

Original Research Article

Chemical composition and insecticidal properties of the essential oil of *Bidens frondosa* L (Asteraceae) against booklice (*Liposcelis bostrychophila*)

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Abstract

Purpose: To investigate contact and fumigant toxicity of the essential oil of *Bidens frondosa* and its isolated constituents against booklice (*Liposcelis bostrychophila*).

Methods: The essential oil of *B. frondosa* was obtained by hydro-distillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) with HP-5MS column. The active constituents were purified from the oil by bioactivity-guided fractionation. Contact (impregnated filter paper method) and fumigant toxicity (sealed space) of the oil and its isolates were determined.

Results: Thirty-two compounds, representing 98.88 % of the total oil, were determined and the major constituents of the essential oil were caryophyllene oxide (20.50 %), borneol (17.66 %), 4-terpineol (17.26 %), and β -cedrene (6.94 %). The essential oil displayed fumigant toxicity against booklice, with a median lethal concentration (LC_{50}) of 507.35 $\mu\text{g/L}$ while the isolated constituents, borneol and 4-terpineol, had LC_{50} values of 2.20 mg/L and 335.24 $\mu\text{g/L}$ against booklice, respectively. The essential oil also exhibited contact toxicity against *L. bostrychophila* with an LC_{50} value of 210.73 $\mu\text{g/cm}^2$. Borneol, caryophyllene oxide, β -cedrene, and 4-terpineol showed acute toxicity against booklice with LC_{50} of 98.04, 84.62, 458.79 and 211.35 $\mu\text{g/cm}^2$, respectively.

Conclusion: The results suggest that the essential oil and its isolates possess potential for cultivation into natural insecticides or fumigants, for control of insects in stored grains.

Keywords: *Bidens frondosa*, *Liposcelis bostrychophila*, Contact toxicity, Essential oil, Booklice, Stored grains, Natural insecticides, Fumigants

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INTRODUCTION

During our screening program for new pesticides from Chinese medicinal herbs and local wild plants, the essential oil of the aerial parts of *Bidens frondosa* L. (Family: Asteraceae) was found to exhibit acute toxicity against booklice (*Liposcelis bostrychophila* Badonnel). Common beggar-ticks (*B. frondosa*) are annual, erect, herbaceous plants arising from a taproot. This herb is usually about 20 to 60 centimeters high,

but it can reach 1.8 meters. It is weedy in appearance and extremely prolific.

This species originated from North America and has now become a common invasive plant in China where it is often found in moist woods, meadows, thickets, fields, roadsides, railroads, borders of streams, ponds, sloughs, swamps and ditches [1]. It is presently distributed in Guangdong, Jiangsu, Shanghai, Jiangxi, Zhejiang and Liaoning province, China [1,2].

Fresh leaves and stems of *B. frondosa* can be used as human food [3]. Chinese traditional medicine made use of the aerial parts of *B. frondosa* to treat virtual fatigue, night sweat hemoptysis, infantile malnutritional and dysentery [4]. Various polyacetylenes, acylated glucosides, flavonoids, phenylpropanoids, terpenoids, and sterols were isolated from this plant in the previous phytochemical investigations [5,6]. The composition of the essential oil derived from the leaves of *B. frondosa* has also been determined previously [3]. However, a literature survey has shown that there is no previous report on the volatile constituents and insecticidal activity of the essential oil of the aerial parts of *B. frondosa*. The aims of the present project were to determine the composition of the essential oil of *B. frondosa* and its insecticidal properties against booklice, and to isolate active constituents from the essential oil.

