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Insecticidal Components from the Essential Oil of Chinese Medicinal Herb, *Ligusticum chuanxiong* Hort

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Abstract: Essential oil of Chinese medicinal herb, *Ligusticum chuanxiong* dried rhizome was found to possess insecticidal activity against maize weevils, Sitophilus zeamais. The main components of *L. chuanxiong* essential oil were Z-3-butylidene-phthalide (20.56%), *Z*-ligustilide (19.61%), 4-terpinenol (8.82%), 4-vinylguaiacol (6.81%) and *α*-selinene (6.01%). Bioactivity-guided chromatographic separation of the essential oil on repeated silica gel columns led to isolate three compounds namely 3-butylidenephthalide, *Z*-ligustilide and 4-vinylguaiacol. *Z*-liqustilide and *Z*-3-butylidenephthalide showed pronounced toxicity against *S. zeamais* (LD_{50} = 10.23 and 15.81 μg/adult respectively) and were more toxic than 4-vinylguaiacol (LD_{50} = 63.75 μg/adult). The crude essential oil also possessed contact toxicity against *S. zeamais* (LD_{50} = 13.09 μg/adult).

Keywords: *Ligusticum chuanxiong*, *Sitophilus zeamais*, Contact toxicity, *Z*-ligustilide, 3-Butylidenephthalide.

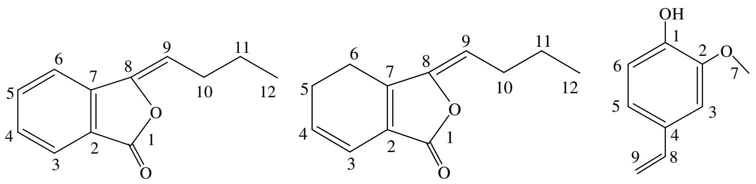
Introduction

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, essential oil derived from dried rhizome of *Ligusticum chuanxiong* Hort. (Family: Umbelliferae) was found to possess strong insecticidal activity against maize weevils, *Sitophilus zeamais* Motsch.

Ligusticum chuanxiong is mainly distributed in southwest China, especially in Sichuan Province. The rhizomes of *L. chuanxiong* have been used as an important crude drug since antiquity. They were used for activating blood circulation and acesodyne, as well as for the treatment of cerebro- and cardio-vascular diseases[2]. Chemical composition of the essential oil of *L. chuanxiong* dried rhizome has been widely studied[3-5]. However, insecticidal activity of *A. chinensis* essential oil against stored product insects was not measured. In this report, three insecticidal compounds were isolated and identified from the essential oil of *A. chinensis* by bioassay-directed fractionation.

Experimental

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 [300MHz (^{1}H)] and AMX500 [500MHz (^{1}H)] instruments using deuterochloroform ($CDCl_3$) as the solvent with tetramethylsilane (TMS) as the internal standard. Electron impact ionone mass spectra (EIMS) were determined on a Micromass VG7035 mass spectrometer at 70 eV (probe). The crude essential oil (25 mL) was chromatographed on a silica gel (Merck 9385, 1,000 g) column (85 mm i.d., 850 mm length) by gradient elution with a mixture of solvents (*n*-hexane, *n*-hexane-ethyl acetate, and acetone). Fractions of 500 mL were collected and concentrated at 40 °C and similar fractions according to TLC profiles were combined to yield 25 fractions. Each fraction was tested with contact toxicity bioassay to identify the bioactive fractions (Fraction 6, 15 and 19). Fractions that possessed contact toxicity, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until to obtain three pure compounds for determining structure (Figure 1). The spectral data of 3-butylidenephthalide (**1**) (1.7 g) matched with the previous report[6]. The data of *Z*-ligustilide (**2**) (1.4 g) matched with the previous report[7]. The spectral data were identical to the published data of 4-vinylguaiacol (**3**) (0.4 g)[8].



