

Original Article

Evaluation of Essential Oil and its Three Main Active Ingredients of Chinese *Chenopodium ambrosioides* (Family: Chenopodiaceae) against *Blattella germanica*

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Abstract

Background: The efficacy of essential oil of *Chenopodium ambrosioides* flowering aerial parts and its three main active ingredients was evaluated against *Blattella germanica* male adults.

Methods: Composition of essential oil was determined by GC-MS. Topical application bioassay was used to evaluate contact toxicity of essential oil and three main components. Fumigant toxicity of essential oil and its main components was measured using a sealed space method.

Results: Twenty-two components were identified in the essential oil and the main components were (*Z*)-ascaridole (29.7%), isoascaridole (13.0%), ρ -cymene (12.7%) and piperitone (5.0%). The essential oil and (*Z*)-ascaridole, isoascaridole and ρ -cymene possessed fumigant toxicity against male German cockroaches with LC₅₀ values of 4.13, 0.55, 2.07 and 6.92 mg/L air, respectively. Topical application bioassay showed that all the three compounds were toxic to male German cockroaches and (*Z*)-ascaridole was the strongest with a LD₅₀ value of 22.02 μ g/adult while the crude oil with a LD₅₀ value of 67.46 μ g/adult.

Conclusion: The essential oil from Chinese *C. ambrosioides* and its three main active ingredients may be explored as natural potential insecticides in the control of cockroaches.

Keywords: *Blattella germanica*, *Chenopodium ambrosioides*, Essential oil, Fumigant, Contact toxicity

Introduction

German cockroach, *Blattella germanica* (L) is an important pest of homes, restaurants, and commercial food processing facilities worldwide. They are a major public health concern in hospitals, kitchens, and food manufacturing plants because they are able to carry a variety of bacteria and other pathogenic organisms. They are the mechanical vectors to a few pathogens that can cause disease such as food poisoning, typhoid, pneumonia and asthma (Brenner 1995). Body parts, cast skins, and feces of cockroaches are human allergens, 2nd in importance only to the house dust mites. Currently, control of cockroach pop-

ulations primarily depends on continued applications of residual insecticides, such as propoxur, acephate, dimethyl 2, 2-dichlorovinyl phosphate (dichlorvos, DDVP), and pyrethroids and stomach poisons, such as hydramethylnon and sulfluramid. However, the repeated application of these insecticides may possess undesirable side effects, such as the disruption of natural biological control systems, and the development of resistance (Chang and Ahn 2001). There are also serious concerns about human health. These problems have highlighted the need for the development of new types of selective cockroach-control alternatives.

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There has been growing interest in the use of plant oils for protection of agricultural products and control of public health insects because they are often of low mammalian toxicity, readily biodegradable and pose low danger to the environment if used in small amounts (Rajendran and Srianjini, 2008). During our screening program for new agrochemicals from Chinese medicinal herbs, *Chenopodium ambrosioides* L (Family: Chenopodiaceae) was found to possess strong insecticidal activity against German cockroaches (Fig. 1). *Chenopodium ambrosioides* is an aromatic herb that grows in Central and South America and now distributed throughout the tropical parts of the world (Duke et al. 2002). It has also been employed by empirical herbalists and healers against intestinal parasites (especially small tapeworms and round worms) throughout Latin America, as well as in the West Indies (Quinlan et al. 2002). The plant is also distributed in the southern provinces of China. The aerial parts of this plant have been used as condiment, traditional purgative for intestinal worms and acesodyne and in the Chinese traditional medicine. This herb can expel wind, treat rheumatism (Jiangsu New Medical College 1977). Essential oil of *C. ambrosioides* has been shown to possess insecticidal and repellent activities against several stored product insects (Su 1991, Tapondjou et al. 2002) and medical important insect pests (Toloza et al. 2006, Gillij et al. 2008).

In the present study, insecticidal activity *C. ambrosioides* essential oil and three main active ingredients against the German cockroaches were investigated.

