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Chemical composition and toxicity of the essential oil of *Cayratia japonica* against two grain storage insects

Zhi Long Liu[a]*, Kai Yang[a], Fan Huang[a], Qi Zhi Liu[a], Ligang Zhou[b] and Shu Shan Du[c]

[a]*Department of Entomology;* [b]*Department of Plant Pathology, China Agricultural University, Beijing, 100193, China;* [c]*State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, Beijing, 100875, China*

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During our screening program for new agrochemicals from Chinese medicinal herbs, essential oil of *Cayratia japonica* aerial parts was found to possess strong insecticidal activities against *Sitophilus zeamais* and *Tribolium castaneum*. A total of 37 components of the essential oil were identified by gas chromatography (GC) and GC–mass spectrometry (GC–MS). Linalool (19.4%), *trans*-α-ionene (11.4%), α-terpineol (7.9%), dihydroactinolide (7.8%) and geranial (5.8%) were the main components of the essential oil. The essential oil exhibited strong fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 10.05 and 15.67 mg/L air, respectively. *Cayratia japonica* essential oil also possessed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 32.06 and 44.49 mg/adult, respectively. The essential oil *C. japonica* may have potential to be developed as a new natural fumigant/insecticide for the control of stored-product insects.

Keywords: *Cayratia japonica*; *Sitophilus zeamais*; *Tribolium castaneum*; contact toxicity; essential oil composition

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 design of target-specific molecules (1). During the screening program for new agrochemicals from Chinese medicinal herbs, the essential oil of *Cayratia japonica* (Thunb.) Gagnepain (Family: Vitaceae) aerial parts was found to possess strong insecticidal toxicity against the maize weevils, *Sitophilus zeamais* (Motschulsky) and red flour beetles, *Tribolium castaneum* Herbst. *Sitophilus zeamais* and *T. castaneum* are the most widespread and destructive primary insect pests of stored cereals (2). Infestations not only cause significant losses due to the consumption of grains, they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (3). The control of this insect relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users. An alternative to synthetic pesticides is the use of natural compounds such as essential oils that result from secondary metabolism in plants. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects (4).

Bushkiller or sorrel vine (*C. japonica*) is an herbaceous vine that climbs by means of branched tendrils. *Cayratia japonica* and is native to a wide area of temperate and southeast Asia. It has been reported from Japan, southern China, Indo-China, the Philippines, Taiwan, New Guinea and Queensland (5). It aggressively spreads from an aggressive and invasive root system. A single plant can quickly cover a large area, including the existent vegetation. Aerial parts and roots of *C. japonica* are used in traditional Chinese medicine to relieve swelling and heat, and to enhance diuresis and detoxification (6, 7). Roots of *C. japonica* are also used in Malaysia and Thailand traditionally to treat cancer (8). Acetone extracts of *C. japonica* aerial parts showed contact toxicity against the diamond back moth, *Plutella xylostella* (9). Previous phytochemical studies on *C. japonica* resulted in the identification of flavonoids, anthocyanin, phenolic acids, triterpenoids (10–13) and alkaloids was reported in the roots of this species of plants (7). The chemical composition of *C. japonica* essential oil was also studied previously (14). The essential oil has been demonstrated to possess antiviral and anti-bacterial activity (14, 15). However, no reports on insecticidal activity of *C. japonica* essential oil against stored-product insects were available so far. This study analyses the chemical composition and toxicity of essential oil of *C. japonica* against stored-product insects.

*Corresponding author. Email: zhilongliu@cau.edu.cn

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Experimental

Plant material

Ten kilograms of aerial parts of *C. japonica* were harvested from Baiwangshan National Forest Park, Haidian District (Beijing 100094, China). The species was identified, and the voucher specimens (BNU-Liuzhilong-Vit-10-211) were deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The plant was air-dried and first ground to a powder using a grinding mill (Retsch Muhle, Germany). Each 600-g portion of powder ground was mixed in 1800 mL of distilled water and soaked for 3 hours. The mixture was then boiled in a round-bottom flask, and steam distilled for 6–8 hours. Volatile essential oil from distillation was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separatory funnel, using the non-polar solvent, *n*-hexane. The solvent was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-124, Switzerland). The sample was dried over anhydrous Na_2SO4 and kept in a refrigerator (4°C) for subsequent experiments.

