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ORIGINAL PAPER

Chemical composition and insecticidal activities of the essential oil of *Perilla frutescens* (L.) Britt. aerial parts against two stored product insects

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Abstract The chemical composition of the essential oil of *Perilla frutescens* (L.) Britt. aerial parts and its insecticidal activity against *Tribolium castaneum* and *Lasioderma serricorne* were investigated. The essential oil of *P. frutescens* was obtained by hydrodistillation and a total of 34 components in the essential oil were identified with GC–MS. It was found that the main compounds included 2-furyl methyl ketone (71.83 %), decahydro-1-methyl-2-methylene-naphthalene (10.47 %), limonene (5.16 %) and caryophyllene (1.66 %). With a further isolation, the two active constituents were obtained from the essential oil and identified as 2-furyl methyl ketone, limonene. In the progress of assay, it showed that the essential oil and 2-furyl methyl ketone exhibited stronger contact and fumigant activities against the two stored product insects than limonene. Moreover, the essential oil and its constituents exhibited the comparable repellency against the two stored product insects, relative to the positive control, DEET. The results indicate that the essential oil of *P. frutescens* aerial parts and its isolated compounds have potential for development into natural insecticides or fumigants as well as repellents for control of insects in stored grains.

Keywords *Tribolium castaneum* · *Lasioderma serricorne* · *Perilla frutescens* (L.) Britt. · Contact toxicity · Fumigant toxicity · Essential oil composition

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Introduction

The red flour beetle (*Tribolium castaneum* Herbst) and cigarette beetle (*Lasioderma serricorne*) are two serious pest species of stored botanicals worldwide. They cause significant damage during storage [1]. The red flour beetle not only causes significant losses because of the consumption of grains, but also results in increased temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species [2]. The cigarette beetle occurs frequently in tropical and subtropical areas. It is usually found in perishable food products such as cereals, legumes, tobacco and traditional Chinese medicinal materials in warehouses [3]. Currently, recommended pest control measures in durable stored products rely heavily on the use of synthetic insecticides or fumigants that pose possible health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence [4]. These

problems have necessitated a search for alternative ecologically safe insect pest control methods [5]. The use of essential oils or their constituents with low mammalian toxicity can effectively prevent insect pest especially in storage [6]. Investigations in several countries confirm that some plant essential oils not only repel insects, but also possess contact and fumigant toxicity against stored product pests as well as exhibiting feeding inhibition or harmful effects on the reproductive system of insects [7]. Essential oils and their constituents of many plants including medicinal herbs, spices and fruits have been evaluated successfully for insecticidal or repellent activity against stored product insects; they have been proven more effective than traditionally used pesticides in some cases [8–17].

Besides insecticidal and repellent activities, essential oils from different plant sources have exhibited several biological activities, including antibacterial and antifungal [18, 19], nematicidal [20–22], larvicidal [23–25] and acaricidal activities [26]. As a consequence, this vast arsenal of bioactive compounds has attracted significant and crescent attention of researchers in recent years [27]. During our screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *P. frutescens* aerial parts and its constituents were found to possess insecticidal toxicity against *T. castaneum* and *L. serricorne*. As a genus of annual herb in the Lamiaceae family, *Perilla frutescens* (L.) Britt is a commonly used traditional Chinese medicine in China for more than 1000 years. It is an edible plant. Its fresh leaves are usually used as vegetables and commonly used for seasoning pickles or as garnish for raw fish dishes in Japan. It is a popular leafy vegetable in Korea, consumed as a pickle or wrapping with roasted meats. The seeds are ground and added to soup for seasoning in Korea. There are both green-leafed and purple-leafed varieties of *P. frutescens*, which are generally recognized as separate species by botanists, and their Chinese names are Baisu and Zisu, respectively. There are significant differences in the morphology and chemistry between Baisu and Zisu. Both Baisu and Zisu were recorded in the "Dictionary of Chinese Crude Drug" with different clinical usage. However, in the Pharmacopoeia of People's Republic of China, 2010, Baisu was not recorded separately, just being used as Zisu [28–30]. Baisu has significant antiallergic, anti-inflammatory and strong anti-tumor-promoting activities [31]. And a literature survey has shown that there is no report on insecticidal of Baisu essential oil and 2-furyl methyl ketone against *T. castaneum* and *L. serricorne*; thus, we decided to investigate the insecticidal activity of the essential oil of Baisu aerial parts for the first time and to isolate any active compounds from the essential oil.

