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Research Article

Isolation of Insecticidal Constituents from the Essential Oil of *Ageratum houstonianum* Mill. against *Liposcelis bostrychophila* Badonnel

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The aim of this research was to determine chemical composition and insecticidal activities of the essential oil of *Ageratum houstonianum* Mill. aerial parts against booklice, *Liposcelis bostrychophila* Badonnel, and to isolate any insecticidal constituents from the oil. Essential oil of *A. houstonianum* was obtained by hydrodistillation and analyzed by GC-MS. A total of 35 components in the essential oil were identified. The major compounds were precocene II (62.68%), precocene I (13.21%), and β-caryophyllene (7.92%). Based on bioactivity-guided fractionation, precocene II and precocene I were isolated and identified as the active constituents. The essential oil exhibited contact toxicity against *L. bostrychophila* with an LC_{50} value of 50.8 μg/cm^2. Precocene II (LC_{50} = 30.4 μg/cm^2) exhibited stronger acute toxicity than precocene I (LC_{50} = 64.0 μg/cm^2) against the booklice. The essential oil and the two isolated constituents also possessed strong repellent activity against *L. bostrychophila*. The results indicated that the essential oil and its constituent compounds have potential for development into natural insecticides or repellents for control of insects in stored grains.

1. Introduction

Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for the design of target-specific molecules. During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *Ageratum houstonianum* (Family: Compositae) aerial parts was found to possess strong insecticidal toxicity against the booklice, *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae). *A. houstonianum* is an annual erect ornamental shrub of 30–70 cm height. It is commonly known as floss flower and native to southeastern Mexico, Central America. Now it is cultured as an ornamental flower and also naturalized as an invasive weed in Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Jiangsu, Nanhai Zhudao, Shandong, Sichuan, Taiwan, Yunnan, and Zhejiang Province, China [1]. The whole plant of *A. houstonianum* is used medicinally in traditional Chinese medicine to clear away heat and toxic materials. People in Central America (Ecuador) use this plant as an antiphlogistic to relieve swelling and pain in the throat [1]. In the previous reports, various flavonoids, triterpenoids, steroids, pyrrolizidine alkaloids, and benzofuran derivatives (chromenes) have been isolated and identified in the plant [2–7]. The chemical composition of the essential oil of *A. houstonianum* has been studied previously [8–14]. It is reported that essential oil and extracts derived from the aerial parts (leaves) of *A. houstonianum* exhibit antifungal, antimicrobial, acaricidal, and mosquitocidal activity as well as repellency against mosquitoes [11–19]. However, a literature survey has shown that there is no report on insecticidal and repellent activity of the essential oil of *A. houstonianum* against *L. bostrychophila*. The present research was therefore undertaken to investigate the chemical constituents and insecticidal and repellent activity of the essential oil against the booklice for the first time and to isolate active constituent compounds from the essential oil.

2. Materials and Methods

2.1. Plant and Essential Oil Extract. Fresh aerial parts of *A. houstonianum* (15 kg) at flowering stage were harvested from Lishui City (27.54°N latitude and 119.20°E longitude, Zhejiang

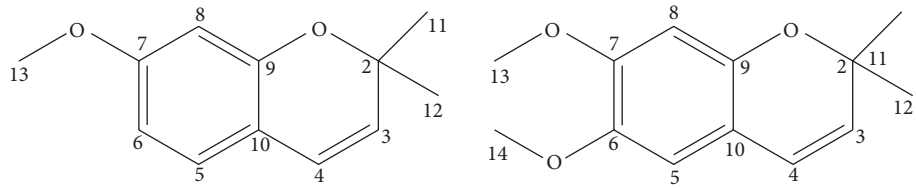

Figure 1: Precocenes isolated from *Ageratum houstonianum* essential oil.

Province, China) in August 2013. The plant was identified, and a voucher specimen (CMH-Xiongercao-Zhejiang-2013-08) was deposited at the herbarium of Department of Entomology, China Agricultural University. The sample was air-dried and ground to powder using a grinding mill (Retsch Muhle, Haan, Germany) and was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. Essential oils were stored in airtight containers in a refrigerator at 4°C for subsequent experiments.