Analysis of the essential oils

Capillary gas chromatography (GC) was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector; fused silica capillary column HP-5 (5% diphenyl and 95% dimethylpolysyloxane, 30 m × 0.25 mm, 0.25 μm film thickness); helium as carrier gas (1 mL/minure); and temperature programming from 60° to 280°C (2°C/minute); injector temperature 270°C and detector temperature 300°C. Components of the essential oil were separated and identified by GC–mass spectrometry (GC–MS) using an Agilent 6890N gas chromatograph hooked to an Agilent 5973N mass selective detector, equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60°C for 1 minute and ramped at 10°C/minute to 180°C for 1 minute, and then ramped at 20°C/minute to 280°C for 15 minutes. The injector temperature was maintained at 270°C. The samples (1 μL) were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL/minute. Spectra were scanned from 20 to 550 *m*/*z* at two scans per second. Most constituents were identified by GC by comparison of their retention indices with those of the literature (14) 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 08 (19) and Wiley 275 libraries or with mass spectra from literature (16). Component relative percentages were calculated based on GC peak areas without using correction factors.

Insects

Sitophilus zeamais and *T. castaneum* were obtained from laboratory cultures maintained for the last 10 years in the dark in incubators at 27–29°C and 70–80% relative humidity. *Sitophilus zeamais* adults were reared on whole wheat at 12–13% moisture content while *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w:w). Unsexed adults of the two species of insects used in all the experiments were about 2 weeks old.

Fumigant toxicity

A Whatman filter paper (diameter 2.0 cm, CAT No. 1001020) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 mL). Range-finding studies were run to determine the appropriate testing concentrations. Ten microliters of 5.0–25.0% of essential oil (V:V, six concentrations) was added to the filter paper. The solvent was allowed to evaporate for 15 seconds before the cap was placed tightly on the glass vial (with 10 unsexed insects) to form a sealed chamber. Fluon (ICI America Inc.) was used inside glass vial to prevent insects from touching the treated filter paper. *n*-Hexane was used as controls. Six replicates were used in all treatments and controls and they were incubated at 27–29°C and 70–80% relative humidity for 24 hours. The insects were then transferred to clean vials with some culture media and kept in an incubator. The mortality of insects was observed daily until end-point mortality was reached 1 week after treatment. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values (17).

Contact toxicity using topical application

The contact toxicity of essential oil against *S. zeamais* and *T. castaneum* adults was measured as described by Liu and Ho (2). Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (2.0–30.0%, six concentrations) was prepared in *n*-hexane. Aliquots of 0.5 μl of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Six replicates were used in all treatments and controls. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators. The mortality of insects was observed daily until end-point mortality was reached 1 week after treatment. The LD_{50} values were calculated by using Probit analysis (17).

Results and discussion

The steam distillation for 3 hours of aerial parts of *C. japonica* afforded essential oil (yellow) with a yield of 0.06% (v/w) and the density of the concentrated essential oil was determined to be 0.82 g/mL. The GC–MS analysis of the essential oils of the aerial parts of *C. japonica* led to the identification and quantification of a total of 37 major components accounting for 99.0% of the total components present (Table 1). Linalool (19.4%), *trans*-α-ionene (11.4%), α-terpineol (7.9%), dihydroactinolide (7.8%) and geranial (5.8%) were the main components of the essential oil. In a previous report (14), 30 components (e.g. sabinene, 4-terpineol, α-terpineol, piperitone, bornyl acetate) were identified but the percentage content of the components was not provided. However, the two main components (linalool and *trans*-α-ionene) in the present study were not detected in the previous report (14). The above findings suggest that further studies on plant cultivation and essential oil standardization are needed because of great variations in chemical composition of essential oil of *C. japonica* aerial parts.

Table 1. Chemical constituents of volatile oil from *Cayratia japonica* aerial parts.