Results and discussion

Essential oil chemical composition

The essential oil of *P. frutescens* aerial parts was yellow with a yield of 0.24 % (v/w) and density of 0.91 g/mL. A total of 34 components of the essential oil of *P. frutescens* were identified, accounting for 94.07 % of the total oil (Table 1). The main compounds in the essential oil were 2-furyl methyl ketone (71.83 %), followed by decahydro-1-methyl-2-methylene-naphthalene (10.47 %), limonene (5.16 %) and caryophyllene (1.66 %). Monoterpenoids represented 8 of the 34 compounds, corresponding to 6.44 % of the whole oil, while 7 of the 34 constituents (3.23 % of the crude essential oil) were sesquiterpenoids.

The chemical composition of the essential oil of *P. frutescens* aerial parts in the present study was not the same as what had been reported in previous studies. For example, perilla ketone and caryophyllene were the main volatile components of Baisu harvested in June from Yangpu District, Shanghai Province. Moreover, the content and composition of the volatile of Baisu were various with different extraction methods [28]. However, perilla ketone, palmitic acid and β-caryophyllene were the main volatile components of Baisu collected from Shaodong, Hunan Province [32]. These differences of chemical content and composition of the essential oils might have been due to harvest time and local, climatic and seasonal factors, storage duration of medicinal herbs as well as extraction method, and these differences may result in different biological activities.

Insecticidal Activities

The essential oil of *P. frutescens* aerial parts exhibited contact toxicity against *T. castaneum* and *L. serricorne* adults with LD_{50} values of 1.20 μg/adult and 1.46 μg/adult, respectively. When compared with the positive control, pyrethrins (LD_{50} = 0.26 μg/adult, 0.24 μg/adult), the essential oil demonstrated 4.5 and 6 times less toxic against *T. castaneum* and *L. serricorne* adults, respectively. 2-Furyl methyl ketone (LD_{50} = 3.04 μg/adult and 6.67 μg/adult, respectively) exhibited stronger contact toxicity against *T. castaneum* and *L. serricorne* adults than limonene (LD_{50} = 14.97 μg/adult and 13.66 μg/adult, respectively) (Table 2). 2-Furyl methyl ketone had almost 5 and 2 times more toxicity than limonene against *T. castaneum* and *L. serricorne* adults, respectively. It is suggested that 2-furyl methyl ketone is a major contributor to the contact toxicity of the essential oil against *T. castaneum* and *L. serricorne* adults. However, compared with pyrethrins (positive control), 2-furyl methyl ketone showed 12 and 28 times less toxicity against *T. castaneum* and *L. serricorne* adults, respectively. When compared with other

Table 1 Chemical constituents of the essential oil derived from *P. frutescens* aerial parts

Peak No.	RI*	RT**	Compound	Composition (%)
1	1155	10.38	p-Mentha-1(7),3-diene	0.08
2	1157	10.45	Fenchene	0.11
3	1208	14.63	2,2-Dimethyl-heptane	0.09
4	1251	13.05	m-Cymene	0.65
5	1258	13.24	Limonene	5.16
6	1259	17.73	4-Acetoxy-3-methoxystyrene	0.36
7	1309	14.98	4,7-Dimethyl-undecane	0.49
8	1327	15.96	4-Methyl-dodecane	0.03
9	1362	17.90	Linalool propionate	0.30
10	1370	23.98	Undecane	0.06
11	1394	12.23	3′,6-Dimethoxyaurone	0.03
12	1399	15.29	2,7,10-Trimethyl-dodecane	0.24
13	1516	32.12	Hexyl pentyl ether	0.05
14	1520	32.33	Methyl geranate	0.08
15	1571	29.59	α-Methyl-1H-indene-1-methanol acetate	0.05
16	1595	30.95	Decahydro-1-methyl-2-methylene-naphthalene	10.47
17	1731	26.01	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1c-cyclohexanol	0.31
18	1823	27.66	2-Furyl methyl ketone	71.83
19	1858	36.85	Germacrene D	0.50
20	1873	37.57	Caryophyllene	1.66
21	1917	39.64	α-Caryophyllene	0.26
22	1981	42.66	(Z,E)- α-Farnesene	0.45
23	2018	34.28	Eugenol	0.04
24	2030	55.23	2-Methyl-1-undecanol	0.04
25	2067	46.69	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	0.05
26	2072	57.63	2,4,6-Trimethyl-decane	0.06
27	2108	59.60	2,5-Diacetyl-6-methoxy-benzofuran	0.06
28	2148	50.51	(1-Butylpentyl)-benzene	0.07
29	2282	41.60	1R,2c,3t,4t-Tetramethyl-cyclohexane	0.08
30	2343	51.04	(1-Propyloctyl)-benzene	0.03
31	2412	45.20	3-Ethyl-3-methylheptane	0.04
32	2492	47.42	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide	0.28
33	2558	55.73	(1-Butyloctyl)-benzene	0.05
34	2567	56.12	(1-Propylnonyl)-benzene	0.04
			Total identified	94.07
			Monoterpenoids	6.44
			Sesquiterpenoids	3.23
			Others	84.40