2.2. Oil Isolation and Fractionation. The essential oil (25 mL) was chromatographed on a silica gel (Merck 9385, 1,000 g) column by gradient elution with a mixture of solvents (*n*-hexane, *n*-hexane-ethyl acetate, and acetone). Fractions of 500 mL were collected and concentrated at 40°C, and similar fractions according to TLC profiles were combined to yield 16 fractions. Fractions (8-9, 11-12) that possessed contact 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) to obtain the pure compound for determining structure as precocene I (**1**, 1.3 g, 7-methoxy-2,2-dimethylchromene, Figure 1) and 3-precocene II (**2**, 1.9 g, 6,7-dimethoxy-2,2-dimethyl-2-chromene, Figure 1). The spectra data of the two compounds matched with previous report [20].

2.3. Gas Chromatography-Mass Spectrometry (GC-MS). Analyses of volatile constituents were determined using an Agilent 5973 GC-MS system operating in the EI mode at 70 eV (equipped with a 30 m HP-5MS column (0.25 mm × 30 m × 0.25 μm) and coated with 5% phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm) (Agilent J&W Scientific, USA)). The temperature program used for the analysis was as follows: initial temperature at 60°C, held for 1 min, ramped at 4°C/min to 290°C, and held for 0.5 min. Helium was the carrier gas at 1.0 mL/min; the sample (1 μL 1/100, v/v, in acetone) was injected in the split mode (1 : 10). The injector and detector temperatures were performed at 230°C and 300°C, respectively. Most constituents were identified by gas chromatography by comparison of their retention indices with those of 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 the literature [21].

2.4. NMR Analysis. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 (300 MHz (^{1}H)) and AMX500 (500 MHz (^{1}H)) instruments using deuterochloroform ($CDCl_3$) as the solvent with tetramethylsilane (TMS) as the internal standard. Electron impact mass spectra (EIMS) were determined on a Micromass VG7035 mass spectrometer at 70 eV (probe).

2.5. Insects. *L. bostrychophila* was obtained from laboratory cultures in the dark in incubators at 28–30°C and 70–80% r.h. and was reared on a 1 : 1 : 1 mixture, by mass, of milk powder, active yeast, and flour. All containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK). Adult insects used in all the experiments were about one week old.

2.6. Contact Toxicity. Contact toxicity of the oil against the booklice was measured as described by Zhao et al. [22]. The filter paper with 3.5 cm in diameter (Whatman) was treated with 150 μL of the solution (2.0%, 2.4%, 2.9%, 3.5%, 4.2%, and 5.0% in acetone). The treated filter paper after being treated with solid glue (Glue Stick, Jong Ie Nara Co., Ltd., Hong Kong) was placed in a Petri dish (3.5 cm in diameter) and 10 booklice were put on the filter paper. The plastic cover with holes was put and all the Petri dishes were kept in incubators at 27–29C, 70–80% r.h. for 24 h and mortality of insects was observed. Acetone was used as controls and pyrethrum extract was used as a positive control. Pyrethrum extract (25% pyrethrin I and pyrethrin II) was purchased from Fluka Chemie (Buchs, Switzerland).

2.7. Repellent Activity. A commercial repellent, dimethyl phthalate, was purchased from Aladdin-Reagent Company (Shanghai) and used as a positive control. The essential oil and isolated compounds were diluted in acetone to four concentrations (6.4, 3.2 1.6, and 0.8 nL/cm^2). Filter paper (6 cm in diameter) was cut in half and 150 μL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 150 μL of absolute acetone. Both the treated half and the control half were then air dried to evaporate the solvent completely (10 sec). A full disc was carefully remade

Table 1: The scale to be assign repellency of the essential oil and its constituents.

Class	Percent repulsion (%)
0	0.01 to 0.1
I	0.1–20
II	20.1–40
III	40.1–60
IV	60.1–80
V	80.1–100

by attaching the tested half to the negative control half with tape. Each remade filter paper after being treated with solid glue was placed in a Petri dish and 20 insects were released in the center of each filter-paper disc and a cover was placed over the Petri dish. Five replicates were used and the experiment was repeated for three times. Counts of the insects present on each strip were made after 2 h and 4 h. The percent repellency of the oil/compounds was then calculated using the formula

$$\text{PR } (\%) = \left[\frac{(\text{Nc} - \text{Nt})}{(\text{Nc} + \text{Nt})}\right] \times 100, \quad (1)$$

where Nc was the number of insects present in the negative control half and Nt was the number of insects present in the treated half.

The averages were then categorised according to the following scale [23, 24] as shown in Table 1.

