SHORT REPORT

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Insecticidal Activity of *Artemisia frigida* Willd. Essential Oil and Its Constituents Against Three Stored Product Insects

Zhe Zhang[1], Xue Pang[1], Shanshan Guo[1], Juqin Cao[1,2], Yang Wang[1], Zhenyang Chen[1], Yixi Feng[1]Ning Lei[3*] and Shushan Du[1*]

[1]*Beijing Key Laboratory of Traditional Chinese Medicine Protection and Utilization; Faculty of Geographical Science; Beijing Normal University, NO.19 Xinjiekouwai Street, Beijing 100875, P. R. China*

[2] *Medical Chemistry Department; School of Basic Medical Sciences; Ningxia Medical University, NO. 1160 Shengli Street, Xingqing District,Yinchuan 750004, P. R. China*

[3]*Department of Pharmacy; The General Hospital of the PLA Rocket Force, NO.16 Xinjiekouwai Street, Beijing 100875, P. R. China*

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Abstract: In this work, we investigated the chemical constituents of hydrodistillation essential oil from the aerial parts of *Artemisia frigida* Willd. All together 14 components were identified by GC-MS. Moreover, we tested the fumigant and contact activities of the essential oil and its five major individual compounds (terpinen-4-ol, verbenone, camphene, α-terpineol and α-terpinyl acetate) against *Liposcelis bostrychophila*, *Lasioderma serricorne* and *Tribolium castaneum*. In fumigant toxicity tests, α-terpineol possessed the strongest activity (LC_{50} = 3.27 mg/L air) against *L. serricorne*. Terpinen-4-ol exhibited the strongest activity (LC_{50} = 0.08 and 3.74 mg/L air respectively) against *L. bostrychophila* and *T. castaneum.* As for contact toxicity, terpinen-4-ol and α-terpinyl acetate exhibited fair toxicity against *L. bostrychophila* (LD_{50} = 33.10 and 31.80 µg/cm^2 respectively) and *L. serricorne* (LD_{50} = 8.62 and 8.87 µg/adult respectively), and camphene possessed the strongest activity (LD_{50} = 5.13 µg/adult) against *T. castaneum*. The results indicated that *A. frigida* essential oil and its individual compounds had the potential to be developed as natural fumigants and insecticides for control of these three stored-product insects.

Keywords: *Artemisia frigida* Willd.; essential oil; fumigant activity; contact activity; stored-product insects; GC; GC-MS.

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1. Plant Source

Fresh aerial parts of *A. frigida* were collected in August 2016 from Lanzhou City (36°01' N latitude and 103°45' E longitude), Gansu Province, China. The species was identified by Dr. Liu, Q.R.

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[*] Corresponding author: E-Mail: lilyzebra@163.com (N.Lei); dushushan@bnu.edu.cn (S.Du)

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A voucher specimen (BNU-dushushan-20160808) was deposited at the Faculty of Geographical Science Beijing Normal University.

2. Previous Studies

Artemisia frigida Willd., a perennial semi-shrub distributed in the heavily grazed grasslands in Inner Mongolia Autonomous Region and the northern part of China, is a commonly used medicinal material in Mongolian traditional folk medicine to treat joint renal heat, abnormal menstruation, swelling, and sore carbuncle. A literature survey has shown that *A. frigida* extracts have contact activity and fumigant activity against *L. bostrychophila* and *Sitophilus zeamais*, however, the study only tested the bioactivities of essential oil from *A. frigida* and seldom mentioned its bioactive compounds [1]. To the best of our knowledge, the essential oil from *A. frigida* has never been previously reported to have fumigant and contact activities against *L. serricorne* and *T. castaneum* adults.

3. Present Study

Here we analyzed the chemical composition of the essential oil of *A. frigida* aerial parts and further investigated the fumigant and contact activities of its crude oil and its individual constituents against *L. bostrychophila*, *L. serricorne* and *T. castaneum* adults, and tried to find bioactive compounds.

Table 1. Chemical constituents identified from the essential oil of *A. frigida* aerial parts.

