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Original Research Article

Gas Chromatography-Mass Spectrometric Analysis of Nematicidal Essential Oil of *Valeriana amurensis* P Smirn ex Kom (Valerianaceae) Roots and its Activity against *Heterodera avenae*

Yun Chao Li, Hua Ji and Hong Tao Li*
Institute of Genetics and Physiology, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang 050051, PR China

For correspondence: **Email: lihongtao1314@hotmail.com; **Tel:** +86-311-13933107622; **Fax:** +86-311-87652319*

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Abstract

Purpose: *To investigate the chemical composition and nematicidal activity of the essential oil of Valeriana amurensis roots against cereal cyst nematodes (Heterodera avenae).*
Methods: *The essential oil of V. amurensis roots was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromaotography-mass spectrometry (GC-MS). The nematicidal activity of the essential oil and its major constituents was determined against second stage juveniles of H. avenae.*
Results: *A total of 33 components of the essential oil were identified. The major constituents were bornyl acetate (12.5 %), patchoulol (11.6 %), caryophyllene (8.2 %), 3-methylvaleric acid (7.3 %) and isovaleric acid (6.5 %). The essential oil exhibited nematicidal activity against H. avenae with a medium lethal concentration (LC_{50}) value of 311.6 µg/mL. The major constituents, isovaleric acid and 3-methylvaleric acid, exhibited nematicidal activity against H. avenae with LC_{50} of 218.2 and 683.8 µg/mL, respectively.*
Conclusion: *The study indicates that the essential oil of V. amurensis roots and its two major constituents, isovaleric acid and 3-methylvaleric acid, have a potential to be developed to natural nematicides for the control of cereal cyst nematodes.*

Keywords: *Valeriana amurensis, Heterodera avenae, Nematicidal activity, Isovaleric acid, 3-Methylvaleric acid, Essential oil, Cereal cyst nematodes*

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INTRODUCTION

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Valeriana amurensis* P. Smirn. ex Kom. roots was found to possess nematicidal activity against cereal cyst nematodes (*Heterodera avenae* Wollenweber). Nematode management is generally based upon chemical treatments, but environmental concerns and governmental regulations are now resulting in a strong interest in nematicides of natural origin [1] Many plant constituents and metabolites including essential oils have been investigated for activity against plant - parasitic nematodes [2-7]. The results suggest that some of the essential oils tested and selected constituents are potential natural pesticides in the control of nematodes.

The genus *Valeriana* belongs to the family Valerianaceae and is distributed in Asia, Europe,

North and South America with about 300 species in the world, of which 21 species (13 endemic) are distributed in China [8]. *V. amurensis* is a perennial herb (80 - 150 cm tall) and grows in hillside meadows, larch and birch forest in Heilongjiang and Jilin provinces in China as well as Korea, Russia (Far East) [8].

The roots and rhizomes of this plant are widely used in phytotherapy for the preparation of phytomedicines with sedative, antispasmodic, carminative, mild anodyne and hypotensive properties [9-11]. This herb has also been used to treat dementia in Mongolian preparations for a long time [12]. From the roots and rhizomes of *V. amurensis*, iridoid glycosides, monoterpene-glucosides, phenylpropanoids glycosides, sesquiterpenoids, triterpenoids, lignans and phenolic acids have been isolated [9-14]. The chemical composition of the essential oils derived from *V. amurensis* has also been determined [15-18]. However, a literature survey shows that there is no report on nematicidal activity of the essential oil derived from *V. amurensis* roots. Thus, the objective of this study was to investigate the chemical constituents and nematicidal activity of the essential oil of *V. amurensis* roots and its selected major constituents against the cereal cyst nematodes.

EXPERIMENTAL

Plant collection and identification

The roots and rhizomes of *V. amurensis* (10 kg) were from the Great Xing'an Mountains area (Heilongjiang province, China) (50.42^{o} N and 124.12^{o} E) in August 2014 and identified by Dr QR Liu (College of Life Sciences, Beijing Normal University, Beijing 100875, China). A voucher specimen (no. 20140807) was deposited at the herbarium of Institute of Genetics and Physiology, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, China.