2.8. Data Analysis. The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates in contact toxicity were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LD_{50} and LD_{90} values [25]. The percentage repellency data were subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

3. Results and Discussion

3.1. Essential Chemical Composition. The yellow essential oil yield of *A. houstonianum* was 0.67% (v/w based on dry weight) while the density of the concentrated essential oil was 0.90 g/mL. A total of 35 components from the essential oil of *A. houstonianum* were identified, accounting for 97.92% of the total oil. Precocenes only represented 2 of the 35 compounds, corresponding to 75.89% of the whole oil, while 18 of the 35 constituents were sesquiterpenoids (20.61% of the crude essential oil) (Table 2). The major constituents of *A. houstonianum* essential oil were precocene II (62.68%), precocene I (13.21%), and β-caryophyllene (7.92%). The results are similar to the previous reports [8–14] although there are some variations in chemical composition of the essential oils of *A. houstonianum*. For example, the essential oil of *A. houstonianum* leaves harvested from Cameroon contained precocene I and precocene II in almost similar amounts (32% and 24%, resp.) [8]. However, the essential oil of *A. houstonianum* flowers collected from Cameroon mainly contained precocene I (48.01%), precocene II (36.55%), and β-caryophyllene (8.37%) [12]. Precocene II (43.99%), precocene I (23.34%), and β-caryophyllene (9.18%) were identified as major constituents of the essential oil of *A. houstonianum* harvested from Jammu Region of India [9]. In another report [10], precocene II (52.64%), precocene I (22.45%), and β-caryophyllene (9.66%) represented the major constituents in the essential oil of *A. houstonianum* aerial parts also collected from India.

Table 2: GC-MS analysis of essential oil of *Ageratum houstonianum* aerial parts.

No.	Compound	RI	Peak area (%)
1	α-Pinene*	939	0.19
2	β-Pinene*	974	0.11
3	Sabinene	975	0.06
4	β-Myrcene*	991	0.09
5	β-Phellandrene	1030	0.18
6	1,8-Cineole*	1032	0.11
7	Linalool*	1094	0.32
8	Isoborneol	1160	0.18
9	4-Terpineol*	1177	0.07
10	α-Terpineol*	1189	0.11
11	Isobornyl acetate	1284	0.07
12	Bornyl acetate*	1287	0.43
13	Piperitenone	1338	0.27
14	α-Cubebene	1345	0.34
15	α-Terpineol acetate	1349	0.21
16	Eugenol*	1356	0.16
17	Copaene	1375	2.45
18	β-Cubebene	1388	0.32
19	(Z)-Caryophyllene	1409	0.75
20	β-Caryophyllene*	1420	7.92
21	γ-Elemene	1433	0.11
22	β-Gurjunene	1434	0.51
23	α-Caryophyllene	1454	0.25
24	(E)-β-Famesene	1457	0.28
25	Precocene I	1467	13.21
26	γ-Muurolene	1473	2.87
27	Germacrene D	1485	0.91
28	α-Muurolene	1495	0.13
29	γ-Cadinene	1513	2.18
30	δ-Cadinene	1523	0.17
31	Cadine-1,4-diene	1532	0.22
32	Spathulenol	1578	0.09
33	Caryophyllene oxide	1583	0.23
34	Precocene II	1656	62.68
35	β-Bisabolol	1673	0.88
	Total identified		97.92
	Monoterpenoids		1.26
	Sesquiterpenoids		20.61
	Precocenes		75.89
	Others		0.16

RI, retention index on a HP-5MS column. *Identification by coinjection of authentic compounds.

Table 3: Contact toxicity of *Ageratum houstonianum* essential oil and its isolated compounds against *Liposcelis bostrychophila*.

Treatment	LD_{50} ($\mu g/cm^2$) (95% FL*)	LD_{90} ($\mu g/cm^2$) (95% FL*)	Slope ± SD	Chi-square (χ^2)
Essential oil	50.8 (45.7–55.6)	176.3 (148.6–194.2)	3.85 ± 0.36	10.98**
Precocene I	64.0 (57.8–70.3)	211.9 (189.8–232.5)	7.19 ± 0.66	8.67**
Precocene II	30.4 (27.6–33.2)	105.8 (94.1–116.4)	8.65 ± 0.81	7.45**
Pyrethrum extract	19.0 (17.6–20.9)	68.5 (62.1–75.8)	2.21 ± 0.17	4.27**

*Fiducial limits, **significant at $P < 0.05$ level.

Table 4: Repellency (PR) after two exposure times for *Ageratum houstonianum* essential oil and its isolated compounds against *Liposcelis bostrychophila*.