NO.	**RI exp.**[a]	**RI lit.**[b]	**Compounds**	**Peak area (%)**	**Identified Method**[c]
1	941	951	Camphene	3.7	MS; RI
2	997	996	Yogomi alcohol	6.5	MS; RI
3	1032	1031	1,8-Cineole	14.4	MS; RI
4	1086	1080	Artemisia alcohol	3.1	MS; RI
5	1117	1122	*p*-2-Menthen-1-ol[d]	1.1	MS; RI
6	1124	1124	6-Camphenol	3.6	MS; RI
7	1145	1146	Camphor	45.0	MS; RI
8	1156	1152	Borneol	3.5	MS; RI
9	1177	1172	Terpinen-4-ol	5.1	MS; RI
10	1188	1190	α-Terpineol	2.6	MS; RI
11	1206	1195	Verbenone	1.6	MS; RI
12	1350	1367	α-Terpinyl acetate	2.0	MS; RI
13	1493	1491	Bicyclogermacrene	1.0	MS; RI
14	1506	1506	β-Bisabolene	1.9	MS; RI
			Monoterpene	92.2	
			Sesquiterpene	2.9	
			Total	**95.1**	

[a] RI exp., retention index as determined on HP-5MS column using the homologous series (C_9-C_{17}) of n-hydrocarbons. [b] RI lit., retention index taken from literatures. [c] MS = based on comparison of mass spectra with those listed in the NIST 05 and Wiley 275 libraries and with published data. [d] cis- or trans- *p*-2-Menthen-1-ol cannot be identified

Chemical components determination was carried out through GC-MS and GC-FID analysis. The essential oil was extracted from the aerial parts of *A. frigida* with the yield of 0.4% (v/w) and the density of 0.938 g/mL. The GC-MS and GC-FID analysis results for the *A. frigida* essential oil were summarized in Table 1. Total 14 components were identified in *A. frigida* essential oil and the sum of these components was accounted for 95.1%. The main components were camphor (45.0%), 1,8-cineole

(14.4%), yogomi alcohol (6.5%), terpinen-4-ol (5.1%), camphene (3.7%), 6-camphenol (3.6%), borneol (3.5%), artemisia alcohol (3.1%), α-terpineol (2.6%), α-terpinyl acetate (2.0%), β-bisabolene (1.9%) and verbenone (1.6%). Among these compounds, we selected five major compounds (terpinen-4-ol, verbenone, camphene, α-terpineol and α-terpinyl acetate) to test the bioactivities against three stored product insects along with *A. frigida* essential oil.

These results were different to those reported in the published literatures. For example, *A. frigida* essential oil collected from Inner Mongolia province mainly contained cis-ρ-menth-2-en-1-ol (20.8%), 1,8-cineole (12.0%), borneol (10.2%), lavandulol (9.3%) and camphor (6.9%) [1]. The main components of *A. frigida* essential oil collected from Central Alberta Prairies, Canada, were camphor (20.6%), 1,8-cineole (25.1%), chrysanthenone (7.4%), borneol (8.1%) and camphene (4.1%) [2]. And the essential oil of *A. frigida* collected on the catchment area of Lake Baikal showed that samples from different populations all have these components: 1,8-cineole (6.6-23.4%), camphor (3.6-35.9%), borneol (6.1-7.0%), terpinen-4-ol (4.2-14.1%), bornyl acetate (1.1-6.0%) and germacrene D (1.4-5.0%) [3]. It was believed that the variety of harvest time, regional habitat conditions and growing years might result in an obvious difference in the composition of the volatile oil. On the other hand, those studies also found some common compounds, such as camphor and 1,8-cineole, the finding was corresponded with previous research that bornane derivatives and 1,8-cineole were major characteristic components of many essential oils of *Artemisia* species [4].

Table 2. Fumigant toxicity of *A. frigida* essential oil and individual compounds against *L. bostrychophila* (LB), *L. serricorne* (LS), and *T. castaneum* (TC) adults

Insects	**Samples**	**LC_{50} [a] (mg/L air)**	**95% FL (mg/L air)**	**Slope ± SE**	**Chi-square**	***P-value***
LB	The oil	0.52	0.46-0.58	1.42 ± 0.19	10.02	0.931
	Terpinen-4-ol	0.08	0.07-0.08	4.68 ± 0.81	15.76	0.865
	Verbenone	0.14	0.13-0.15	14.79 ± 1.51	14.76	0.903
	Camphene	–	–	–	–	–
	α-Terpineol	0.58	0.52-0.65	4.66 ± 0.55	10.40	0.918
	α-Terpinyl acetate	0.39	0.37-0.42	2.96 ± 0.41	13.98	0.730
	Dichlorvos[b]	1.35×10^{-3}	$(1.25\text{-}1.47) \times 10^{-3}$	6.90 ± 0.60	10.40	–
LS	The oil	4.53	3.91-5.12	4.05 ± 0.54	18.15	0.446
	Terpinen-4-ol[c]	6.90	6.04-7.84	–	19.84	–
	Verbenone	–	–	–	–	–
	Camphene	8.78	5.75-11.15	2.47 ± 0.45	10.53	0.987
	α-Terpineol[c]	3.27	3.17-3.38	12.12 ± 1.51	19.09	0.986
	α-Terpinyl acetate	–	–	–	–	–
	Phosphine[d]	9.23×10^{-3}	$(7.13\text{-}11.37) \times 10^{-3}$	2.10 ± 0.30	12.00	–
TC	The oil	6.79	6.08-7.58	4.72 ± 0.57	7.41	0.986
	Terpinen-4-ol[e]	3.74	3.30-4.26	–	12.44	–
	Verbenone	7.09	6.00-10.05	3.54 ± 0.53	6.97	0.904
	Camphene	4.10	3.55-4.68	3.51 ± 0.47	14.02	0.698
	α-Terpineol	–	–	–	–	–
	α-Terpinyl acetate	–	–	–	–	–
	MeBr[f]	1.75	–	–	–	–

