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Nematicidal Constituents from the Essential Oil of *Chenopodium Ambrosioides* Aerial Parts

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Abstract: Essential oil of Chinese medicinal herb, *Chenopodium ambrosioides* aerial parts was found to possess nematicidal activity against the root-knot nematodes, *Meloidogyne incognita*. The essential oil of *C. ambrosioides* was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 27 components of the essential oil were identified. The principal compounds in *C. ambrosioides* essential oil were (*Z*)-ascaridole (27.27%), ρ-cymene (19.05%), isoascaridole (14.75%), α-pinene (6.33%) and α-terpinene (5.12%). Bioactivity-guided chromatographic separation of the essential oil on repeated silica gel columns led to isolate three volatile components ((*Z*)-ascaridole, ρ-cymene and isoascaridole) from the essential oil. The essential oil and (*Z*)-ascaridole exhibited strong nematicidal activity against *M. incognita* with LC_{50} values of 49.55 µg/mL and 32.79 µg/mL, respectively. ρ-Cymene and isoascaridole also possessed nematicidal activity against *M. incognita* with LC_{50} values of 435.89 µg/mL and 1323.51 µg/mL, respectively but weaker than the crude essential oil.

Keywords: *Chenopodium ambrosioides*, *Meloidogyne incognita*, (*Z*)-Ascaridole, ρ-Cymene, Isoascaridole, Essential oil composition

Introduction

Plant-parasitic nematodes are responsible for substantial economic loss to agricultural crops. Nematode management is generally based upon chemical treatments, but environmental concerns and governmental regulations[1] are now resulting in a strong interest in nematicides of natural origin[1,2]. One alternative is to screen naturally occurring compounds in plants, which are known as plant secondary compounds. Many plant constituents and metabolites including essential oils have been investigated for activity against plant-parasitic nematodes[3-6]. A series of nematicidal substances of plant origin such as triglycerides, sesquiterpenes, alkaloids, steroids, diterpenes and flavonoids have been identified[2]. During

our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, essential oil derived from aerial parts of Chinese *Chenopodium ambrosioides* L. (Family: Chenopodiaceae) was found to possess strong nematicidal activity against the root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood. *M. incognita*, is the most economically important and widely distributed nematode throughout China and a considerable crop loss is caused by this nematode.

C. ambrosioides, commonly known as wormseed, Mexican tea or epazote, is a native of Central and South America and now distributed throughout the tropical parts of the world. This species of plant is also distributed in the southern provinces of China[7]. 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 and treat rheumatism[7]. Chemical composition of the essential oil of *C. ambrosioides* from different parts of the world has been widely studied such as from Brazil[8], Cuba[9], Mexico[10], Cameroon[11], Nigeria[12], Rwanda[13], China[14, 15] and India[16, 17].

A botanical based on *C. ambrosioides* var. *ambrosioides* essential oils (UDA-245) has been commercialized to control greenhouse insects and mites[18], but as far as we know, there are no reports about isolation of active components against nematodes from this essential oil. In this report, the essential oil was evaluated for toxicity against *M. incognita* and three active compounds were isolated and identified from the essential oil of *C. ambrosioides* 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 (20 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 (petroleum ether, ethyl acetate and ethanol). Fractions of 500 mL were collected and concentrated at 40 °C and similar fractions according to TLC profiles were combined to yield 30 fractions. Each fraction was tested with nematicidal toxicity bioassay to identify the bioactive fractions (Fraction 4, 16 and 18). Fractions that possessed nematicidal 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 *Z*-ascaridole (**1**) (0.8 mL) matched with the previous report[19,20]. The data of ρ-cymene (**2**) (0.6 mL) matched with the previous report[19,21]. The spectral data were identical to the published data of iso-ascaridole (**3**) (0.2 mL)[20].

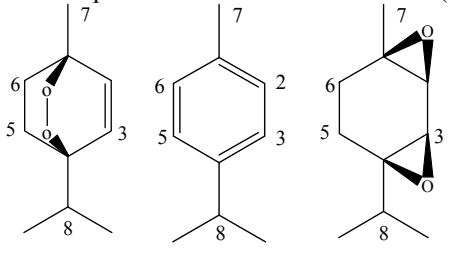

Figure 1. Compounds isolated from *chenopodium ambrosioides* essential oil

Chinese medicinal herb and hydrodistillation

Fresh aerial parts (10 kg of leaves, stems and flowers) of *C. ambrosioides* were harvested in July 2010 from Fuzhou City (Latitude: 26.06° N, Longitude: 119.28° E) Fujian province, China. The aerial parts were air-dried for one week and ground to a powder. The species was identified and the voucher specimens were deposited at 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 in a refrigerator at 4 °C.

