SUPPLEMENTARY MATERIAL

Repellence of the Main Components from the Essential Oil of *Glycosmis lucida* Wall. ex Huang Against Two Stored Product Insects

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Abstract: A screening of Chinese medicinal herbs and wild plants for agrochemicals was carried out, the essential oil of *Glycosmis lucida* leaves was found to possess significant repellent activity against *Tribolium castaneum* and *Liposcelis bostrychophila*. It was found that the main components included elixene (19.81%), spathulenol (10.68%), anethole (12.05%), verbenone (10.32%) followed by β -caryophyllene (6.87%). The essential oil, anethole and verbenone were strongly repellent against *T. castaneum* (96%, 86% and 94%, respectively, at 15.73 nL cm⁻²) and *L. bostrychophila* (100%, 68% and 72%, respectively, at 31.58 nL cm⁻²) after 2 h treatment. The results indicate that anethole and verbenone had the potential to be developed as natural repellents for control of stored product insects.

Keywords: *Glycosmis lucida*; *Tribolium castaneum*; *Liposcelis bostrychophila*; repellence; essential oil; chemical composition

1. Experimental Section

1.1. Plant material and extractions

The leaves of *Glycosmis lucida* were collected from Xishuangbanna Yunnan China (northern latitude: 21°08'~22°36'; east longitude: 99°56'~101°50'). The species were identified by Dr. Zhang, L.X., Yunnan Branch of the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences, Yunnan, China, and a voucher specimen (BNU-Dushushan-2011-11-16-06) were deposited at the Herbarium (BNU) of the College of Resources Science and Technology, Beijing Normal University. The 2 kg of dried leaf was ground to powders using a grinding mill (High speed grinder 6202, Beijing huanya tianyuan machinery technology co., LTD, Beijing, China). The powder of *G. lucida* was subjected to hydrodistillation using a modified Clevenger-type apparatus for 10 h, extracted with hexane, and the essential oil was dried (anh. Na₂SO₄) and stored in airtight containers in a refrigerator at 4°C.

1.2 Insects cultures

The red flour beetle, *T. castaneum* was obtained from laboratory cultures maintained for the last 2 years in the dark, in incubators at 28-30°C and 70-80% relative humidity. The beetles were reared on wheat flour mixed with yeast (10:1, w/w) at 12-13% moisture content. Mixed-sex adult beetles used in the experiments were about 2 weeks old.

The booklouse, *L. bostrychophila* was obtained from laboratory cultures maintained for the last 2 years in the dark, in incubators at 29-30°C and 70-80% relative humidity and was reared on a 1:1:1 mixture, by mass, of milk powder, active yeast and wheat flour. All containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Sino-Rich, Beijing, China). Insects used for the bioassay were 1 week old.

1.3 Gas chromatography and mass spectrometry

GC-MS analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and an 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⁻¹ to the temperature of 150 °C and held for 2 min, and then increased at 10 °C min⁻¹ until the final temperature of 250 °C was reached, where it was held for 5 min. The injector temperature was maintained at 250 °C. The samples (1 µL) were diluted to 1% with *n*-hexane. The carrier gas was helium at flow rate of 1.0 mL min⁻¹. Spectra were scanned from 50 to 550 *m/z*. Most constituents were identified by gas chromatography by comparison of their retention indices with those of 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 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from literature (Adams, 2001; Laribi et al., 2010; Samojlik et al., 2010). Component relative percentages were calculated based on GC peak areas, without using correction factors.

1.4 Purification and characterization of four individual compounds

The essential oil (5 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (30 mm i.d., 500 mm length) by

gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate (from 100:1 to 1:1), and last with ethyl acetate to obtain 20 fractions. Based on repellent test, fraction 4, 9 and 12 were chosen for further fractionation. With repeated silica gel columns and PTLC, three compounds were obtained and analysed by ^1H NMR and ^{13}C NMR. NMR experiments were performed on Bruker Avance DRX 500 instrument using CDCl_3 as solvent with tetramethylsilane (TMS) as internal standard.

1.5 Repellent tests

The repellent effects of the essential oil against *T. castaneum* and *L. bostrychophila* were assessed by using assays on petri dishes (Zhang et al., 2011). Petri dishes (9 cm in diameter) were used to confine beetles during the experiment. The essential oils were prepared in *n*-hexane (39.32, 7.86, 1.57, 0.31 and 0.06 nL cm^{-2}), and absolute *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 absolute *n*-hexane. Both the treated half and the control half were then air-dried for 30 s to evaporate the solvent completely. A full disk was carefully remade by attaching the tested half to the negative control half with tape. Each reassembled filter paper 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. Five replicates were used, and the experiment was repeated three times. Counts of the insects present on each strip were made at 2 and 4 h after treatments. The percent repellence (PR) of each volatile oil was then calculated using the formula:

$$\text{PR (\%)} = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. The averages were then assigned to different classes (0 to V) using the following scale (percentage repellency) (Liu and Ho 1999). Class, % repellency: 0, >0.01 to <0.1 ; I, 0.1-20.0; II, 20.1-40.0; III, 40.1-60.0; IV, 60.1-80.0; and V, 80.1-100.