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

** *RT* retention time

essential oils, *P. frutescens* essential oil possessed stronger contact toxicity against *T. castaneum* adults, e.g., essential oils of *Dracocephalum moldavica* (LD_{50} = 18.28 μg/adult) [33], *Murraya exotica* (LD_{50} = 20.94 μg/adult) [34], *Evodia lepta* (LD_{50} = 166.94 μg/adult) [35], *Heracleum moellendorffii* (LD_{50} = 23.01 μg/adult) [36], *Cayratia japonica* (LD_{50} = 44.49 μg/adult) [37].

2-Furyl methyl ketone (LC_{50} = 1.32 mg/L air and 0.86 mg/L air, respectively) exhibited stronger fumigant toxicity against *T. castaneum* and *L. serricorne* adults than limonene (LC_{50} = 6.21 mg/L air and 14.07 mg/L air, respectively), while the crude essential oil of *P. frutescens* aerial parts showed LC_{50} values of 4.10 mg/L air and 1.21 mg/L air against *T. castaneum* and *L. serricorne* adults

Table 2 Contact toxicity of the essential oil of *P. frutescens* aerial parts and its constituents against *T. castaneum* and *L. serricorne* adults

Insects	Treatment	LD_{50} (μg/adult)	95 % FL*	Slope ± SE	Chi square (χ^2)
Tribolium castaneum	*P. frutescens*	1.20	1.14–1.26	4.04 ± 0.55	7.96
	2-Furyl methyl ketone	3.04	2.84–3.25	2.97 ± 0.37	19.52
	Limonene	14.97	12.88–17.04	3.33 ± 0.42	20.01
	Pyrethrins	0.26	0.22–0.30	3.34 ± 0.32	13.11
Lasioderma serricorne	*P. frutescens*	1.46	1.30–1.63	1.77 ± 0.23	18.89
	2-Furyl methyl ketone	6.67	6.27–7.10	3.33 ± 0.38	19.77
	Limonene	13.66	11.63–16.18	3.21 ± 0.54	11.04
	Pyrethrins	0.24	0.16–0.35	1.31 ± 0.20	17.36

* Fiducial limits

Table 3 Fumigant toxicity of the essential oil of *P. frutescens* aerial parts and its constituents against *T. castaneum* and *L. serricorne* adults

Insects	Treatment	LC_{50} (mg/L air)	95 % FL*	Slope ± SE	Chi square (χ^2)
Tribolium castaneum	*P. frutescens*	4.10	3.92–4.29	4.35 ± 0.96	16.91
	2-Furyl methyl ketone	1.32	1.29–1.36	7.65 ± 0.91	17.84
	Limonene	6.21	5.38–7.05	3.14 ± 0.39	13.34
	MeBr**	1.75	–	–	–
Lasioderma serricorne	*P. frutescens*	1.21	1.15–1.28	3.57 ± 0.71	16.49
	2-Furyl methyl ketone	0.86	0.80–0.93	2.72 ± 0.31	17.79
	Limonene	14.07	12.41–15.76	4.26 ± 0.51	22.08
	Phosphine	9.23×10^{-3}	7.13×10^{-3}–11.37×10^{-3}	2.12 ± 0.27	11.96

* Fiducial limits. ** Data from Liu and Ho [38]

