

Original Research Article

Gas Chromatography-Mass Spectrometry Analysis of Insecticidal Essential Oil Derived from Chinese *Ainsliaea fragrans* Champ ex Benth (Compositae)

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Abstract

Purpose: To investigate the chemical composition and insecticidal activity of the essential oil of the aerial parts of *Ainsliaea fragrans* against maize weevils (*Sitophilus zeamais*).

Methods: The essential oil of *A. fragrans* aerial parts was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Contact toxicity of the essential oil and its major constituents was determined by topical application against *S. zeamais*.

Results: A total of 30 components of the essential oil were identified. The major constituents were myristicin (41.3 %), elemicine (11.9 %), cis-isosafrole (11.5 %), borneol (9.1 %) and caryophyllene (8.8 %). The essential oil of *A. fragrans* exhibited contact toxicity against *S. zeamais* with LC_{50} value of 50.7 $\mu\text{g}/\text{adult}$. Elemicine possessed the strongest contact toxicity ($LC_{50} = 13.5 \mu\text{g}/\text{adult}$) while cis-isosafrole, myristicin, caryophyllene and borneol had LC_{50} values of 31.2 $\mu\text{g}/\text{adult}$, 43.4 $\mu\text{g}/\text{adult}$, 57.9 $\mu\text{g}/\text{adult}$, and 98.4 $\mu\text{g}/\text{adult}$, respectively.

Conclusion: The study indicates that the essential oil of *A. fragrans* aerial parts and its major constituents have a potential for development into natural insecticides for the control of grain storage insects.

Keywords: *Ainsliaea fragrans*, *Sitophilus zeamais*, Contact toxicity, Elemicine, Essential oil, Grain storage

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INTRODUCTION

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Ainsliaea fragrans* Champ. ex Benth. aerial parts was found to possess insecticidal toxicity against maize weevils (*Sitophilus zeamais* Motsch.). The genus *Ainsliaea* belongs to the family Compositae (Asteraceae) and is distributed in east part of Asia with about 50 species in the world, of which 40 species (28 endemic) are distributed in China [1]. *A. fragrans* is a perennial

herb (25 - 60 cm tall) distributed in Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Taiwan, Yunnan, and Zhejiang Provinces in China [1]. The whole herb of this plant is widely used in traditional Chinese medicine for the treatment of coughing up blood, jaundice with damp-heat pathogen, edema, and superficial infection [2].

The aqueous extract of *A. fragrans* aerial parts is widely applied in treating cervicitis, endometritis, and pelvic inflammation, and its clinical effects are significant in China [3]. From the aerial parts

of *A. fragrans*, sesquiterpenoids, sesquiterpene lactones, sesquiterpene lactone glycosides, coumarins, flavonoids, triterpenoids, lignans and phenolic acids have been isolated [2-5]. The chemical composition of the essential oils derived from *A. fragrans* has also been determined [6,7]. However, literature survey shows that there is no report on insecticidal activity of the essential oil derived from *A. fragrans* aerial parts. Thus, the objective of this study was to investigate the chemical constituents and insecticidal activity of the essential oil of *A. fragrans* aerial parts and its major constituents against maize weevils.

EXPERIMENTAL

Plant collection and identification

The aerial parts of *A. fragrans* (10 Kg) were harvested from Lishui City, Zhejiang Province, China (27.54° N and 119.20° E) in September 2014. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU-Compositae-Xinxiangtuerfeng-016) was deposited at the museum of Department of Entomology, China Agricultural University, Beijing, China.

Extraction and isolation of essential oils

The sample was cut into small pieces and subjected to hydro distillation using a modified Clevenger-type apparatus for 6 h. Anhydrous Na₂SO₄ was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Analysis of the essential oils

Gas chromatographic analysis 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 oils 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. 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 NIST 05 and Wiley 275 libraries or with mass spectra from literature [8]. Relative percentages of the oil components were calculated based on GC peak areas without using correction factors.