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 with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature[22]. Component relative percentages were calculated based on GC peak areas without using correction factors.

Nematicidal assay

Egg masses of *M. incognita* obtained from tomato roots with aid of a stereomicroscope were maintained in Petri dishes during 24 h in distilled H_2O for the juvenile eclosion. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of *C. ambrosioides* essential oil (5 concentrations, 0.14-0.50%) and pure compounds (5 concentrations, 0.05-0.50%) was prepared in H_2O solution with 2% DMSO. 20 μL portions of H_2O containing ca. 30 juveniles (J_2) were transferred to vials to which 980 μL of the solution containing essential oil or pure compounds was added. The vials were kept on a hood at 25°C. The counting of the inactive nematodes was performed at every 24 h for 72 h. After the last counting, the inactive juveniles were maintained in distilled H_2O for 24 h to observe their revival. Six repetitions for each treatment were performed using H_2O and a 2% DMSO in H_2O solution as control. The experiments were repeated in three times. Results from all replicates for the pure compounds and essential oil were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} (median lethal concentration) values and their 95% confidence intervals (CI 95%)[23].

Results and Discussion

Chemical constituents of the essential oil

Hydrodistillation of flowering aerial parts of *C. ambrosioides* yielded 2.12 % essential oil (v/w). A total of 27 components were identified in the essential oil, accounting for 94.68% of

the total oil (Table 1). The main constituents of the essential oil were (*Z*)-ascaridole (27.27%), *ρ*-cymene (19.05%), isoascaridole (14.75%), *α*-pinene (6.33%) and *α*-terpinene (5.12%). The essential oil of *C. ambrosioides* is characterized by a high content of monoterpenoids (90.34%) and sesquiterpenoids comprise only 1.78% of the essential oil. The results of chemical composition of the essential oil were quite different from the previous reports. For example, α-terpinyl acetate (73.9%) and *ρ*-cymene are major constituents of *C. ambrosioides* essential oil from Mexico and content of ascaridole is only 2%[9]. In another report[10], limonene (32.5%), *trans*-pinocarveol (26.7%) and geranial (5.0%) were main components of *C. ambrosioides* essential oil from Mexico. However, the *C. ambrosioides* essential oil from Nigeria contained *α*-terpinene (56.0%), *α*-terpinyl acetate (15.7%) and *ρ*-cymene (15.5%) 26 and no ascaridole was detected in the oil[12].

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

Peak	Compounds	RI*	Formula	RA**, %
1	*α*-Pinene	931	$C_{10}H_{16}$	6.33
2	*β*-Pinene	981	$C_{10}H_{16}$	1.21
3	*δ*-4-Carene	1002	$C_{10}H_{16}$	2.67
4	*α*-Terpinene	1017	$C_{10}H_{16}$	5.12
5	*ρ*-Cymene	1024	$C_{10}H_{14}$	19.05
6	*ρ,α*-Dimenthylstyrene	1118	$C_{10}H_{12}$	0.07
7	*trans*-*ρ*-2,8-menthadien-1-ol	1128	$C_{10}H_{16}O$	0.35
8	2-Ethylcyclohexanone	1158	$C_8H_{14}O$	0.09
9	*γ*-Terpinene	1179	$C_{10}H_{16}$	1.43
10	*α,α*-4-Trimethylbenzyl alcohol	1182	$C_{10}H_{14}O$	1.75
11	*p*-Cymen-8-ol	1186	$C_{10}H_{14}O$	0.59
12	*α*-Terpineol	1191	$C_{10}H_{18}O$	1.21
13	*cis*-Piperitol	1196	$C_{10}H_{16}$	0.23
14	(*Z*)-Ascaridole	1245	$C_{10}H_{16}O_2$	27.27
15	Piperitone	1250	$C_{10}H_{16}O$	1.09
16	Piperitone oxide	1253	$C_{10}H_{16}O_2$	1.21
17	3,4-Epoxy-*ρ*-menthan-2-one	1276	$C_{10}H_{16}O_2$	0.34
18	Thymol	1292	$C_{10}H_{14}O$	2.11
19	Isoascaridole	1295	$C_{10}H_{16}O_2$	14.75
20	Carvacrol	1298	$C_{10}H_{14}O$	3.56
21	Precocene II	1368	$C_{13}H_{16}O_3$	0.21
22	Elemicin	1554	$C_{12}H_{16}O_3$	0.11
23	Caryophyllene oxide	1584	$C_{15}H_{24}O$	1.33
24	Allyltetramethoxybenzene	1591	$C_{13}H_{14}O_4$	0.23
25	Asarone	1678	$C_{12}H_{16}O_3$	1.27
26	Geranyl tiglate	1700	$C_{15}H_{24}O_2$	0.45
27	Phytol	2119	$C_{20}H_{40}O$	0.65
	Total identified			94.68
	Monoterpenoids			90.34
	Sesquiterpenoids			1.78
	Other			2.56