(Table 3). 2-Furyl methyl ketone showed 3 and 1.5 times stronger fumigant toxicity than the essential oil against *T. castaneum* and *L. serricorne* adults. However, compared with the positive control, MeBr (LC_{50} = 1.75 mg/L air), phosphine ($LC_{50} = 9.23 \times 10^{-3}$ mg/L air), 2-furyl methyl ketone exhibited almost equal toxicity to *T. castaneum* adults and 93 times less toxicity to *L. serricorne* adults, and the crude essential oil exhibited almost 2 and 131 times less toxicity to *T. castaneum* and *L. serricorne*, respectively. However, compared with the other essential oils in the literature, the essential oil of *P. frutescens* aerial parts possessed stronger fumigant toxicity against *T. castaneum* adults, e.g., essential oils of *Illicium difengpi* (LC_{50} = 16.22 mg/L air) [39], *Illicium pachyphyllum* (LC_{50} = 15.08 mg/L air) [40] and *Aster ageratoides* (LC_{50} = 12.14 mg/L air) [11]. While the essential oil also showed stronger fumigant toxicity against *L. serricorne* adults, e.g., the essential oil of *Agastache foeniculum* (LC_{50} = 21.57 μL/L air) [41]. As currently used fumigants are synthetic insecticides and the most effective fumigants (e.g., phosphine and methyl bromide) are also highly toxic to humans and other non-target organisms, the essential oil of *P. frutescens* aerial parts and its two isolated compounds show potential to be developed as possible natural fumigants or insecticides for the control of *T. castaneum* and *L. serricorne*. However, for the practical application of the essential oil and the isolated constituents as novel insecticides or fumigants, 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 costs.

Repellency

The results of repellency assays for *P. frutescens* essential oil and isolated constituents against *T. castaneum* and *L. serricorne* are presented in Figs 1 and 2. Data showed that at tested concentration of 39.32 nL/cm^2, crude essential oil showed 98 % repellency (Class V) against *T. castaneum* and 74, 72 % repellency (Class IV) against *L. serricorne* at 2 and 4 h after exposure. At tested concentration of 39.32 nL/cm^2, 2-furyl methyl ketone showed 98, 92 % repellency (Class V) against *T. castaneum* and 70, 76 % (Class IV) against *L. serricorne* at 2 and 4 h after exposure. Moreover, at tested concentration of 39.32 nL/cm^2, limonene exhibited 84 % repellency (Class V) against *T. castaneum* and 78 % repellency (Class IV) against *L. serricorne* at 2 h after exposure, whereas limonene showed 78, 74 % repellency (Class IV) against *T. castaneum* and *L. serricorne* at 4 h after exposure. At the lowest assayed concentration (0.06 nL/cm^2), crude essential oil showed 80,