Materials and Methods

Test insects

Male adults (5–10 days old) were collected from a synchronously reared laboratory colony of insecticide-susceptible German cockroaches. Cockroaches were supplied ad libi-

tum with Purina No 5012 Rat Chow (Laboratory Animal Centre, Chinese Academy of Medicinal Sciences, Beijing 100021), and water was provided in glass tubes with cotton stoppers. All colonies were kept in plastic tanks at room temperature.

Plants

Fresh aerial parts (15 kg of leaves, stems and flowers) of *C. ambrosioides* were harvested in August 2008 from Fuzhou (26.08° North latitude and 119.28° East longitude), Fujian Province (Fuzhou 350013), PR China. The aerial parts were air-dried for one week and ground to a powder. The species was identified and the voucher specimens (CMH-TuJingJie-FuJian-2008-08) were deposited at the Department of Entomology, China Agricultural University, Beijing 100094.

Extraction of essential oil

The ground powder of *C. ambrosioides* aerial parts was subjected to hydrodistillation using a modified Clevenger-type apparatus (XWD-C-1000, Shanghai XinWangDe Laboratory Equipment Co, China) for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. Essential oil was stored in an airtight container in a refrigerator at 4 °C.

Gas chromatography and mass spectrometry

Gas chromatographic analysis was performed on the Agilent 6890N while the essential oil was identified on a mass spectrometer Agilent Technologies 5973N. They were equipped with a flame ionization detector and capillary column with HP-5MS (30m× 0.25mm× 0.25µm). 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 (Adams 2001). Component relative percentages were calculated based on GC peak areas without using correction factors.

(*Z*)-Ascaridole and isoascaridole were isolated by using bioassay-directed fractionation on repeated silica column from the essential oil of *C. ambrosioides* and confirmed by MS, $^1\text{H-NMR}$ and $^{13}\text{C-HNM}$ data. *p*-Cymene (98%) was purchased from Aladdin Reagent (China) Co., Ltd, Shanghai 201206, PRChina. Positive control, pyrethrum extract (25% pyrethrin I and pyrethrin II) was purchased from Fluka Chemie.

Fumigant toxicity

A serial dilution of *C. ambrosioides* essential oil (20–1.3%, 5 concentrations) and pure compounds (2.5–0.3% for (*Z*)-ascaridole, 20–0.6% for another two compounds, 5 concentrations) was prepared in *n*-hexane. A Whatman filter paper (diameter 6.0 cm) were each impregnated with 50 μl dilution, and then placed on the underside of the screw cap of a glass vial (diameter 7.2 cm, height 19.0 cm, volume 750 ml). The solvent was allowed to evaporate for 30 s before the cap was placed tightly on the glass vial, each of which contained 10 male cockroaches inside to form a sealed chamber. Fluon (ICI America Inc) was used inside glass vial to prevent

insects from contacting the treated filter paper. Preliminary experiments demonstrated that 30 s were sufficient for the evaporation of solvents. *n*-Hexane was used as controls. Five replicates were carried out for all treatments and controls, and they were incubated at 24–26 °C, 75% RH, 12:12 LD photoperiod for 24 h and then mortality was recorded. Mortality was defined as inability to move when placed on the dorsal side and inability to respond to prodding. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values (Sakuma 1998).

Topical application bioassay

Groups of ten adult male cockroaches were anaesthetized with carbon dioxide for 15 seconds before treatment. A serial dilution of the essential oil (7.0–1.3%, 5 concentrations) and pure compounds (10–0.6%, 6 concentrations) was prepared in acetone. Aliquots of 2 μl of the solution were dispensed from an Arnold Automatic Micro-applicator (Burkard, Ricksmanworth, England) and applied to the dorsal thorax of individual insects. Controls were determined using acetone. Both treated and control cockroaches were then transferred to glass vials (10 insects/ vial) and kept in incubators (24–26 °C, 75% RH, 12:12 LD photoperiod). Mortality of cockroaches was observed at 24 h post-treatment. Five replicates were carried out for all treatments and controls. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LD_{50} values (Sakuma 1998).

