was dissolved in dimethyl sulfoxide (DMSO, 0.3 ml) and suspended in dechlorinated tap water (9.7 ml) to make a 10 ml test solution (1,000 μg/ml). Immersion tests were performed in 12-well tissue culture plates (3.0 ml/well) (Falcon, Inc.). The 3rd and 4th instars of *P. grimmii* and *Ae. albopictus* were introduced into the wells and kept unfed at 20°C under a photoperiod condition of 12L:12D. Six to eight larvae of both species were used for each test and control (3.0% DMSO in water) solution.

The survival numbers were counted daily for three days. Larvae were counted as dead when they showed no movement on stimulation with a needle. In each experiment, the tests were repeated three times and the mean mortality rates were obtained in each experiment. Mortality data for each larvicidal concentration were used to determine the LC$_{50}$ and LC$_{90}$ using the probit method (Bliss, 1934).
RESULTS AND DISCUSSION

Alantolactone and isoalantolactone showed more rapid, strong larvicidal activity in 1,000 μg/ml against both species, and these sesquiterpenes produced 100% mortality within 24 h at the same concentration. Dihydroisoalantolactone showed larvicidal activity of 84% against Ae. albopictus; however, no mortality was observed against P. grimmii within 72 h at 1,000 μg/ml. An eudesmanolide, α-santonin, showed lower activity than alantolactones at 500 μg/ml (Table 1).

We then investigated the more detailed larvicidal activity of alantolactone and isoalantolactone against Ae. albopictus and P. grimmii.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (μg/ml)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ae. a.</td>
<td>P. g.</td>
<td>Ae. a.</td>
</tr>
<tr>
<td>Alantolactone</td>
<td>1,000</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Isoalantolactone</td>
<td>1,000</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Dihydroisoalantolactone</td>
<td>1,000</td>
<td>30.2</td>
<td>0</td>
<td>73.8</td>
</tr>
<tr>
<td>α-Santonin</td>
<td>500</td>
<td>5.6</td>
<td>0</td>
<td>38.9</td>
</tr>
</tbody>
</table>

*No mortality was observed in controls (3% DMSO). Tests were repeated three times and mortality is shown as the mean value. Ae. a.: Aedes albopictus. P. g.: Paratanytarsus grimmii.*

In alantolactone, all mosquito larvae died at 100 μg/ml within 48 h and showed 90% mortality at 25 μg/ml within 48 h. Alantolactone also showed significant larvicidal activity (mean mortality of 80% or more) at 48 h against mosquito larvae in 10 μg/ml (Fig. 2). On the other hand, isoalantolactone showed perfect mortality at 100 μg/ml against *Ae. albopictus* within 48 h, as did alantolactone, and showed a significant effect (mortality of 75%) against mosquito larvae in 20 μg/ml (Fig. 2).

Against *P. grimmii* larvae, alantolactone and isoalantolactone showed 100% mortality at 20 μg/ml and 100 μg/ml, respectively, and moreover, both compounds showed 70% and nearly 80% mortality at 10 μg/ml within 48 h (Fig. 3). There was no mortality in the 3% DMSO control.
The LC₅₀ values (95% confidence limits) and LC₉₀ values of alantolactone and isoalantolactone against mosquito and midge larvae were calculated (Table 2). The LC₅₀ values of both compounds for mosquito larvae were 2.7 μg/ml and 11.9 μg/ml after 48 h, respectively. The LC₅₀ values of both compounds for midge larvae were 5.1 μg/ml and 4.1 μg/ml after 48 h, respectively. These results suggest that the activities of both compounds were stronger than α-santonin (LC₅₀ = 849, 1,051 μg/ml) against larvae of *Ae. albopictus* and *P. grimmii*, but alantolactone and isoalantolactone did not show selective toxicity against both species (Table 2).

Alantolactone and isoalantolactone possessing the α-methylene-γ-lactone moiety showed high larvicidal activity against the larvae of insects as well as antiproliferative activity against tumor cells (Konishi et al., 2002); however, dihydroisoalantolactone, which have a saturated γ-lactone moiety in the molecule, did not show significant larvicidal activity against the larvae of both insects. α-Santonin with saturated γ-lactone moiety in the molecule showed the same result as dihydroisoalantolactone. These results suggest that the α-methylene-γ-lactone moiety of these compounds contributed to the high larvicidal activity.

This evidence indicates that conjugation with the carbonyl group in the lactone ring appeared to play an important role in larvicidal activity. Arnason et al. (1985) reported that alantolactone and isoalantolactone possessing the α-methylene-γ-lactone moiety showed higher toxicity than compounds lacking this moiety, but that the order of toxicity of sesquiterpene lactones was related to other structural factors than the α-methylene-γ-lactone moiety. Dupuis and Brisson (1976) reported that the cytotoxicity of alantolactone and dihydroalantolactone against leukocytes in *in vitro* culture was not due to the presence of unsaturated γ-lactone.

In the present study, alantolactone and isoalantolactone showed strong larvicidal activity against a mosquito, *Ae. albopictus* and chironomid, *P. grimmii*. Dihydroisoalantolactone and α-santonin did not show high larvicidal activity against either insect. We appreciate that the presence of the α-methylene-γ-lactone moiety contributed to the larvicidal activity.

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