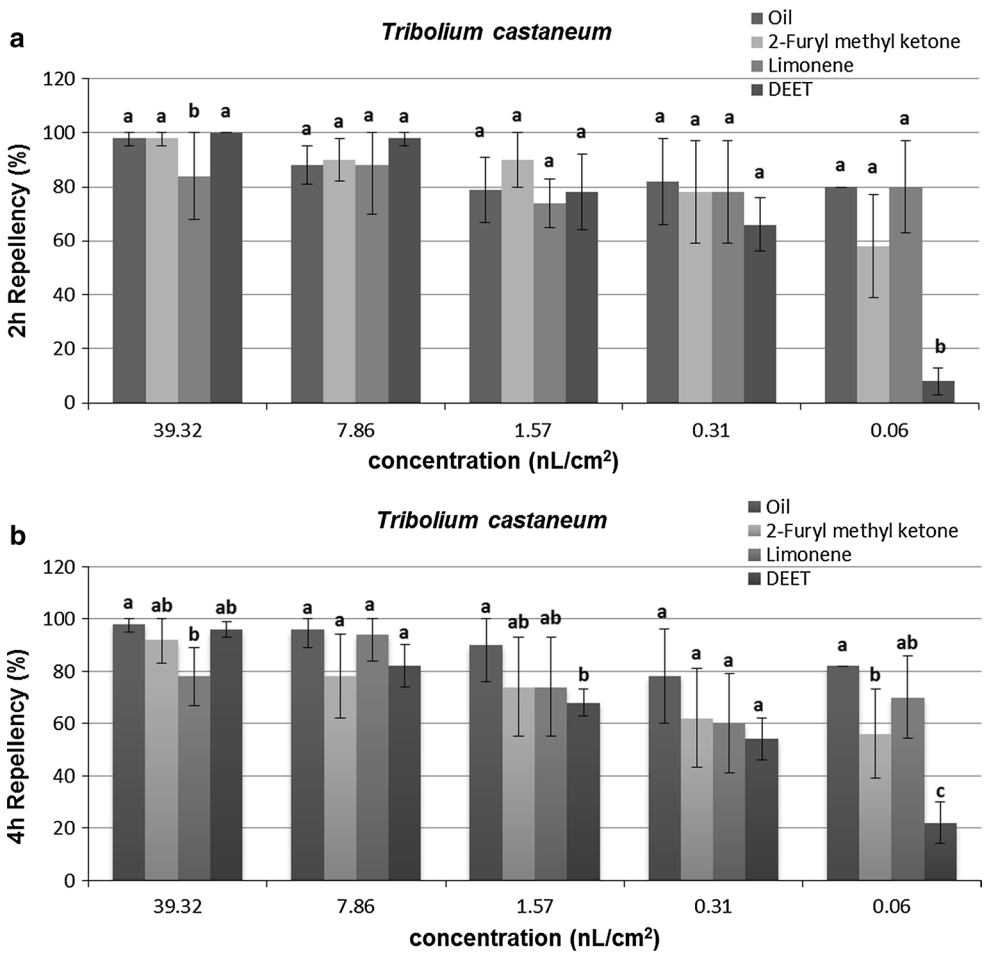

Fig. 1 Percentage repellency (PR) of the essential oil from *P. frutescens aerial parts* and its constituents against *T. castaneum* at 2 h (**a**) and 4 h (**b**) after exposure. *a* Means in the same column followed by the *same letters* do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests

82 % repellency (Class V) against *T. castaneum* at 2 and 4 h after exposure, while limonene showed 80 % repellency (Class V) against *T. castaneum* at 2 h after exposure and 70 % repellency (Class IV) against *T. castaneum* at 4 h after exposure.

Compared with the positive control, DEET, crude essential oil and 2-furyl methyl ketone exhibited stronger repellency against *T. castaneum* at 2 and 4 h after exposure. It is due to that at the concentrations of 39.32, 7.86 and 1.57 nL/cm^2, crude essential oil and DEET ($P = 0.565$, 0.657, $P = 0.120$, 0.082 and $P = 0.268$, 0.056) exhibited the same level of repellency against *T. castaneum* at 2 and 4 h after exposure; moreover, 2-furyl methyl ketone and DEET ($P = 0.565$, 0.696, $P = 0.240$, 0.800 and $P = 0.328$, 0.547) exhibited the same level of repellency against *T. castaneum* at 2 and 4 h after exposure. However, at the concentration of 1.57 nL/cm^2, crude essential oil exhibited stronger repellency than DEET ($P = 0.025$) against *T. castaneum* at 4 h after exposure. At the concentration of 0.06 nL/cm^2, crude essential oil ($P = 0.000$, 0.000) and 2-furyl methyl ketone ($P = 0.003$, 0.017) exhibited stronger repellency than DEET against *T. castaneum* at 2 and 4 h after exposure. In addition, limonene exhibited stronger repellency than DEET against *T. castaneum* at 4 h after exposure. It is because that at the concentrations of 39.32, 7.86, 1.57 and 0.31 nL/cm^2, limonene and DEET ($P = 0.051$, 0.107, 0.611 and 0.671) exhibited the same level of repellency against *T. castaneum*, whereas at the concentration of 0.06 nL/cm^2, limonene exhibited stronger repellency than DEET ($P = 0.001$) against *T. castaneum*.