EXPERIMENTAL

Plant material and essential oil

Fresh aerial parts of *B. frondosa* (20 kg) at flowering stage were collected from Lishui City, Zhejiang Province, China (27.54° N and 119.20° E) in September 2014. The plant was classified by Dr. Liu (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU-Compositae-Dalangba-161) was saved at the museum of Department of Entomology, China Agricultural University, Beijing, China. The plant sample was chopped into small pieces and subjected to hydro distillation for 6 h using a modified Clevenger-type apparatus. *n*-Hexane was used as a solvent to extract the essential oil from the distilled solution and water was removed from the extract by using anhydrous Na₂SO₄. The essential oil was then saved in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Analysis of the essential oils

GC analysis was carried out using a Hewlett-Packard 5890 gas chromatograph equipped with HP-5 column (5 % diphenyl and 95 % dimethylpolysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness) with nitrogen as the carrier gas and flow rate of 1 mL min⁻¹. Oven temperature was programmed from 60 to 280 °C (at a rate of 2 °C min⁻¹); injector and detector temperatures were 270 °C and 300 °C, respectively. GC-MS analysis was performed using a Agilent 6890N gas chromatograph connected to Agilent 5973N mass selective detector at 70 eV ionization energy, equipped with an HP-5MS capillary

column (5 % diphenyl and 95 % dimethylpolysiloxane, 30m × 0.25mm × 0.25μm). The carrier gas was helium at a flow rate of 1.0 ml min⁻¹. Oven temperature was programmed as follows: the initial temperature was detained at 60 °C for 1 min and increased at 10 °C min⁻¹ to 180 °C, remained at 180 °C for 1 min, and then ramped at 20 °C min⁻¹ to 280 °C and held there for 15 min. The injector temperature was kept at 270 °C. The samples (1 μL, 1/100, v/v, in acetone) were instilled, with a split ratio of 1:10. Spectra were scanned over the range 20 to 550 m/z at 2 scans s⁻¹. Most constituents were identified by comparison of their retention indices with those reported in the literature or with those of authentic compounds available in our laboratories. Retention indices were determined using retention times of *n*-alkanes (C₈–C₂₄) under the same chromatographic conditions. Further identification was made by comparing the NIST 05 and Wiley 275 library data of the peaks with those reported in literature, mass spectra of the peaks with literature data [7]. Relative percentages of the oil constituents were estimated based on GC peak areas without using correction factors.

Purification and characterization of four constituents

The crude essential oil of *B. frondosa* (30 ml) was subjected to column chromatography over silica gel, eluting with a gradient of increasing ethyl acetate in *n*-hexane (0-100 %, v/v). Fractions of 300 mL each were gathered and distilled at 35 °C, and related fractions as indicated by their TLC profiles were merged to produce 15 fractions. Based on contact bioassay, fractions (4, 6, 9 and 13) were further separated by preparative silica gel column chromatography (PTLC) until to obtain four pure compounds borneol, 4-terpineol, β-cedrene and caryophyllene oxide. The structure of the compounds was determined by using nuclear magnetic resonance. ¹H and ¹³C NMR spectra were detected on Bruker Avance DRX 500 instruments using CDCl₃ as solvent with TMS as internal standard.

Insects

The booklice (*L. bostrychophila*) were provided from a laboratory colony in the dark in incubators at 28-30 °C and 70 - 80 % relative humidity and the artificial diet (1: 1: 1 mixture of milk powder, active yeast, and flour) was provided. All the containers accommodating the insects and the Petri dishes utilized in experiments were made escape-proof with a coating of polytetrafluoroethylene (Fluon[®], Blades

Biological, Edenbridge, UK). Laboratory bioassays were performed within one week after adult collections.

Contact toxicity test

In a preliminary experiment, suitable testing concentration range of the essential oil of *B. frondosa* and pure compounds was determined. Acetone was used to dilute the essential oil and compounds. The solution (150 μ L, 2.0, 2.4, 2.9, 3.5, 4.2 and 5.0 % in acetone) was applied to a filter paper (Whatman, 3.5 cm in diameter). Then the treated filter paper was treatment with a solid glue (Glue Stick, Jong le Nara Co Ltd, Hong Kong) and put in a petri dish (3.5 cm in diameter). Ten booklice were left on the Petri dish. The cover of the Petri dish with several drilled holes drilled into it was placed over it and all the Petri dishes were kept in an incubator at 27 - 29 °C, 70 - 80 % RH for 24 h. Acetone was used as a control while pyrethrum extract (25 % pyrethrin I and pyrethrin II, obtained from Fluka Chemie) was adopted as a positive control. Five concentrations and five replicates of each concentration were taken in all treatments and controls. Mortality of insects was examined.