[a] 50% of lethal concentration. [b] Date from Zhao et al [7]. [c] Date from Zhang et al [8]. [d] Date from You et al [9]. [e] Date from Zhang et al [10]. [f] Date from Liu and Ho [11].

We have tested the bioactivities of *A. frigida* essential oil and five major individual compounds (terpinen-4-ol, verbenone, camphene, α-terpineol and α-terpinyl acetate). The results of fumigant assays for the oils are presented in Table 2. In fumigant toxicity tests, the essential oil extracted from the aerial parts of *A. frigida* showed pronounced toxicity against *L. bostrychophila*, *L. serricorne* and *T. castaneum* with LC_{50} values of 0.52, 4.53 and 6.79 mg/L air respectively. When it came to *L. bostrychophila* adults, terpinen-4-ol exhibited the strongest fumigant toxicity of LC_{50} = 0.80 mg/L air. Verbenone was 1.7 times less toxic than terpinen-4-ol and α-terpinyl acetate was 4.8 times less toxic than terpinen-4-ol, however, these two compounds were not so much effective against *L. serricorne* in our fumigant toxicity measure range and their LC_{50} values could not be calculated (LC_{50} > 50.0 mg/L air). Although α-terpineol has weakest fumigant against *L. bostrychophila*, it has the strongest activity against *L. serricorne* (LC_{50} = 3.27 mg/L air). Compared with the positive control (dichlorvos, LC_{50} = 1.35×10^{-3} mg/L air; phosphine, LC_{50} = 9.23×10^{-3} mg/L air), the fumigant toxicities of *A. frigida* essential oil and its compounds against *L. bostrychophila* and *L. serricorne* were weaker. However, compared with the fumigant activity of the other oils reported in the literature which were tested using a similar bioassay, the essential oil obtained in the present study exhibited the same or stronger fumigant toxicity against *L. bostrychophila* and *L. serricorne*, e.g. the *A. frigida* essential oil possessed the same level of fumigant toxicity to *L. bostrychophila* as that of *Ajania fruticulosa* essential oil (LC_{50} = 0.65 mg/L air) and the same level of fumigant toxicity to *L. serricorne* as that of *Artemisia mongolica* essential oil (LC_{50} = 6.08 mg/L air) [5,6]. When it came to *T. castaneum* adults, the essential oil and its components exhibited fair toxicity and they had fumigant activity from LC_{50} = 3.74 mg/L air to 7.09 mg/L air. Although terpinen-4-ol also exhibited the strongest fumigant toxicity (LC_{50} = 3.74 mg/L air) which was just 2.14 times less toxic than the positive control (MeBr, LC_{50} = 1.75 mg/L air), it was not obviously different from camphene (LC_{50} = 4.10 mg/L air) since their 95% confidence limit values overlap with each other.

The results of contact assays for the essential oil and its compounds against *L. bostrychophila*, *L. serricorne* and *T. castaneum* adults are presented in Table 3. Compared to the fumigant toxicity, the contact toxicity was more remarkable against *L. bostrychophila* and *L. serricorne*. Except for camphene and α-terpineol with negligible toxic effects (LC_{50} could not be calculated under the tested concentrations in the preliminary test) against *L. bostrychophila* and *T. castaneum* respectively, all of the other compounds analyzed showed toxic effects against these three stored product pests. The crude oil was always less contact toxic than its compounds against these three stored product pests. When it came to *L. bostrychophila* adults, terpinen-4-ol and α-terpinyl acetate showed similar contact activity (LD_{50} = 33.10 and 31.80 μg/cm^2) since their 95% confidence limit values overlap with each other and they were just about 1.7 times less toxic than the positive control (pyrethrins, LD_{50} = 18.72 μg/cm^2). As for *L. serricorne*, terpinen-4-ol and α-terpinyl acetate exhibited fair contact activities of LD_{50} = 8.62 μg/adult and 8.87 μg/adult respectively. Except for α-terpineol, other four major individual compounds had activities from LD_{50} = 5.13 to 9.60 μg/adult against *T. castaneum* adults and camphene exhibited the strongest contact toxicity (LD_{50} = 5.13 μg/adult).