Crude essential oil and 2-furyl methyl ketone exhibited stronger repellency against *L. serricorne* at 2 h after exposure. It is due to that at the concentrations of 39.32, 7.86, 0.31 and 0.06 nL/cm^2, crude essential oil and DEET ($P = 0.142$, 0.811, 0.067 and 0.059) exhibited the same level of repellency against *L. serricorne*. At the concentrations of 39.32 and 7.86 nL/cm^2, 2-furyl methyl ketone and DEET ($P = 0.094$ and 0.083) exhibited the same level of repellency against *L. serricorne*. However, at the concentration of 1.57 nL/cm^2, crude essential oil exhibited stronger repellency than DEET ($P = 0.011$) against *L. serricorne.* At the concentrations of 1.57, 0.31 and 0.06 nL/cm^2, 2-furyl methyl ketone exhibited stronger repellency than DEET ($P = 0.026$, 0.011 and 0.001) against *L. serricorne*. In addition, at the concentrations of 39.32, 7.86, 1.57, 0.31 and 0.06 nL/cm^2, limonene and DEET ($P = 0.601$, 0.737,

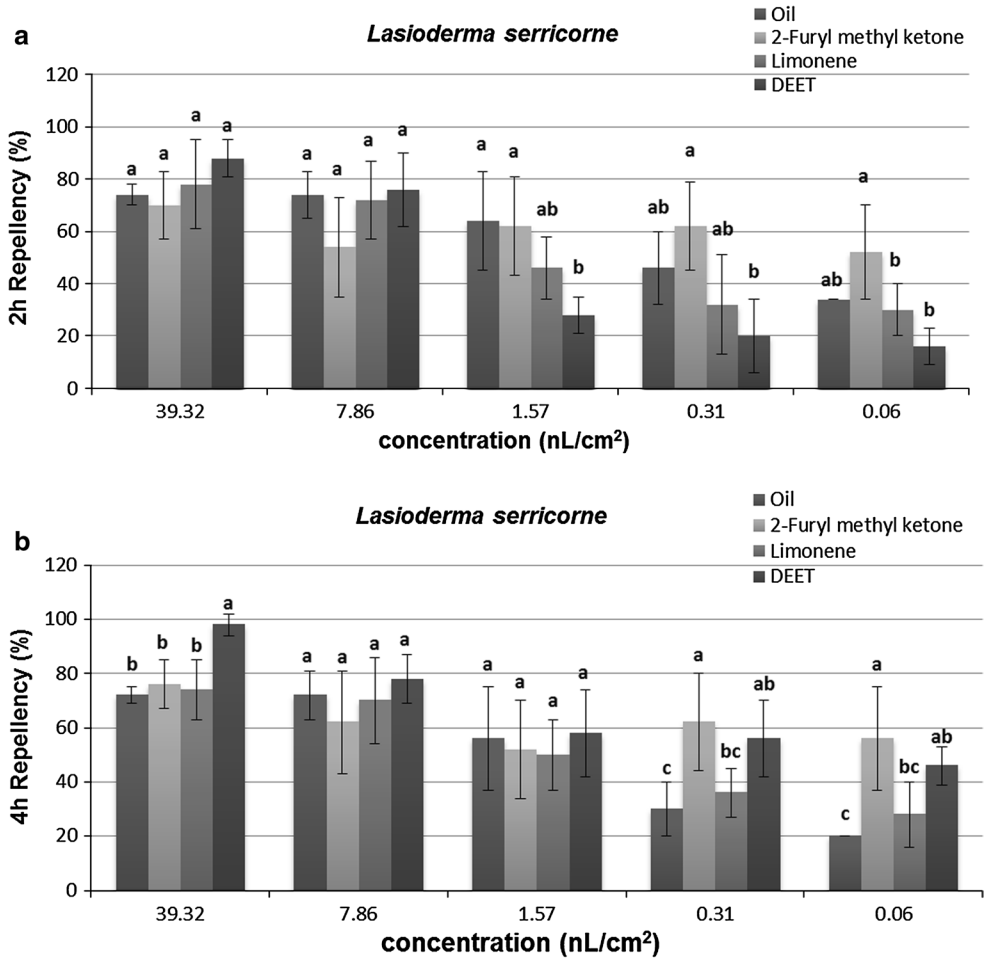

Fig. 2 Percentage repellency (PR) of the essential oil from *P. frutescens aerial parts* and its constituents against *L. serricorne* at 2 h (**a**) and 4 h (**b**) after exposure. *a* Means in the same column followed by the *same letters* do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests

0.224, 0.387 and 0.121) exhibited the same level of repellency against *L. serricorne* at 2 h after exposure. Many other essential oils and their constituents also have been evaluated for repellency against insects [42, 43].