Fumigant toxicity bioassay

The appropriate test concentrations of the essential oil/pure compounds were estimated by range-finding experiments. Ten adults of the booklice were transferred into a small glass bottle (8 mL) by using a fine goat hair brush. Acetone was utilized to dilute the essential oil/compounds. A 10 μ L solution (3.1, 3.4, 3.7, 4.1, 4.5 and 5.0 % in acetone) was evenly applied to a filter paper strip (3.5 cm \times 1.5 cm). The impregnated filter paper was then laid in the bottom cover of a big glass bottle (250 mL). The small bottle was placed in a big bottle, and covered leaving a sealed space. The booklice were exposed for 24 h and the mortality of insects was determined. All the treatments were replicated five times. Acetone was utilized as control and dichlorvos was employed as positive control. Positive control, dichlorvos (99.9 %) was purchased from Aladdin-reagent Company (Shanghai).

Statistical analysis

The LC₅₀ values of the essential oil/compounds and their 95 % confidence intervals were estimated by using Probit Program V1.6.3 [8]. Samples for which the 95 % fiducial limits did not overlap were regarded as significantly different.

RESULTS

The content of essential oil of the aerial parts of *B. frondosa* at flowering stage was 0.03 % (yellow, v/w based on fresh weight) while its density was 0.89 g/ml. A total of 32 components from the essential oil of *B. frondosa* were detected, representing 98.88 % of the crude essential oil (Table 1). The principal constituents of *B. frondosa* essential oil were caryophyllene oxide (20.50 %), borneol (17.66 %), 4-terpineol (14.56 %), α -terpineol (6.28 %), β -cedrene (6.94 %), spathulenol (4.07 %) and caryophyllene (3.97 %) (Table 1). Sesquiterpenoids represented 16 of the 32 constituents, corresponding to 52.14 % of the essential oil while monoterpenoids characterized 12 of the 32 compounds (46.06 % of the crude essential oil).

Borneol. Colorless oil. ¹H-NMR (CDCl₃, 500 MHz) δ ppm: 4.04 (1H, m, H-1), 2.28 (1H, m, H-5), 1.90 (1H, m, H-6a), 1.70 (2H, m, H-6b, H-4a), 1.62 (1H, t, -OH), 1.25 (2H, m, H-4b, H-3a), 0.95 (1H, dd, H-3b), 0.87 (3H, s, 8-C H₃), 0.86 (3H, s, 9-C H₃), 0.85 (3H, s, 10-C H₃). ¹³C-NMR (CDCl₃, 125MHz) δ ppm: 77.7 (C-1), 49.9 (C-2), 48.2 (C-8), 45.2 (C-5), 39.2 (C-6), 28.5 (C-4), 26.2 (C-3), 20.5 (C-9), 18.9 (C-10), 13.4 (C-7). The spectra data are in agreement with earlier reports [9,10].

4-Terpineol. Colorless oil. ¹H-NMR (CDCl₃, 500 MHz) δ ppm: 5.32 (1H, m, H-5), 2.17 (2H, m, H-6), 1.94 (2H, m, H-3), 1.71 (3H, s, 4-CH₃), 1.67 (1H, m, H-7), 1.58 (2H, m, H-2), 0.97 (3H, d, J = 7.0 Hz, 8-CH₃), 0.94 (3H, d, J = 7.0 Hz, 9-CH₃). ¹³C-NMR (CDCl₃, 125MHz) δ ppm: 133.9 (C-4), 118.5 (C-5), 71.8 (C-1), 36.8 (C-7), 34.6 (C-2), 30.8 (C-6), 27.1 (4-CH₃), 23.3 (C-3), 16.8 (C-8, 9). The spectra data are consistent with the previous reports [9,10].