Chemicals

Borneol, caryophyllene, elemicine, *cis*-isosafrole, and myristicin were purchased from purchased from Aladdin-Reagent Company (Shanghai, China). Pyrethrum extract (25 % pyrethrin I and pyrethrin II) was purchased from Fluka Chemie and used as a positive control.

Insects

S. zeamais was obtained from laboratory cultures in the dark in incubators at 27 - 29 °C and 70 - 80 % relative humidity. Adult *S. zeamais* insects were reared on whole wheat at 12 - 13 % moisture content in glass jars (diameter 85 mm, height 130 mm). Laboratory bioassays were done within one week after unsexed adult collection. All containers housing insects and the Petri dish used in the experiments were made escape-proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK).

Contact toxicity test

The contact toxicity of the essential oil of *A. fragrans* aerial parts against *S. zeamais* adults was measured as described in the previous reports [9]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (6 concentrations, 3.5 - 10.0 %, v/w) was prepared in *n*-hexane. Aliquots of 0.5 µL of the dilutions were applied topically to the dorsal thorax of the

insects, using a Burkard Arnold microapplicator. Controls were determined using n-hexane. Both treated and control insects were then transferred to glass vials (10 insects per vial) with culture media and kept in incubators for 24 h. Then mortality of insects was observed.

Statistical analysis

The results from all replicates in contact toxicity tests were subjected to Probit analysis [10] using PriProbit Program V1.6.3 to determine LC₅₀ values and their 95 % confidence intervals [11]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The essential oil of *A. fragrans* was yellow with a yield of 0.06 % (v/w) and density of 0.82 g/ml. A total of 30 components of the essential oil were

identified, accounting for 98.7 % of the total oil. The major compounds in the essential oil were myristicin (41.3 %), elemicine (11.9 %), *cis*-isosafrole (11.5 %), borneol (9.1 %) and caryophyllene (8.8 %) (Table 1). Phenylpropanoids represent 4 of 30 compounds, corresponding to 65.2 % of the whole oil while 12 and 14 of the 32 constituents were monoterpenoids and sesquiterpenoids, respectively (13.3 % and 21.2 % of the whole essential oil, respectively) (Table 1).

The essential oil of *A. fragrans* exhibited contact toxicity against *S. zeamais* with a LC₅₀ value of 50.7 µg/adult (Table 2). Among 5 major constituents, elemicine possessed strongest contact toxicity (LC₅₀ = 13.5 µg/adult) while *cis*-isosafrole, myristicin, caryophyllene and borneol had LC₅₀ values of 31.2 µg/adult, 43.4 µg/adult, 57.9 µg/adult, and 98.4 µg/adult, respectively (Table 2).

Table 1: The main compounds of the essential oil of *Ainsliaea fragrans* aerial parts

Peak no.	Compound	Retention index	(%)
	<u>Monoterpenoids</u>		12.3
1	α-Pinene	931	0.1
2	β-Pinene	981	0.1
3	β-Myrcene	991	0.5
4	Limonene	1029	0.1
5	1,8-Cineole	1031	0.6
6	(Z)-β-Ocimene	1037	0.2
7	Linalool	1094	0.4
8	Camphor	1143	0.1
9	Borneol	1174	9.1
10	4-Terpineol	1176	0.2
11	α-Terpineol	1188	0.6
12	Bornyl acetate	1287	0.3
	<u>Sesquiterpenoids</u>		21.2
13	α-Cubebene	1349	0.9
14	Copaene	1375	2.2
15	Caryophyllene	1420	8.8
16	α-Caryophyllene	1449	3.2
17	(E)-β-Farnesene	1457	0.7
18	Alloaromadendrene	1462	1.5
19	Aristolene	1484	1.1
20	Germacrene D	1486	0.1
21	δ-Cadinene	1525	0.1
22	trans-Nerolidol	1564	0.6
23	Spathulenol	1574	0.3
24	Caryophyllene oxide	1578	1.5
25	τ-Cadinol	1642	0.1
26	β-Eudesmol	1648	0.1
	<u>Phenylpropanoids</u>		65.2
27	Eugenol	1356	0.5
28	<i>cis</i> -Isosafrole	1306	11.5
29	Myristicin	1523	41.3
30	Elemicine	1554	11.9