Treatment	2 hr (nL/cm^2)*								4 hr (nL/cm^2)*							
	6.4		3.2		1.6		0.8		6.4		3.2		1.6		0.8	
Essential oil	93 ± 4^a	V	86 ± 9^a	V	82 ± 6^a	V	70 ± 8^{ab}	IV	86 ± 7^a	V	80 ± 6^a	V	71 ± 7^b	V	61 ± 6^b	IV
Precocene I	96 ± 5^a	V	87 ± 4^a	V	84 ± 7^a	V	74 ± 9^{ab}	IV	91 ± 4^a	V	81 ± 6^a	V	83 ± 5^a	V	70 ± 8^{ab}	IV
Precocene II	97 ± 5^a	V	85 ± 8^a	V	79 ± 8^a	IV	67 ± 7^b	IV	89 ± 9^a	V	82 ± 7^a	V	78 ± 5^{ab}	IV	63 ± 1^b	IV
Dimethyl phthalate	98 ± 4^a	V	91 ± 3^a	V	89 ± 4^a	V	81 ± 5^a	V	90 ± 4^a	V	85 ± 4^a	V	84 ± 4^a	V	77 ± 4^a	IV

*Means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

3.2. Contact Toxicity. The essential oil of *A. houstonianum* aerial parts exhibited contact toxicity against *L. bostrychophila* with an LC_{50} value of 50.8 $\mu g/cm^2$ (Table 3). Precocene II (LC_{50} = 30.4 $\mu g/cm^2$) exhibited stronger acute toxicity than precocene I (LC_{50} = 64.0 $\mu g/cm^2$) against the booklice. Precocene II shows almost 2 times stronger toxicity than the oil while precocene I exhibits less toxicity than the oil. Thus it seems that contact toxicity of the oil may be mainly attributed to precocene II. However, compared with the positive control, pyrethrum extract (LC_{50} = 18.99 $\mu g/cm^2$), *A. houstonianum* essential oil, and precocene II showed 2.7 and 1.6 times less toxicity to *L. bostrychophila*, respectively. When compared with the other essential oils in the previous studies using the same bioassay, the essential oil of *A. houstonianum* exhibited stronger or the same level of acute toxicity against the booklice, for example, essential oils of *Acorus calamus* [26], *Artemisia rupestris* and *A. frigida* [24, 27], *Curcuma wenyujin* [28], *Foeniculum vulgare* [22], and *Valeriana jatamansi* [29].

3.3. Repellent Activity. The essential oil of *A. houstonianum* showed strong repellent activity (Class V) against the booklice at a concentration of 1.6 nL/cm^2 and higher concentration after 4 hr of exposure (Table 4). Moreover, the essential oil of *A. houstonianum* still exhibited the same level (class IV) of repellent activity against the booklice as commercial repellent, dimethyl phthalate, at a concentration of 0.8 nL/cm^2 after 4 hr of exposure. Precocene I possessed the same strong repellency against the booklice, while precocene II exhibited class IV repellent activity at a concentration of 1.6 nL/cm^2 after 4 hr of exposure. Compared with commercial repellent, dimethyl phthalate, precocene I showed the same level of repellent activity against the booklice, while precocene II exhibited less active at lower concentrations (0.8 nL/cm^2 and 1.6 nL/cm^2) (Table 4).

This study demonstrates that the essential oil of *A. houstonianum* had contact toxicity and repellent activity to the booklice. Furthermore, the two isolated constituent compounds, precocene II and precocene I, also exhibited insecticidal and repellent activity against *L. bostrychophila*. In the previous studies, precocene II and precocene I demonstrated to inhibit the synthesis of juvenile hormone in a number of insects. Consequently, this inhibition can disturb the embryonic development, induce premature metamorphosis, decrease the reproductive potential, and affect the insect behavior including the antifeedant and repellent effect [19, 30–35]. The above findings suggest that the essential oil and the two isolated constituent compounds show a potential to be developed as possible natural insecticides/repellents for the control of grain storage insects. It seems that this plant is quite safe to human consumption because it has been used as a medicinal herb for clearing away heat and toxic materials [1]. However, *A. houstonianum* is reported to be toxic to grazing animals, causing liver lesions [36]. Moreover, no information on toxicity of the essential oil and the isolated constituents to human were available. Thus, to develop a practical application for the essential oil and the isolated constituents as novel natural insecticides, further research into the safety of the essential oil/compounds to humans is needed. Additional studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

4. Conclusion

This study indicates that the essential oil of *A. houstonianum* aerial parts and its isolated constituent compounds

have potential for development into natural insecticides and repellents for control of insects in stored grains.

Conflict of Interests

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

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