β -Cedrene. Colorless oil. ¹H-NMR (CDCl₃, 500 MHz) δ ppm: 4.58 (1H, t, J = 2.2 Hz, H-15a), 4.51 (1H, t, J = 2.2 Hz, H-15b), 2.32 (2H, m, H-4), 2.19 (1H, d, J = 4.4 Hz, H-2), 1.87 (1H, H-8a), 1.82 (1H, H-10), 1.78 (1H, H-1a), 1.69 (1H, H-7), 1.55 (1H, H-9a), 1.48 (1H, H-5a), 1.43 (1H, H-9b), 1.38 (1H, H-5b), 1.30 (1H, H-8b), 1.20 (1H, H-1b), 0.97 (3H, s, 12-CH₃), 0.94 (3H, s, 13-CH₃), 0.85 (3H, d, J = 6.9 Hz, 14-CH₃). ¹³C-NMR (CDCl₃, 125MHz) δ ppm: 151.8 (C-3), 107.6 (C-15), 60.7 (C-2), 56.5 (C-10), 55.4 (C-6), 45.1 (C-1), 42.3 (C-11), 37.0 (C-8), 33.7 (C-8), 33.7 (C-3), 29.7 (C-4), 26.6 (C-12), 25.9 (C-13), 15.7 (C-9), 15.5 (C-14). The spectra data are in agreement with the reported data [10].

Table 1: The main compounds of the essential oil of *Bidens frondosa*

Peak no.	Compound	Retention index	(%)
	Monoterpenoids		46.06
1	α -Pinene	939	0.11
2	β -Pinene	981	0.56
3	β -Phellandrene	1027	1.26
4	1,8-Cineole	1031	1.13
5	(Z)- β -Ocimene	1037	0.71
6	Linalool	1094	0.67
7	Camphor	1146	1.31
8	Borneol	1174	17.66
9	4-Terpineol	1177	17.26
10	α -Terpineol	1189	3.58
11	Verbenone	1205	1.11
12	Pulegone	1236	0.72
	Sesquiterpenoids		52.14
13	β -Cubebene	1387	1.17
14	Caryophyllene	1420	3.97
15	β -Cedrene	1424	6.94
16	β -Gurjunene	1434	0.71
17	α -Caryophyllene	1454	2.69
18	Germacrene D	1487	3.18
19	Eremophilene	1489	0.71
20	γ -Cadinene	1514	0.61
21	Calamenene	1520	0.89
22	δ -Cadinene	1523	2.68
23	<i>trans</i> -Nerolidol	1564	1.18
24	Spathulenol	1578	4.07
25	Caryophyllene oxide	1583	20.50
26	Humulene oxide II	1608	0.21
27	β -Eudesmol	1648	1.69
28	α -Cadinol	1654	0.94
	Others		0.66
29	Eugenol	1356	0.22
30	Methyleugenol	1403	0.11
31	β -Ionone	1485	0.21
32	Phytol	2119	0.12
	Total identified		98.88

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

Caryophyllene oxide. Colorless oil. ^1H NMR (CDCl_3 , 500MHz) δ : 5.00 (1H, s, H-12), 4.88 (1H, s, H-12), 2.90 (1H, *dd*, $J = 4.1$ and 10.7 Hz, H-9), 2.64 (1H, *d*, $J = 9.1$ Hz, H-2), 2.33 - 2.37 (1H, *m*, H-11), 2.28 (1H, *dd*, $J = 3.6$ and 8.4 Hz, H-10), 2.09 - 2.15 (2H, *m*, H-7, H-11), 1.72 - 1.74 (1H, *m*, H-5), 1.69 (1H, *br. s.*, H-3), 1.65 - 1.67 (1H, *m*, H-6), 1.62 (1H, *br. s.*, H-3), 1.44 (1H, *d*, $J = 2.8$ Hz, H-6), 1.36 - 1.39 (1H, *m*, H-10), 1.23 (3H, s, H-15), 1.03 (3H, s, H-13), 1.01 (3H, s, H-14), 0.99 (1H, *br. s.*, H-7). ^{13}C NMR (CDCl_3 , 125MHz) δ : 151.8 (C-1), 112.8 (C-12), 63.8 (C-9), 59.9 (C-8), 50.7 (C-5), 48.9 (C-2), 39.7 (C-3), 39.1 (C-7), 34.0 (C-4), 30.2 (C-10), 29.9 (C-14), 29.8 (C-11), 27.2 (C-6), 21.6 (C-13), 17.0 (C-15). The spectra data matched with the previous report [10].