*RI, retention index as determined on a HP-5MS column using the homologous series of n-hydrocarbons

Table 2: Contact toxicity of *Ainsliaea fragrans* essential oil and its major constituents against adult *Sitophilus zeamais*

Treatment	LC ₅₀ (µg/adult)	95% fiducial limits	Slope ± SD	Chi-square value
Essential oil	50.7	47.4-54.1	3.2 ± 0.2	13.2
Borneol	98.4	91.7-106.4	4.6 ± 0.4	10.9
Caryophyllene	57.9	53.5-62.3	4.7 ± 0.4	8.3
Elemicine	13.5	12.5-14.6	3.4 ± 0.2	15.8
cis-Isosafrole	31.2	29.3-33.7	3.9 ± 0.4	13.9
Myristicin	43.4	40.1-46.9	2.7 ± 0.2	12.2
Pyrethrum extract	4.5	4.1-4.8	0.7 ± 0.1	13.5

DISCUSSION

The main constituents of the essential oil of *A. fragrans* aerial parts were myristicin, elemicine, cis-isosafrole, borneol and caryophyllene. Its chemical composition is noticeably different from that collected from other locations in China. For example, it varies substantially from the chemical compositions of the essential oils derived from five *A. fragrans* samples collected from Jiangxi Province, China [6]. This suggests that great variations in chemical composition of essential oil of *A. fragrans* aerial parts may be due to harvest time, geographical location, climatic and seasonal factors, as well as storage duration of the medicinal herbs. Thus, essential oil standardization is needed before *A. fragrans* essential oil prior to releasing the products for to the market.

The essential oil of *A. fragrans* aerial parts exhibited contact toxicity against *S. zeamais*. However, compared with positive control (Pyrethrum extract), *A. fragrans* essential oil showed 11 times lower acute toxicity to maize weevils. However, compared with contact toxicity of the other essential oils reported in the literature and which were tested using a similar bioassay, the essential oil obtained in the present study exhibited the same or stronger contact toxicity against maize weevils [12-16]. Among the five major constituents, elemicine exhibited the strongest contact toxicity against maize weevils and three phenylpropanoids possessed stronger toxicity than the crude oil. Caryophyllene showed the same level of toxicity as the essential oil, and borneol was less toxic than the essential oil. It appears that the acute toxicity of the essential oil may be due to the three phenylpropanoids.

In a previous report, elemicine exhibited acaricidal activity against larval cattle tick, *Rhipicephalus (Boophilus) microplus* [17]. Moreover, myristicin was shown to have contact and fumigant toxicity against several insects and mites, including houseflies (*Musca domestica*) [20], hairy caterpillars (*Spilarctia obliqua*) [18], armyworms (*Pseudaletia unipuncta*) [19], and house dust mites (*Dermatophagoides farinae*

and *D. pteronyssinus*) and mould mites (*Tyrophagus putrescentiae*) [20]. Myristicin was also demonstrated to exhibit strong synergistic activity because it has been demonstrated to possess strong inhibitory effects on many P450s [22].

The foregoing suggest that the essential oil of *A. fragrans* and its five major components especially three phenylpropanoids have some promise as a possible natural insecticide for the control of grain storage insects. However, to develop a practical application for the essential oil and its major constituents as novel insecticides, further research into their safety in humans is needed. Additional studies on the development of formulations are also necessary to improve efficacy and stability as well as to reduce cost.

CONCLUSION

The essential oil of *A. fragrans* aerial parts and its major constituents possess some activity against maize weevils but needs to be further evaluated for safety in humans and to enhance its insecticidal activity.

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