Compounds	RI[a]	RI[b]	Peak area (%)
α-Pinene	931	934	0.4
Camphene	952	949	0.5
Sabinene	979	975	2.2
β-Pinene	981	975	0.6
6-Methyl-5-hepten-2-one	986	985	2.6
β-Myrcene	991	990	0.9
β-Phellandrene	1026	1030	0.
Limonene	1032	1035	1.8
γ-Terpinene	1057	1060	0.8
Acetophenone	1066	1065	1.4
cis-Linalool oxide	1076	1074	1.7
Linalool	1094	1098	19.4
Camphor	1143	1145	2.7
p-Mentha-1,5-dien-8-ol	1168	1170	1.3
Terpinen-4-ol	1175	1177	2.4
α-Terpineol	1189	1189	7.9
Verbenone	1205	1204	1.2
β-Cyclocitral	1215	1219	3.6
Neral	1235	1238	0.7
Geranial	1269	1270	5.8
Phellandral	1281	1278	0.3
Bornyl acetate	1285	1285	3.4
Piperitenone	1340	1343	0.8
α-Copaene	1374	1376	2.6
β-Geranyl acetate	1380	1381	2.7
β-Bourbonene	1385	1388	0.5
β-Elemene	1391	1390	2.9
trans-α-Ionene	1426	1430	11.4
β-Gurjunene	1432	1431	0.4
γ-Selinene	1470	1470	1.1
trans-β-Ionone	1482	1488	1.6
δ-Selinene	1491	1493	0.7
γ-Cadinene	1513	1514	0.7
δ-Cadinene	1523	1523	3.6
Dihydroactinolide	1531	1532	7.8
δ-Cadinol	1640	1640	0.5
Phytol	2119	1949	0.1
Total identified			99.0
Monoterpenoids			58.5
Sesquiterpenoids			28.5
Other			12.0

Notes: [a]RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons. [b]RI, relative retention index taken from Adams (2007) and/or NIST 08 (2008).

The essential oil of *C. japonica* aerial parts exhibited contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 32.06 and 44.49 µg/adult, respectively (Table 2). Linalool, one of main constituents of the essential oil, also possessed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 13.90 and 8.12 µg/adult, respectively (20). However, the essential oil showed weak acute toxicity against the two species of insects when compared with the positive control (pyrethrum extract, 25% pyrethrine I and pyrethrine II) because the pyrethrum extract possessed acute toxicity to maize weevils and red flour beetles with LD_{50} values of 4.29 and 0.36 µg/adult, respectively (18).

The essential oil of *C. japonica* aerial parts also possessed strong fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 7.54 and 15.67 mg/L air, respectively (Table 2). Linalool also showed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 10.46 and 9.34 mg/L air, respectively (20). The currently used grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 and 1.75 mg/L air, respectively (2). The essential oil was 11 and nine times less toxic to the maize weevils and red flour beetles compared with the commercial fumigant MeBr. When compared with the other essential oils in the literature, the essential oils *C. japonica* aerial parts exhibited stronger or the same level of fumigant toxicity against the maize weevils, e.g. essential oils of *Murraya exotica* (LC_{50}=8.29 mg/L) (21), *Artemisia lavandulaefolia* (LC_{50}=11.2 mg/L) (18), *Artemisia vestita* (LC_{50}=13.42 mg/L) (22), *Illicium simonsii* (LC_{50}=14.95 mg/L) (23) and *sieversiana sieversiana* (LC_{50}=15.0 mg/L) (18). Considering the currently used fumigants are synthetic insecticides, fumigant activity of the essential oil of *C. japonica* is quite promising. These findings, considered together, suggest that the essential oil of *C. japonica* shows potential to be developed as a natural fumigant for control of stored-product insects. However, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost. The isolation and identification of the bioactive compounds in the essential oil of *C.*

Table 2. Insecticidal activities of the essential oil of *Cayratia japonica* against *Sitophilus zeamais* and *Tribolium castaneum* adults.

Insects	Essential oil	Contact toxicity		Fumigant toxicity	
		LD_{50} (μg/adult)	95% fiducial limits	LC_{50} (mg/L air)	95% fiducial limits
S. zeamias	*C. japonica*	32.06	29.29–34.85	7.54	6.74–8.52
	Linalool[a]	13.90	13.05–14.83	10.46	9.58–11.55
	Pyrethrum extract[b]	4.29	–	–	–
	MeBr[c]	–	–	0.67	–
T. castaneum	*C. japonica*	44.49	40.78–47.69	15.67	–
	Linalool[a]	8.12	7.43–9.09	9.34	8.64–10.17
	Pyrethrum extract[b]	0.36	–	–	
	MeBr[c]			1.75	

Notes: [a]Data from Wang et al. (20). [b]Data from Liu et al. (18). [c]From Liu and Ho (2).

japonica aerial parts are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited.