Results

The results of GC-MS of *C. ambrosioides* essential oil are presented in Table 1. A total of 22 active ingredients were identified in the essential oil, accounting for 88.6% of the total oil (Table 1). The main components were (*Z*)-ascaridole (29.7%), isoascaridole (13.0%),

and ρ -cymene (12.7%) followed by piperitone (5.0%), isothymol (4.9%), and 3, 4-epoxy- ρ -menthan-2-one (4.1%).

(Z)-Ascaridole, isoascaridole and ρ -cymene possessed fumigant toxicity against male German cockroaches with LC₅₀ values of 0.55, 2.07 and 6.92 mg/L air, respectively while the crude essential oil with a LC₅₀ value of 4.13 mg/L air (Table 2). (Z)-Ascaridole also showed the strongest contact toxicity to

German cockroaches with a LD₅₀ value of 22.02 μ g/adult (based on the LD₅₀ values, with no overlap in 95% fiducial limits) by using topical application bioassay while the crude essential oil with a LD₅₀ value of 64.47 μ g/adult (Table 3). Isoascaridole and ρ -cymene also had weak contact toxicity against German cockroaches with LD₅₀ values of 96.28 and 119.90 μ g/adult, respectively.

Table 1. Chemical constituents of essential oil derived from *Chenopodium ambrosioides*

Compound	RI*	Chemical Formula	Relative Area (%)
α -Pinene	931	C ₁₀ H ₁₆	1.3
β -Pinene	981	C ₁₀ H ₁₆	0.3
δ -4-Carene	1002	C ₁₀ H ₁₆	1.9
α -Terpinene	1017	C ₁₀ H ₁₆	1.1
ρ -Cymene	1024	C ₁₀ H ₁₄	12.7
ρ,α -Dimenthylstyrene	1118	C ₁₀ H ₁₂	0.7
trans- ρ -Mentha-2,8-dienol	1126	C ₁₁ H ₁₈ O ₂	0.6
trans- ρ -2,8-Menthadien-1-ol	1139	C ₁₀ H ₁₆ O	0.6
2-Ethylcyclohexanone	1158	C ₈ H ₁₄ O	0.9
α,α -4-Trimethylbenzyl alcohol	1182	C ₁₀ H ₁₄ O	2.8
cis-Piperitol	1196	C ₁₀ H ₁₆	0.5
(Z)-Ascaridole	1245	C ₁₀ H ₁₆ O ₂	29.7
Piperitone	1250	C ₁₀ H ₁₆	5.0
3,4-Epoxy- ρ -menthan-2-one	1276	C ₁₀ H ₁₆ O ₂	4.1
Thymol	1292	C ₁₀ H ₁₄ O	1.1
Carvacrol	1298	C ₁₀ H ₁₄ O	4.9
Isoascaridole	1295	C ₁₀ H ₁₆ O ₂	13.0
Precocene II	1368	C ₁₃ H ₁₆ O ₃	1.5
Caryophyllene oxide	1584	C ₁₅ H ₂₄ O	2.2
Geranyl tiglate	1700	C ₁₅ H ₂₄	0.8
Hexahydrofarnesyl acetone	1842	C ₁₈ H ₃₆ O	1.7
Phytol	2119	C ₂₀ H ₄₀ O	1.2
Total			88.6

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

Table 2. Fumigant toxicity of essential oil and components from *Chenopodium ambrosioides* against male cockroach adults

Compounds	LC ₅₀ (mg/L air)	95% fiducial limits	Slope \pm SE	Chi square (χ^2)
Ascaridole	0.55	0.47–0.63	7.25 \pm 0.69	10.15
ρ -Cymene	6.92	6.11–7.85	7.06 \pm 0.45	8.23
Isoascaridole	2.07	1.78–2.43	6.17 \pm 0.49	9.46
Crude oil	4.13	3.62–4.74	5.06 \pm 0.40	11.21
DDVP	0.01*	-	-	-

*Data from Jang et al. (2005)

Table 3. Contact toxicity of essential oil and components from *Chenopodium ambrosioides* against male cockroach adults