3-Butylidenephthalide (**1**) Ligustilide (**2**) 4-Vinylguaiacol (**3**)

Figure 1. Compounds isolated from *Ligusticum chuanxiong* essential oil

Chinese medicinal herb and hydrodistillation

Ten kilograms of dried rhizome of *L. chuanxiong* were purchased from Anguo Chinese Herbs Market (Hebei Province 071200, China). A voucher specimen (CMH-ChuangXiong-SiChuan-2008-08) was deposited in the Department of Entomology, China Agricultural University. To obtain volatile essential oil, the medicinal herb was first ground to a powder then soaked in water at a ratio of 1:4 (w/v) for 1 h, prior to hydrodistillation using a round bottom container over a period of 6 h. The volatile essential oil was collected in a specific receiver, measured, dried over anhydrous sulfate, weighed and stored in airtight containers.

Analysis of the essential oil

Components of the essential oil were separated and identified by gas chromatography–mass spectrometry (GC–MS) Agilent 6890N gas chromatography hooked to Agilent 5973N mass selective detector. They 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 min and ramped at 10 °C min^{-1} to 180 °C for 1 min and then ramped at 20 °C min^{-1} to 280 °C for 15 min. 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 min^{-1}. Spectra were scanned from 20 to 550 *m/z* at 2 scans s^{-1}. 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 (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature[9]. Component relative percentages were calculated based on GC peak areas without using correction factors.

Contact toxicity assay[10]

The maize weevil was obtained from stock culture maintained by Insect Toxicity Laboratory, China Agricultural University and reared on whole wheat at 12-13% moisture content in a constant temperature and humidity incubator maintained at 28-30 °C, 65-75% RH on a 12:12 darkness and light photoperiod. Unsexed adult weevils used in all the experiments were about 2 weeks old. A serial dilution of the essential oil/isolated 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. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators (28-30°C, 65-75% RH). Mortality of insects was observed after 24 h. The experiments were repeated in three times. The LD_{50} values were calculated by using Probit analysis[11].

Results and Discussion

Chemical constituents of the essential oil

Hydrodistillation of dried rhizome of *L. chuanxiong* yielded 0.13% essential oil (v/w). The results of GC-MS of *L. chuanxiong* essential oil are presented in Table 1. A total of 26 components were identified in the essential oil, accounting for 92.57% of the total oil (Table 1). The main components of the essential oil were 3-butylidenephthalide (20.56%), *Z*-ligustilide (19.61%), 4-terpinenol (8.82%), 4-vinylguaiacol (6.81%) and *α*-selinene (6.01%). The chemical composition of *L. chuanxiong* essential oil was different from that reported in other studies. For example, the essential oil of *L. chuanxiong* from stream distillation contained ligustilide (69.1%), senkyunolide (7.5%) and butylidenephthalide (6.9%) while ligustilide (50.7%), senkyunolide (14.5%) and dibutylphthalate (7.3%) were main constituents of essential oil from CO_2 supercritical fluid extract[5]. Zhang *et al.*[4] found that the essential oil of *L. chuanxiong* dried rhizome from stream distillation have ligustilide (24.0%), butylidenephthalide (18.5%), fenipentol (6.4%) and eudesma-4(14), 11-diene (6.2%). However, the main components of essential oil of *L. chuanxiong* cultivated in the GAP demonstrative base of chinese medicinal materials were *Z*-ligustilide (21.1%), 4,5-dihydro-3β-butylphthalide (6.3%), 3-butylidenephthalide (5.9%) and 4-terpinenol (4.8%)[12]. In all the cases, liguistilide was one of main constituents of the essential oil of *L. chuanxiong*.