By the structural analysis, terpinen-4-ol, α-terpineol and α-terpinyl acetate had the similar structures, however, the insecticidal activities of them are quite different. In fumigant tests, α-terpinyl acetate possessed weaker activity than terpinen-4-ol and α-terpineol which might due to its ester fragment. Moreover, in our previous work, oxygen-containing groups had been found to enhance the insecticide activities of essential oils [15], e.g. camphor, which has a similar structure to camphene, was reported to possess a fumigant activity of LC_{50} = 0.43 mg/L air against *L. bostrychophila* by Liang et al [5]. Comparing camphor to camphene, the former had stronger fumigant toxicity and it had a ketone group. In our tests, terpinen-4-ol, verbenone and α-terpinyl acetate all had oxygen-containing groups and they have been certified to possess fair contact activity.

Table 3. Contact toxicity of *A. frigida* essential oil and individual compounds against *L. bostrychophila* (LB), *L. serricorne* (LS) and *T. castaneum* (TC) adults

Insects	Samples	LD$_{50}$ (ug/cm^2 for LB; µg/adult for TC and LS)	95% FL	Slope ± SE	Chi-square	*P-value*
LB	The oil	78.18	74.52-81.98	7.24 ± 0.90	15.55	0.624
	Terpinen-4-ol	33.10	30.59-35.74	6.61 ± 0.71	8.01	0.928
	Verbenone	35.15	33.79-36.51	18.99 ± 2.00	16.23	0.845
	Camphene	–	–	–	–	–
	α-Terpineol	37.76	33.60-41.17	7.32 ± 0.99	4.25	0.994
	α-Terpinyl acetate	31.80	30.13-33.41	12.82 ± 1.78	12.10	0.520
	Pyrethrins[a]	18.72	17.60-19.92	2.98 ± 0.40	10.56	0.987
LS	The oil	16.84	14.26-19.50	3.70 ± 0.56	14.42	0.971
	Terpinen-4-ol[b]	8.62	7.38-9.85	–	12.65	–
	Verbenone	12.10	10.93-13.33	4.61 ± 0.50	19.77	0.656
	Camphene	17.73	12.24-21.94	17.87 ± 0.35	18.80	0.526
	α-Terpineol[b]	11.99	10.42-13.42	3.12 ± 0.43	18.96	0.624
	α-Terpinyl acetate	8.87	7.61-10.25	3.10 ± 045	13.75	0.745
	Pyrethrins	0.24	0.16-0.35	1.31 ± 0.2	17.36	–
TC	The oil	25.22	21.39-29.17	3.70 ± 0.56	14.42	0.345
	Terpinen-4-ol[c]	7.65	6.75-8.55	2.21 ± 0.33	18.77	0.715
	Verbenone	9.60	8.13-11.17	3.54 ± 0.53	6.97	0.904
	Camphene	5.13	4.32-5.91	2.76 ± 0.38	16.82	0.496
	α-Terpineol	–	–	–	–	–
	α-Terpinyl acetate	9.28	6.78-11.35	2.37 ± 0.43	15.71	0.613
	Pyrethrins[d]	0.26	0.22-0.30	3.34 ± 0.32	13.11	0.950

[a] Date from Liu et al [12]. [b] Date from Zhang et al [8]. [c] Date from Wang et al [13]. [d] Date from Guo et al [14].

It was reported that essential oils from plants and their constituents can be useful alternatives to conventional insecticides and fumigants due to no residues dangerous of stored food treated with such products for human health and environmentally safe [16-18]. This work indicates that the *A. frigida* essential oil and its individual constituents have potential to be developed into natural insecticides for the control of insects in stored products. However, further studies also should be focus on evaluating the efficacy, safety and cost of the essential oil and its compounds in a wide range of practical storage applications.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID

Zhe Zhang: 0000-0002-6956-3456
Xue Pang: 0000-0002-5544-1968
Shanshan Guo: 0000-0002-2981-939X
Juqin Cao: 0000-0001-6095-236X
Yang Wang: 0000-0003-1364-5142
Zhenyang Chen: 0000-0002-5130-7331
Yixi Feng: 0000-0002-3376-4512
Ning Lei: 0000-0002-4920-2127

Shushan Du: 0000-0003-0037-2480

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