**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)*

The essential oil of *C. ambrosioides* collected from India contained α-terpinene (47.4%), ρ-cymene (25.8%) and ascaridole (14.8%)[16]. The essential oil of *C. ambrosioides* harvested from China comprised bornylene (42.63%), benzene, 1-methyl-4-(1-methylethyl) (21.84%), ascaridole (18.36%) and α-terpinene (11.75%)[14]. The essential oils of *C. ambrosioides* were suggested to be split into three categories[13]: oils rich in ascaridole (70-90%) (North American); oils in which ascaridole is the main components (20-67%) alongside hydrocarbons such as ρ-cymene, limonene or β-myrcene (India, Argentina, Brazil, Russia); oils rich in α-pinene (30-32%) accompanied by pinocarveol (40-42%) and/or pinocarveol (62-65%) (Japan). The essential oils of the Chinese *C. ambrosioides* belong to the second categories with about 30% ascaridole. However, besides due to species variety, these differences of chemical composition of the essential oils might have been due to harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herbs. For practical use, it is necessary to standardize the essential oil of the Chinese *C. ambrosioides* because chemical composition of the essential oil varies greatly with the plant population.

Nematicidal toxicity of isolated compounds and essential oil

Three isolated compounds and the crude oil exhibited nematicidal toxicity against the root-knot nematodes, *M. incognita* (Table 2). (*Z*)-Ascaridole (LC_{50} = 32.79 µg/mL) exhibited stronger nematicidal activity against *M. incognita* than the crude essential oil (LC_{50} = 49.55 µg/mL). The other two isolated constituents, ρ-cymene and isoascaridole also possessed nematicidal activity against *M. incognita* with LC_{50} values of 435.89 µg/mL and 1323.51 µg/mL, respectively but weaker than the crude essential oil. (*Z*)-Ascaridole shows almost 13 and 40 times more toxic against *M. incognita*, respectively, compared with ρ-cymene and isoascaridole. It is suggested that -*O*-*O*- in (*Z*)-ascaridole maybe a functional group for nematicidal activity against the root-knot nematodes. In the previous report, (*Z*)-ascaridole was also found to possess nematicidal activity against *M. incognita* with LC_{50} values of 52 µg/mL[25] and 87.36 µg/mL[24]. Compared with the other monoterpenoids tested in the previous reports[5,6], (*Z*)-ascaridole shows stronger nematicidal activity against the root-knot nematodes, *M. incognita*. For example, the activity of the nematicidal monoterpenoids was found to decrease in the order *L*-carvone, pulegone, *trans*-anethole, geraniol, eugenol, carvacrol, thymol, terpinen-4-ol and the respective EC_{50} values (24 h) against *M. incognita* were calculated in the range of 115-392 µg/mL. However, compared with a commercial nematicide (carbofuran), the three compounds possessed 10-426 times less toxic against the root-knot nematodes because carbofuran exhibited nematicidal activity against the root-knot nematodes, *M. incognita* with a LC_{50} value of 3.1 µg/mL[26].

Table 2. Nematicidal toxicity of the essential oil of *Chenopodium ambrosioides* and isolated compounds against *Meloidogyne incognita*

Compounds	LC_{50} µg/mL	95% Confidence intervals	Chi square (χ^2)
(*Z*)-Ascaridole	32.79	20.87-47.14	5.16
ρ-Cymene	435.89	341.13-585.31	5.09
Isoascaridole	1323.51	870.59-3052.25	3.08
Essential oil	49.55	34.70-74.68	0.21
Carbofuran*	3.1	-	-

**data from Shakil et al.[26]*

Considering the currently used nematicides are synthetic, nematicidal activity of the crude essential oil and the three isolated compounds are quite promising and they show potential to be developed as possible natural nematicides for control of the root-knot nematodes. However, little has been done on mechanisms of action of these three monoterpenoids against nematodes. In addition, further testing is necessary to evaluate the spectrum of nematicidal activity against other plant parasitic and free-living nematodes and their phytotoxicity to crops, and to develop formulations to improve the efficacy and stability and to reduce cost.

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