Contact toxicity of isolated compounds against maize weevils

Z-Liqustilide (**2**) and 3-butylidenephthalide (**1**) showed pronounced insecticidal activity against *S. zeamais* (LD_{50} = 10.23 and 15.81 µg/adult respectively) while the crude essential oil with a LD_{50} value of 13.09 µg/adult (Table 2). The two compounds and crude essential oil were more toxic to *S. zeamais* than another compound, 4-vinylguaiacol (**3**) (LD_{50} = 63.75 µg/adult). Compared with the famous botanical insecticide, pyrethrum extract, *Z*-liqustilide and 3-butylidenephthalide as well as crude essential oil were 2-3 times less active against the maize weevils because pyrethrum extract displayed a LD50 value of 4.29 µg/adult[13]. However, 4-vinylguaiacol was 15 times less active against the maize weevils compared to pyrethrum extract (Table 2).

Table 1. Chemical constituents of essential oil from *Ligusticum chuanxiong*

No.	Compounds	RI	Formula	RA, %
1	*α*-Pinene	931	$C_{10}H_{16}$	0.81
2	Sabinene	977	$C_{10}H_{16}$	1.56
3	*ρ*-Cymene	1024	$C_{10}H_{14}$	3.94
4	*γ*-Terpinene	1057	$C_{10}H_{16}$	2.11
5	*δ*-2-Carene	1002	$C_{10}H_{16}$	1.92
6	Limonene	1020	$C_{10}H_{16}$	0.92
7	*cis-β*-Terpineol	1060	$C_{10}H_{18}O$	1.15
8	*trans*-Sabinene hydrate	1097	$C_{10}H_{18}O$	1.20
9	*cis-p*-Menth-2-en-1-ol	1126	$C_{10}H_{18}O$	0.28
10	Camphor	1143	$C_{10}H_{16}O$	0.48
11	4-Terpinenol	1179	$C_{10}H_{18}O$	8.82
12	*ρ*-Cymen-8-ol	1182	$C_{10}H_{14}O$	1.18
13	*α*-Terpineol	1191	$C_{10}H_{18}O$	1.06
14	4-Vinylguaiacol	1323	$C_9H_{10}O_2$	6.81
15	Eugenol	1356	$C_{10}H_{12}O_2$	1.12
16	*β*-Elemene	1393	$C_{15}H_{24}$	0.35
17	*β*-Selinene	1483	$C_{15}H_{24}$	6.02
18	*α*-Selinene	1492	$C_{15}H_{24}$	1.72
19	Spathulenol	1578	$C_{15}H_{24}O$	4.47
20	3-Butylphthalide	1658	$C_{12}H_{14}O_2$	2.98
21	3-Butylidenephthalide	1678	$C_{12}H_{12}O_2$	20.56
22	Senkyunolide A	1729	$C_{12}H_{16}O_2$	0.87
23	Neocnidilide	1735	$C_{12}H_{18}O_2$	0.75
24	*Z*-Ligustilide	1741	$C_{15}H_{26}O$	19.61
25	Hexahydrofarnesyl acetone	1842	$C_{18}H_{36}O$	0.73
26	Phytol	2119	$C_{18}H_{32}O2$	1.15
Total				92.57

RI= retention index as determined on a HP-5MS column using the homologous series of n-hydrocarbons; RA= relative area (peak area relative to total peak area)

Table 2. Insecticidal activity of *Ligusticum chuanxiong* essential oil against *Sitophilus zeamais* adults by using topical application*

Compounds	LD_{50}, μg/adult	95% fiducial limits
Z-Liqustilide	10.23	8.16-12.58
Z-Butylidenephthalide	15.81	14.11-21.25
4-Vinylguaiacol	63.75	58.14-69.19
Crude oil	13.09	10.71-17.34