Extraction of essential oils

The sample was cut into small pieces and subjected to hydro distillation using a Clevenger-type apparatus for 6 h. Anhydrous Na_2SO_4 was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Analysis of the essential oils

Gas chromatography was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector and fused silica capillary column HP-5MS (5 % diphenyl and 95 % dimethylpolysyloxane, 30 m × 0.25 mm, 0.25 µm film thickness), at a flow rate of 1 mL min^{-1}. Temperature was programmed from 60 to 280 °C (at a rate of 20 °C min^{-1}); injector and detector temperatures were 270 and 300 ^{o}C, respectively. The components of the essential oils were separated and identified by gas chromatography–mass spectrometry (GC–MS) using Agilent 6890N gas chromatography coupled to Agilent 5973N mass selective detector. The system was equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 µm). GC settings were as follows: the initial oven temperature was held at 60 ^{o}C for 1 min and ramped at 10 ^{o}C min^{-1} to 280 ^{o}C and held there for 15 min. The injector temperature was maintained at 270 ^{o}C. The samples (1 µL, diluted to 100:1 with acetone) were injected, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. Spectra were obtained over the scan range 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography-mass spectrometry by comparison of their retention indices with those published in 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 and Wiley 275 libraries or with mass spectra from literature [19]. Relative percentages of the oil components were calculated based on GC peak areas without using correction factors.

Chemicals

Bornyl acetate, caryophyllene, isovaleric acid, 3-methylvaleric acid and patchoulol were purchased from Aladdin-Reagent Company (Shanghai, China). Fosthiazate was purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as positive control.

Nematodes

Cysts of *H. avenae* were extracted from rhizosphere soil of wheat roots collected in Zhengzhou city (34.44^{o}N and 112.56^{o} E), Henan Province, China. Cyst masses were stored at 4 ^{o}C for a month firstly and were maintained on wet filter paper in Petri dishes at 15 ^{o}C for 3 - 7 days for the juvenile eclosion. Only freshly hatched second stage juveniles (J_2) were used in the experiments.

Nematicidal bioassay

Range - finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (six concentrations, 50 - 1,600 µg/mL) was prepared in H_2O solution. Dimethyl sulphoxide (DMSO) was used to firstly dissolve the essential oil and the final concentration of DMSO was 0.2 %. Aliquots of H_2O (20 µL) containing ca. 100 juveniles (J2) were transferred to vials containing 980 µL of the solution containing the essential oil. The vials were kept on a 100-block box and the box was put into an incubator at 20 ^oC. The nematodes were observed at 48 h. The nematodes were considered dead if the nematodes did not move and were stiff after addition of 1 - 2 drops of 1 % NaOH solution. Six repetitions for each treatment were performed using H_2O and a 0.2 % DMSO in H_2O solution as a control. The experiments were repeated three times.

Statistical analysis

The results from all replicates were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values and their 95 % confidence intervals [20]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The essential oil of *V. amurensis* roots and rhizomes was yellow with a yield of 0.21 % (v/w) and density of 0.91 g/mL. A total of 33 components of the essential oil were identified accounting for 98.2 % of the total oil.

The major compounds in the essential oil were bornyl acetate (12.5), patchoulol (11.6 %), caryophyllene (8.2 %), 3-methylvaleric acid (7.3 %) and isovaleric acid (6.5 %) (Table 1). Sesquiterpenoids represent 19 of 33 compounds, corresponding to 52.3 % of the whole oil while 13 of the 33 constituents were monoterpenoids (30.5 % of the whole essential oil) (Table 1).