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References

1. M.B. Isman, *Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world.* Ann. Rev. Entomol., **51**, 45–66 (2006).
2. Z.L. Liu and S.H. Ho, *Bioactivity of the essential oil extracted from* Evodia rutaecarpa *Hook f. et Thomas against the grain storage insects*, Sitophilus zeamais *Motsch. and* Tribolium castaneum *(Herbst)*. J. Stored Prod. Res., **35**, 317–328 (1999).
3. N. Magan, R. Hope, V. Cairns and D. Aldred, *Postharvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain.* Eur. J. Plant Pathol., **109**, 723–730 (2003).
4. S. Rajendran and V. Srianjini, *Plant products as fumigants for stored-product insects control.* J. Stored Prod. Res., **44**, 126–135 (2008).
5. L.E. Brown, Cayratia japonica *(Vitaceae) and* Paederia foetida *(Rubiaceae) adventive in Texas*. Phytologia, **72**, 45–47 (1992).
6. Jiangsu New Medical College, *Dictionary of Chinese Herbal Medicine*. Shanghai Science & Technology Press, Shanghai, China, 472–474 (1977).
7. J. Gong, J. Zhang, S.F. Ni, Y.F. Wu, Y. Tong and R.F. Luo, *Pharmaceutical research on* Cayratia Juss. *plants produced in China*. J. Anhui Agri. Sci., 37, 3031–3032 (2009).
8. C.C. Lee and P. Houghton, *Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer.* J. Ethnopharmacol., **100**, 237–243 (2005).
9. M. Li, X.X. Gao, Z.J. Gao, W. Zhao and Z.W. Sun, *Insecticidal activity of extracts from forty-eight plants including* Xanthium sibiricum *Patrin*. J. Plant Resour. Environ., **17**, 33–37 (2008).
10. N. Ishikura and M. Shibata, *Cayratinin, a new anthocyanin from the pericarp of* Cayratia japonica. Bot. Mag. Tokyo, **83**, 179–183 (1970).
11. H.Y. He and L.Q. Ling, *Studies on chemical constituents of* Cayratia japonica. Chin. Tradit. Patent Med., **4**, 30–32 (1987).
12. J.M. Li, J.P.E. Wang and L.M. Yuan, *Studies on chemical constituents of* Cayratia japonica. Acta Chin. Med. Pharm., **2**, 52–53 (1995).
13. X.H. Han, S.S. Hong, J.S. Hwang, M.K. Lee, B.Y. Hwang and J.S. Ro, *Monoamine oxidase inhibitory components from* Cayratia japonica. Arch. Pharm. Res., **30**, 13–17 (2007).
14. L. Ruo, S.X. Liao and H.Q. Liang, *Constituents and antivirus activity of essential oil from* Cayratia japonica. Acad. J. Sec. Mil. Med. Univ., **13**, 169–173 (1992).
15. M.L. Zhao, *The observation on bacteriostasis of* Cayratia japonica. Chin. Med. J. Metallurgical Ind., **12**, 75–77 (1995).
16. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry,* 4th edn. Allured Publ. Corp., Carol Stream, IL (2007).
17. M. Sakuma, *Probit analysis of preference data.* Appl. Entomol. Zool., **33**, 339–347 (1998).
18. Z.L. Liu, Q.R. Liu, S.S. Chu and G.H. Jiang, *Insecticidal activity and chemical composition of the essential oils of* Artemisia lavandulaefolia *and* Artemisia sieversiana *from China*. Chem. Biodiv., **7**, 2040–2045 (2010).
19. NIST 08, *Mass spectral library (NIST/EPA/NIH)*. National Institute of Standards and Technology, Gaithersburg, MD (2008).
20. C.F. Wang, K. Yang, H.M. Zhang, J. Cao, R. Fang, Z.L. Liu, S.S. Du, Y.Y. Wang, Z.W. Deng and L. Zhou, *Components and insecticidal activity against the maize weevils of* Zanthoxylum schinifolium *fruits and leaves*. Molecules, 16, in press (2011).
21. W.Q. Li, C.H. Jiang, S.S. Chu, M.X. Zuo and Z.L. Liu, *Chemical composition and toxicity against* Sitophilus zeamais *and* Tribolium castaneum *of the essential oil of* Murraya exotica *aerial parts*. Molecules, **15**, 5831–5839 (2010).
22. S.S. Chu, Q.R. Liu and Z.L. Liu, *Insecticidal activity and chemical composition of the essential oil of* Artemisia vestita *from China against* Sitophilus zeamais. Biochem. Syst. Ecol., **38**, 489–492 (2010).
23. S.S. Chu, S.L. Liu, G.H. Jiang and Z.L. Liu, *Composition and toxicity of essential oil of* Illicium simonsii *Maxim (Illiciaceae) fruit against the maize weevils*. Rec. Nat. Prod., **4**, 205–210 (2010).