Compounds	LD ₅₀ (µg/adult)	95% fiducial limits	Slope±SE	Chi square (χ ²)
Ascaridole	22.02	19.92–24.43	6.12±0.73	9.48
ρ-Cymene	119.90	102.13–143.51	5.23±0.47	13.40
Isoascaridole	96.28	78.04–117.93	3.44±0.35	14.67
Crude oil	64.47	59.21–70.46	4.09±0.43	7.40
Pyrethrum extract	1.70	1.16–3.78	4.23±0.47	6.80



Fig. 1. Flowering *Chenopodium ambrosioides* (Orginal)

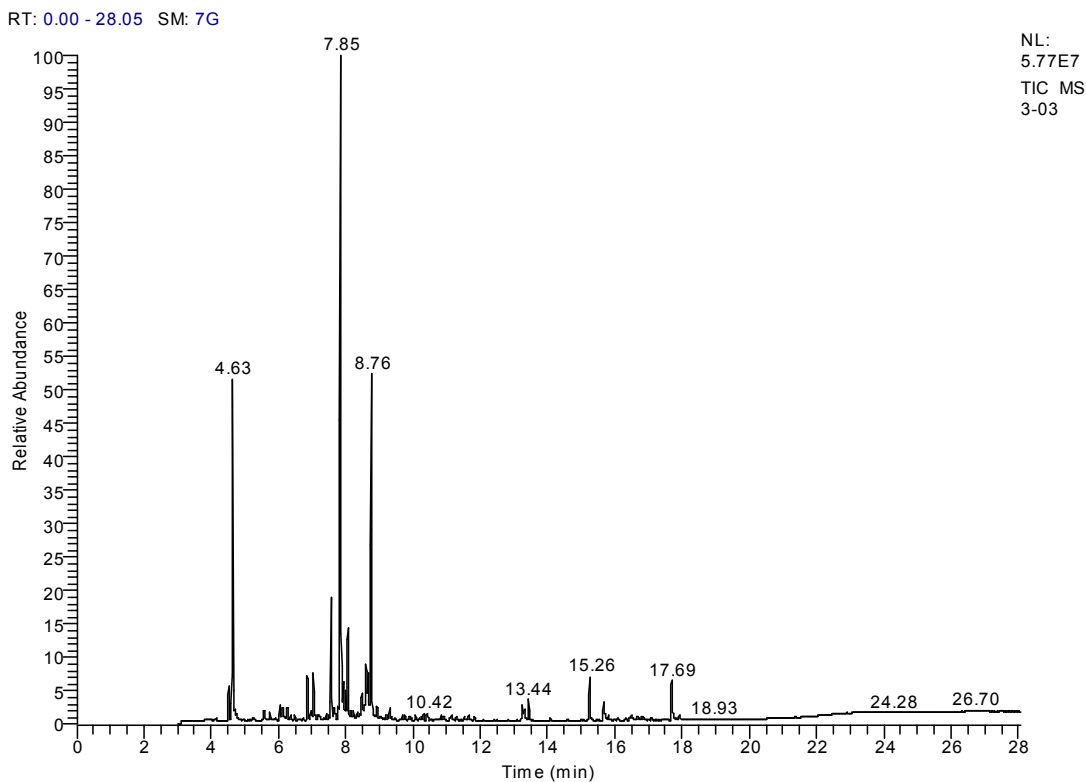


Fig. 2. GC-MS graph of *Chenopodium ambrosioides* essential oil profiles

Discussion

Among the three isolated compounds, (*Z*)-ascaridole was proved to be the most active (fumigant) compound because it showed 7 times more toxic to the German cockroaches compared with the crude essential oil (Table 2). Isoascaridole also demonstrated stronger fumigant toxicity against German cockroaches than the crude essential oil (based on the LC₅₀ values, with no overlap in 95% fiducial limits). However, *p*-cymene had less toxic to German cockroaches than the crude essential oil. In the previous report (Jang et al. 2005), 41 naturally occurring monoterpenoids was evaluated for fumigant toxicity against German cockroaches and verbenone was most toxic to the German cockroaches with a LD₅₀ value of 11.5 mg/L air. In the present study, (*Z*)-ascaridole was 20 times more toxic to the German cockroaches compared to verbenone. However, fumigant toxicity bioassay demonstrated that all the three isolated compounds and the crude essential oil were less toxic to the cockroaches compared to the commercial insecticide, DDVP because of DDVP with a LC₅₀ value of 0.007 mg/L air.