As for the booklouse, petri dishes and filter papers were changed to 6 cm in diameter and the concentration of the oil used in the experiments were 31.58, 6.32, 1.26, 0.25, 0.05 nL cm⁻². The half filter paper was treated with 150 µl of the solution. As a positive control, a commercial repellent DEET (N, N-diethyl-3-methylbenzamide), was used under the conditions as the oil. Analysis of variance (ANOVA) and Tukey's test were conducted by using SPSS statistics 20 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

2 Structure confirmations of isolated compounds

Anethole (1), colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 1.89 (3H, d, J = 6.5 Hz, Me- γ), 3.83 (3H, s, MeO-4), 6.12 (1H, m, H- β), 6.37 (1H, d, J = 16.0 Hz, H- α), 6.86 (2H, d, J = 8.5 Hz, H-3, 5), 7.29 (2H, d, J = 8.5 Hz, H-2, 6); ¹³C-NMR (125 MHz, CDCl₃) δ ppm: 18.4 (C- γ), 55.3 (MeO-4), 113.9 (C-3, 5), 123.5 (C- β), 126.9 (C-2, 6), 130.3 (C- α), 130.8 (C-1), 158.6 (C-4). The spectral data were in agreement with the reported data (Goez & Eckert, 2006).

Verbenone (2), colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 1.02 (3H, s, Me-9), 1.51 (3H, s, Me-8), 2.03 (3H, s, Me-10), 2.09 (1H, d, J = 9.5 Hz, H-5), 2.43 (1H, t, J = 6.0 Hz, H-5), 2.66 (1H, t, J = 6.0 Hz, H-4), 2.82 (1H, m, H-6), 5.74 (1H, s, H-2); ¹³C-NMR (125 MHz, CDCl₃) δ ppm: 22.1 (C-5), 23.6 (C-10), 26.6 (C-8), 40.9 (C-9), 49.7 (C-7), 54.0 (C-4), 57.6 (C-6), 121.2 (C-2), 170.2 (C-3), 204.1 (C-3). The spectral data were in agreement with the reported data (Kuran & Moglioni, 2014; Moglioni et al., 2000).

β -Caryophyllene (3), colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 5.33 (1H, m, H-5), 4.97 (1H, s, H-12a), 4.85 (1H, s, H-12b), 2.37 (1H, m, H-9), 2.33 (1H, m, H-7b), 2.23 (1H, m, H-7a), 2.11 (1H, m, H-1), 2.02 (1H, m, H-6b), 1.94 (1H, m, H-6a), 1.72 (1H, m, H-2b), 1.69 (1H, m, H-3b), 1.65-1.67 (2H, m, H-10), 1.64 (3H, s, Me-15), 1.60 (1H, m, H-3a), 1.52 (1H, m, H-2a), 1.02 (3H, s, Me-12), 1.00 (3H, s, Me-13); ¹³C-NMR (125 MHz, CDCl₃) δ ppm: 154.74 (C-2), 135.58 (C-6), 124.31 (C-5), 111.67 (C-12), 53.54 (C-9), 48.48 (C-1), 40.33 (C-11), 39.96 (C-7), 34.79 (C-3), 33.02 (C-10),

30.09 (C-13), 29.36 (C-8), 28.38 (C-4), 22.66 (C-14), 16.32 (C-15). The ^1H and ^{13}C -NMR data were consistent with the literature data (Ragasa et al., 2013).

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Table S1. Chemical composition of the essential oil derived from *Glycosmis lucida* leaves

Peak	Compounds	RI ^a	Area%
1	Linalool	1094	0.5
2	Verbenone	1204	10.3
3	p-Propenylanisole	1284	12.1
4	α -Cubebene	1350	0.8
5	Copaene	1374	4.6
6	β -Elemene	1393	3.1
7	β -Cubebene	1394	3.5
8	α -Gurjunene	1406	0.5
9	Santalene	1422	0.8
10	Caryophyllene	1432	6.9
11	α -Bergamotene	1437	0.3
12	α -Caryophyllene	1454	2.0
13	Alloaromadendrene	1458	0.9
14	γ -Muurolene	1483	0.6
15	γ -Gurjunene	1485	0.4
16	Viridiflorene	1492	2.0
17	Elixene	1510	19.8
18	δ -Cadinene	1521	2.7
19	Calamenene	1525	0.4
20	Spathulenol	1578	10.7
21	Caryophyllene oxide	1584	3.1
22	Viridiflorol	1588	1.7
23	β -Eudesmol	1648	0.9
24	Cubenol	1630	0.6
25	τ -Cadinol	1640	1.2
26	α -Cadinol	1652	0.5

27	Phytol	2119	1.3
	Total		92.2

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

Figure S1. Compounds isolated from *Glycosmis lucida* leaves essential oil.