By comparing the repellent levels of five different concentrations, we found the recommended concentration of the essential oil and limonene against *T. castaneum* and *L. serricorne* is 7.86 nL/cm^2, whereas 39.32 nL/cm^2 is the recommended concentration of 2-furyl methyl ketone against *T. castaneum* and *L. serricorne*.

The above results indicated that the bioactivity properties of the essential oil are related to the synergistic effects of its diverse major and minor components. Hence, it is significant to isolate chemical constituents in both high and low percentages for their appreciable bioactivity. In addition, further investigations that focus on efficiency and safety of the pure compounds should be conducted, while structure modification is a considerable method.

Experimental section

Plant material and essential oil extraction

Dried aerial parts (4.5 kg) of *P. frutescens* were harvested in July 2013 from Fenghuangcheng City (40.28°N latitude and 124.02°E longitude), Liaoning Province, China. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (BNU-CMH-Dushuahan-2013-07-09-007) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The aerial parts were air-dried for 1 week and then ground to a powder using a grinding mill (RetschMuhle, Germany). The powder was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulfate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Insects

L. serricorne and *T. castaneum* were obtained from laboratory cultures maintained for the last 2 years in the dark in incubators at 29 ± 1 °C and 70–80 % relative humidity. The insects were reared in glass containers (0.5 L) containing wheat flour at 12–13 % moisture content mixed with yeast (wheatfeed/yeast, 10:1, w/w). Adults used in all the experiments were about 7 ± 2 days old.

Gas chromatography–mass spectrometry

Components of the essential oil of *P. frutescens* aerial parts were identified by gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS) using an Agilent 6890 N gas chromatograph hooked to an Agilent 5973 N mass selective detector. The same column and analysis conditions were used for both GC–FID and GC–MS. They were equipped with a HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column. The column temperature was programmed at 50 °C for 2 min, then increased at 2 °C/min to the temperature of 150 °C and held for 2 min and then increased at 10 °C/min until the final temperature of 250 °C was reached, where it was held for 5 min. The injector temperature was maintained at 250 °C and the volume injected was 0.1 mL of 1 % solution (diluted in *n*-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 m/z. Most constituents were identified by comparison of their retention indices with those reported in the literatures. The retention indices were determined in relation to a homologous series of n-alkanes (C_5–C_{36}) 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 [44]. Relative percentages of the individual components of the essential oil were obtained by averaging the GC–FID peak area % reports.

Purification and Characterization of Two Compounds

The crude essential oil (8 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (45 mm i.d., 500 mm length) by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate and last with ethyl acetate. Fractions (200 mL) were collected and concentrated at 35 °C, and similar fractions according to thin layer chromatography (TLC) profiles were combined to yield 25 fractions. Of these, fractions 5 and 9 were further separated on repeated silica gel columns and PTLC to afford two pure compounds for determining structure as limonene (1, Fig. 3, 0.2 g) and 2-furyl methyl ketone (2, Fig. 3, 1.9 g). The ^{1}H and ^{13}C-NMR data of limonene were in agreement with the reported data [45], and the ^{1}H and ^{13}C-NMR data of 2-furyl methyl ketone were in consistent with the literature data [46]. The purity of limonene and 2-furyl methyl ketone was 91.8 and 93.6 %, respectively. The isolated compounds were elucidated based on nuclear magnetic resonance. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker AMX500 [500 MHz (1H)] instrument using $CDCl_3$ as the solvent with TMS as internal standard. The ^{1}H and ^{13}C-NMR spectra were presented in supplementary material 1.

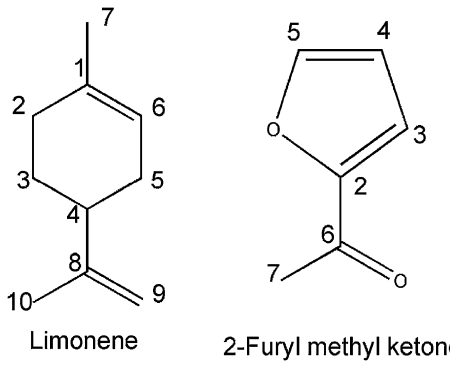

Fig. 3 Constituent compounds isolated from the essential oil of *P. frutescens aerial parts*

Contact toxicity

The contact toxicity of the crude essential oil and isolated compounds against *T. castaneum* and *L. serricorne* adults was measured as described by Liu and Ho [38]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five 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. Ten insects were used for each concentration and control, and the experiment was replicated five times. Both treated and control insects were then transferred to glass vials with culture media and kept in incubators. Mortality was recorded after 24 h and the LD_{50} values were calculated using Probit analysis [47]. The positive control, pyrethrins (pyrethrin 1: 24 %; pyrethrin 2: 13 %; cinnerin 1: 2 %; cinnerin 2: 2 %; jasmolin 1: 1 %; jasmolin 2: 1 %), was purchased from Dr. Ehrenstorfer, Germany.