The three isolated compounds exhibited contact toxicity against the German cockroaches (Table 3). However, compared with the control (pyrethrum extracts, LD₅₀= 1.70 µg/adult), all the three compounds and the essential oil showed less toxic to the German cockroaches in the topical application bioassay. The above findings suggested that the *C. ambrosioides* essential oil and the three components especially (*Z*)-ascaridole may possess potential to be developed as novel natural insecticides, especially fumigants in the control of cockroaches.

In the previous studies, several essential oils have been evaluated and demonstrated to possess insecticidal, antifeedant, and repellent activities against cockroaches, such as mint oil (Appel et al. 2001), catnip essential oil (Peterson et al. 2002), majoram oil (Jang

et al. 2005), garlic and thyme oil (Tunaz et al. 2009), citrus oils (Yoon et al. 2009), and essential oils derived from star anise *Illicium verum* (Chang and Ahn 2001), American peppertree *Schinus molle* (Ferrero et al. 2007), and nutmeg *Myristicla fragrans* (Jung et al. 2007). Moreover, naturally occurring monoterpenoids, components of essential oils were also evaluated for insecticidal and repellent activities against cockroaches (Ngoh et al. 1998, Jang et al. 2005) and cineole, *l*-fenchone, limonene, linalool, menthone, pulegone, and thujone at 50 µg/ml air (14 h exposure) caused 100% mortality of male adult German cockroaches (Lee et al. 2003).

The aerial parts of this plant have been used as traditional purgative for intestinal worms in Chinese medicine (Jiangsu New Medical College 1977). However, the essential oil of *C. ambrosioides* is an irritant to the mucous membrane of the gastrointestinal tract, kidney and liver (Gadano et al. 2006). Overdoses of this oil have caused death in men and rats (Monzote et al. 2006). Intake of 10 mg/kg of the oil has been known to cause cardiac disturbances, convulsions, respiratory disturbances, sleepiness, vomiting and weakness and even death. Moreover, ascaridole is toxic and has a pungent, not very pleasant flavor; in pure form, it is an explosive sensitive to shock (Potawale et al. 2008). For the practical use of ascaridole and the crude essential oil as novel natural fumigants/ insecticides, further studies are necessary on the safety of these materials to human, and on the development of formulations to improve efficacy and stability, and to cut cost as well.

The main constituents of the essential oil were (*Z*)-ascaridole, isoascaridole, *p*-cymene and piperitone (Table1). However, there were great variations in chemical composition of the essential oils of *C. ambrosioides*. For example, *α*-terpinyl acetate (73.9%) and *p*-cymene are major constituents of *C. am-*

ambrosioides essential oil from Mexico and content of ascaridole is only 2% (Pino et al. 2003). In another report, limonene (32.5%), *trans*-pinocarveol (26.7%) and geraniol (5.0%) were main components of *C. ambrosioides* essential oil from Mexico (Sagrero-Nieves and Bartley, 1995). The main components of *C. ambrosioides* essential oil derived from Brazil were (*Z*)-ascaridole (61.4%) and (*E*)-ascaridole (18.6%) (Jardim et al. 2008). However, the *C. ambrosioides* essential oil from Nigeria contained α -terpinene (56.0%), α -terpinyl acetate (15.7%) and p -cymene (15.5%) and no ascaridole was detected in the oil (Muhayimana et al. 1998). The essential oil from India contained α -terpinene (47.4%), p -cymene (25.8%) and ascaridole (14.8%) (Singh et al. 2008). However, (*Z*)-ascaridole (29.7%), isoascaridole (13.0%), and p -cymene (12.7%) are the three main components of the essential oil from the Chinese *C. ambrosioides* (Table 1).

For the practical use of the crude essential oil of Chinese *C. ambrosioides* as a new natural insecticide, standardization of the essential oil is needed.

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