**After 24 h, survival of the adults was recorded and compared with controls*

Z-Ligustilide and 3-butylidenephthalide were reported to exhibit several bioactivities such as acaricidal, insecticidal, phytotoxic, antifungal and antibacterial activities[14]. In the previous reports, *Z*-liqustilide was found to be toxic to brine shrimp (*Artemia salina*)[15]. *Z*-Liqustilide was found to possess insecticidal activity against both larvae and adults of fruit fly *Drosophila melanogaster* and more active than a famous botanical, rotenone[16]. Moreover, it was also a potent deterrent of mosquito feeding[17]. 3-Butylidenephthalide had insecticidal activity against both larvae and adults of fruit fly *D. melanogaster* and more active than rotenone[16]. 3-Butylidenephthalide also showed strong contact toxicity against cat fleas (*Ctenocephalides felis*) and house dust mites (*Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*)[18]. However, no reports on insecticidal activity of 4-vinylguaiacol against insects were available so far. The above results indicated that the three compounds and crude essential oil possess potential to be developed as new natural insecticides. Further studies are needed to assess the insecticidal activity of the compounds and the crude essential oil to other stored product insects and different life stage of insects.

Dried rhizome of *L. chuanxiong* was used as a common Chinese medicinal herb[2]. However, there are no toxicity data for this herb and the three isolated compounds and available on human consumption. Moreover, *Z*-ligustilide was phytotoxic to the monocots bentgrass (*Agrostis stolonifera*) and fescue (*Festuca rubra*)[19]. For the practical use of the three compounds and crude essential oil as novel botanical insecticides, further studies are necessary on the safety of these materials to human, on phytotoxicity to crop seeds and on the development of formulations to improve efficacy and stability and to cut cost as well.

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References

1. Isman M B, *Ann Rev Entomol.,* 2006, **51**, 45-66.
2. Peng C, Xie X F, Wang L, Guo L and Hu T L, *Phytomedicine,* 2009, **16(1)**, 25.
3. Ren D C, Yang N Y, Qian S H, Xie N, Zhou X M and Duan J A, *China J Chinese Materia Medica*, 2007, **32(14)**, 1418-1420.
4. Zhang C, Qi M L, Shao Q L, Zhou S and Fu R N, *J Pharm Biomed Anal.*, 2007, **44(2)**, 464-470.
5. Wu Q and Yang X W, *China J Chinese Materia Medica*, 2008, **33(3)**, 276-280.
6. Kwon J H and Ahn Y J, *J Agric Food Chem.*, 2002, **50(16)**, 4479-4483.
7. Miyazawa M, Tsukamoto T, Anzai J and Ishikawa Y, *J Agric Food Chem.*, 2004, **52(14)**, 4401-4405.
8. Pongpryoon U, Backstroem P, Jacobsson U, Lindstroem M and Bohlin L, *Planta Med.,* 1991, **57**, 515-518.
9. Adams R P, Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy, Allured Publishing Corporation, Carol Stream, 2001.
10. Liu Z L and Ho S H, *J Stored Prod Res.*, 1999, **35**, 317-328.
11. Sakuma M, *Appl Entomol Zool.*, 1998, **33**, 339-347.
12. Hong Z Y, Wang X Z, Le J, Zhang D C, Chai Y F and Liu Z L, *J Chin Pharm Sci.*, 2002, **11**, 31-34.
13. Liu Z L, Liu Q R, Chu S S and Jiang G H, *Chem Biodiv.,* 2010, **7,** 2040-2045.
14. Beck J J and Chou S C, *J Nat Prod.*, 2007, **70(5)**, 891-900.
15. Delgado G, Reza-Garduno R G, Rios M Y and Del Rio F, *Planta Med.*, 1992, **58**, 570-571.
16. Tsukamoto T, Ishikawa Y and Miyazawa M, *J Agric Food Chem.*, 2005, **53(14)**, 5549-5553.
17. Wedge D E, Klun J A, Tabanca N, Demirci B, Ozek T, Baser K H C, Liu Z, Zhang S, Cantrell C L and Zhang J, *J Agric Food Chem.*, 2009, **57(2)**, 464-470.
18. Kwon J H and Ahn Y J, *Pest Manag Sci.*, 2003, **59(1)**, 119-123.
19. Meepagala K M, Sturtz G, Wedge D E, Schrader K K and Duke S O, *J Chem Ecol.*, 2005, **31(7)**, 1567-1578.