Fumigant Toxicity

The fumigant activity of the crude essential oil and isolated compounds against *T. castaneum* and *L. serricorne* adults was tested as described by Liu and Ho [38]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five

concentrations) was prepared in *n*-hexane. A Whatman filter paper (diameter 2.0 cm) was impregnated with 10 μL dilution and then placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL). The solvent was allowed to evaporate for 20 s before the cap was placed tightly on the glass vial, each of which contained ten insects inside to form a sealed chamber. Fluon (ICI America Inc.) was used inside the glass vial to prevent insects from contacting the treated filter paper. Preliminary experiments demonstrated that 20 s was sufficient for the evaporation of solvents. *n*-Hexane was used as a control. Five replicates were carried out for all treatments and controls, and they were incubated under the same conditions as rearing. Mortality was determined after 24 h of treatment, and the LC_{50} values were calculated using Probit analysis [47].

Repellent test

The repellent activity to *T. castaneum and L. serricorne* adults was tested using the area preference method [8]. Petri dishes (9 cm in diameter) were used to confine red flour beetles and cigarette beetles during the experiment. The crude essential oil and the isolated compounds were diluted in *n*-hexane to five concentrations (39.32, 7.86, 1.57, 0.31 and 0.06 nL/cm^2), and *n*-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half and 500 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 500 μL of *n*-hexane. Both the treated half and control half were then air-dried to evaporate the solvent completely (30 s). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Care was taken so that the attachment did not prevent free movement of insects from the one half to the other, but the distance between the filter paper halves remained sufficient to prevent seepage of test samples from one half to the other. Each remade filter paper after treatment with solid glue was placed in a Petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. The procedures of the repellent test were presented in supplementary material 2. Five replicates were used and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compound was then calculated using the formula:

$$\mathrm{PR}\ (\%) = [(\mathrm{Nc} - \mathrm{Nt})/(\mathrm{Nc} + \mathrm{Nt})] \times 100$$

where Nc is the number of insects present in the negative control half and Nt is the number of insects present in the treated half. Analysis of variance (one-way ANOVA and GLM univariate) and Tukey's test were conducted by using SPSS 20.0 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before variance and Tukey's tests. The averages were then assigned to different Classes (0 to V) in Table 4 [38]. A commercial repellent, DEET (N, N-diethyl-3-methylbenzamide), was purchased from Dr. Ehrenstorfer, Germany and used as a positive control.

Table 4 The scale to be assign repellency of the essential oil of *P. frutescens* aerial parts and its constituents

Class	Percent repellency	Class	Percent repellency	Class	Percent repellency
0	>0.01–<0.1	II	20.1–40	IV	60.1–80
I	0.1–20	III	40.1–60	V	80.1–100

Conclusions

The *P. frutescens* aerial parts used as edible plant and traditional Chinese medicine is considered to be less harmful than conventional insecticides. A literature survey has shown that there is no report on insecticidal of *P. frutescens* essential oil against *T. castaneum* and *L. serricorne*. In this paper, we report to isolate two insecticidal and repellent constituents from the essential oil of *P. frutescens* aerial parts against two stored product insects for the first time. The work indicates that the essential oil of *P. frutescens* aerial parts and its two constituents possess significant insecticidal toxicity against *T. castaneum* and *L. serricorne*. As rich in natural resources, *P. frutescens* has a very good perspective of application and should be developed into natural insecticides/fumigants and repellents for control of insects in stored grains. Moreover, further studies on how to improve the insecticidal activity of *P. frutescens* essential oil and its two constituents should be investigated, while combined usage and structure modification are considerable methods.

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Conflict of interest The authors declare no conflict of interest.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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