value of $210.73 \mu\text{g}/\text{cm}^2$ (Table 2). Borneol, caryophyllene oxide, β -cedrene, and 4-terpineol exhibited acute toxicity against the booklice with LC_{50} values of $98.04 \mu\text{g}/\text{cm}^2$, $84.62 \mu\text{g}/\text{cm}^2$, $458.79 \mu\text{g}/\text{cm}^2$, and $211.35 \mu\text{g}/\text{cm}^2$, respectively (Table 2).

Significant fumigant toxicity of the essential oil of *B. frondosa* against booklice (*L. bostrychophila*) was found with LC_{50} value of $507.35 \mu\text{g}/\text{L}$ while the two isolated constituents, borneol and 4-terpineol had LC_{50} values of $2.20 \text{ mg}/\text{L}$ and $335.24 \mu\text{g}/\text{L}$ against the booklice, respectively (Table 2). Caryophyllene oxide and β -cedrene did not show fumigant toxicity against the booklice at the experimental dosage (Table 2).

Contact toxicity of the essential oil of *B. frondosa* was found against *L. bostrychophila* with an LC_{50}

Table 2: Contact and fumigant toxicity of *Bidens frondosa* essential oil and its major constituents against the adults of *Liposcelis bostrychophila*

Toxicity	Treatment	LC ₅₀ (95 % FL)*	Slope ± SD	Chi-square (χ ²)
Contact toxicity (μg/cm ²)	Essential oil	210.73 (191.46-229.76)	7.79 ± 0.77	11.27
	Borneol	98.04 (91.03-106.78)	7.33 ± 0.71	7.84
	Caryophyllene oxide	84.62 (76.24-90.43)	5.54 ± 0.53	13.72
	β-Cedrene	458.79 (419.34-496.98)	6.51 ± 0.62	8.98
	4-Terpineol	211.35 (195.45-229.54)	8.18 ± 0.74	11.56
	Pyrethrum extract	18.99 (17.56-20.06)	7.64 ± 1.05	9.45
Fumigant (μg/L)	Essential oil	507.35 (474.14-545.77)	5.75 ± 0.52	10.09
	Borneol	2200.80 (2065.12-2387.45)	4.08 ± 0.41	9.92
	Caryophyllene oxide	> 10,000	-	-
	β-Cedrene	> 10,000	-	-
	4-Terpineol	335.24 (319.34-361.76)	4.79 ± 0.45	10.34
	Dichlorvos	1.35 (1.25-1.47)	6.87 ± 0.77	4.27

* Fiducial limits

DISCUSSION

The major constituents identified in the essential oil of *B. frondosa* aerial parts were caryophyllene oxide, borneol, 4-terpineol, α-terpineol, β-cedrene, spathulenol and caryophyllene (3.97 %). The results are different from the essential oil of *B. frondosa* leaves that were collected from Kyoungsan city, Republic of Korea [3]. β-Caryophyllene (14.3 %), rosefuran (13.5 %), verbenone (11.5 %), spathulenol (6.02 %), caryophyllene oxide (5.49 %), *cis*-tagetone (5.3 %), α-humulene (4.02 %) and linalool (3.2 %) were determined as the main compounds in the essential oil derived from the leaves of *B. frondosa* [3].

The essential oil of the aerial parts of *B. frondosa* and its isolates possessed acute toxicity toward the booklice. Compared with the positive control, pyrethrum extract (LC₅₀ = 18.99 μg/cm²), the contact toxicity was only 11 times less. However, the essential oil of *B. frondosa* demonstrated stronger contact toxicity against *L. bostrychophila* adults than several essential oils using the same bioassay, e.g. *Artemisia rupestris* [11], *A. frigida* [12], *Curcuma wenyujin* [13], *Foeniculum vulgare* [14], and *Valeriana jatamansi* [15], however, the essential oil of *B. frondosa* shows less contact toxicity than the essential oils of *Illicium henryi* [16] and *Kaempferia galangal* [17]. Among the four isolated constituents, caryophyllene oxide,

and borneol displayed stronger contact toxicity (no overlap in 95 % fiducial limit) than the essential oil against the booklice (*L. bostrychophila*). 4-Terpineol shows the same level of contact toxicity as the oil against booklice but booklice was more susceptible to the essential oil than to β-cedrene (Table 2). Therefore, it is suggested that the contact toxicity of the essential oil may be mainly attributed to caryophyllene oxide and borneol as well as 4-terpineol.

The essential oil of *B. frondosa* and its two isolates, borneol and 4-terpineol were found to display fumigant toxicity against the booklice (*L. bostrychophila*). 4-Terpineol exhibited stronger fumigant toxicity (no overlap in 95 % fiducial limit) than the crude oil (Table 2). When compared with dichlorvos (LC₅₀ = 1.35 μg/L), the essential oil of *B. frondosa* and 4-terpineol showed only 375 times and 248 times less toxicity against *L. bostrychophila*, respectively. However, the essential oil of *B. frondosa* demonstrated a stronger fumigant toxicity against booklice than several essential oils using the same bioassay, e.g., *Artemisia rupestris* (LC₅₀ = 6.67 mg/L air) [11], *A. frigida* (LC₅₀ = 1.25 mg/L) [12], *C. wenyujin* (LC₅₀ = 2.76 mg/L) [13], *V. jatamansi* (LC₅₀ = 6.0 mg/L) [15], and *K. galangal* (LC₅₀ = 1.5 mg/L air) [17], but the oil of *B. frondosa* demonstrated less toxicity than the essential oils of *Allium chinense* (LC₅₀ = 186.5 μg/L air) [18]

and *F. vulgare* fruits ($LC_{50} = 34.07 \mu\text{g/L}$) [14]. However, the currently commercial fumigants (e.g. phosphine and MeBr) are synthetic insecticides and are also extremely poisonous against human beings and other non-target organisms. Thus, it is suggested that fumigant activity of the essential oil of *B. frondosa* aerial parts and the two isolates, especially 4-terpineol is quite promising.

In the previous studies, 4-terpineol had been demonstrated to possess fumigant toxicity against several grain storage insects, such as *Sitophilus granaries*, *S. oryzae*, *S. zeamais*, *Tribolium castaneum*, *T. confusum*, and *Rhyzopertha dominica* as well as German cockroaches, *Blattella germanica* [19]. Borneol was also found to exhibit insecticidal activities against several insects and mites [19]. Moreover, it has been shown that caryophyllene oxide exhibited fumigant and contact toxicity as well as repellent activity against several insects and mites [20]. It also displayed larvicidal activity against *Aedes aegypti* with a LC_{50} value of 125 ppm [21]. However, this is the first time to report contact toxicity of β -cedrene against grain storage insects. The above findings suggest that the essential oil of *B. frondosa* aerial parts and the four isolated constituents, especially 4-terpineol show promise for development into possible natural insecticides/fumigants to control stored product insects.

The aerial parts of *B. frondosa* have been used to treat virtual fatigue, night sweat; hemoptysis, infantile malnutrition and dysentery in traditional Chinese medicine [6] and young leaves of *B. frondosa* are utilized as human food and tea [3]. It seems that the aerial parts of *B. frondosa* are quite safe for human consumption. However, there is no experimental data on toxicity of the essential oil of *B. frondosa* aerial parts and its four isolated constituents to humans to the best of our knowledge.

CONCLUSION

The essential oil of aerial parts of *B. frondosa* demonstrates strong contact and fumigant toxicity against booklice. Four active constituents were isolated and identified from the essential oil. The results suggest that the essential oil of *B. frondosa* aerial parts and the four isolated constituents, especially 4-terpineol have a potential for development into natural insecticides or fumigants for the control of insects in stored grains. However, further evaluation to determine their safety in humans and to optimize their activities is needed.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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