The essential oil of *V. amurensis* roots and rhizomes exhibited nematicidal activity against *H. avenae* with a LC_{50} value of 311.6 µg/mL (Table 2). Among 5 selected major constituents, isovaleric acid and 3-methylvaleric acid possessed activity against *H. avenae* with LC_{50} values of 213.2 µg/mL and 683.6 µg/ml, respectively (Table 2) while the other three constituents did not show any nematicidal activity against the cereal cyst nematodes in the current tested concentrations.

DISCUSSION

The main constituents of the essential oil of *V. amurensis* roots were bornyl acetate, patchoulol, caryophyllene, 3-methylvaleric acid and isovaleric acid. Its chemical composition is quite different from that collected from different populations and different collecting time. For example, the sample essential oil of *V. amurensis* collecting in July in Maoer mountain, Heilongjiang province mainly contained isobornyl acetate (27.6 %), valeranone (7.5 %), butylated hydroxytoluene (7.2 %), α-terpinene (5.6 %) and 9-cedranone (5.5 %) while isobornyl acetate (12.3 %) α-terpinyl acetate (11.6 %), butylated hydroxytoluene (8.8 %), valeranone (6.8 %) α-curcumene (4.3 %), and borneol (4.2 %) were the major components found in the essential oil of *V. amurensis* roots harvested in September [16].

However, the main compounds in the essential oil of *V. amurensis* roots collected from Huma country, Heilongjiang province, China were caryophyllene (28.04 %), 1,2-diethenyl-4-(1-methylethenyl)-cyclohexane (19.83 %) and bornyl acetate (12.08 %) [17]. The above results suggest that great variations in chemical composition of essential oil of *V. amurensis* roots expected due to harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herbs.

Standardization of essential oil is needed before *V. amurensis* essential oil being commercial because chemical composition of the essential oil of *V. amurensis* varies greatly with plant population and depends on time and place of collection.

The essential oil of *V. amurensis* roots exhibited nematicidal toxicity against the cereal cyst nematodes, *H. avenae*. However, compared with the positive control (fosthiazatete, LC_{50} = 84.7 µg/mL), *V. amurensis* essential oil showed 4 times less toxic to the cereal cyst nematodes.

Among the five major constituents, isovaleric acid exhibited stronger nematicidal toxicity against *H. avenae* than the crude essential oil and 3-methylvaleric showed less toxicity to cereal cyst nematodes. It is suggested that nematicidal activity of the essential oil maybe attributed to isovaleric acid.

Table 1: Main compounds of the essential oil of *Valeriana amurensis* roots

Peak no.	Compound	Retention index	(%)
	Monoterpenoids		**30.5**
1	α-Pinene	931	0.9
2	Camphene	945	3.1
3	β-Pinene	981	0.7
4	β-Myrcene	991	1.4
5	Limonene	1029	2.6
6	1,8-Cineole	1031	0.6
7	Linalool	1094	2.8
8	Camphor	1143	0.2
9	Borneol	1174	1.9
10	α-Terpineol	1188	0.7
11	Methyl thymol ether	1228	1.6
12	**Bornyl acetate**	1287	**12.5**
13	Myrtenyl acetate	1328	1.5
	Sesquiterpenoids		**53.2**
14	Copaene	1375	0.6
15	β-Patchoulene	1382	2.3
16	β-Elemene	1392	1.9
17	α-Gurjunene	1406	3.5
18	**Caryophyllene**	1420	**8.2**
19	Calarene	1433	3.2
20	α-Guaiene	1437	2.7
21	α-Caryophyllene	1449	1.1
21	Alloaromadendrene	1462	0.3
22	β-Selinene	1482	1.6
23	Germacrene D	1486	0.8
24	δ-Cadinene	1525	0.2
25	Germacrene B	1552	0.6
26	Spathulenol	1574	3.3
27	Caryophyllene oxide	1578	3.7
25	τ-Cadinol	1642	0.9
26	β-Eudesmol	1648	0.2
27	α-Cadinol	1655	2.8
28	**Patchoulol**	1660	**11.6**
29	Valeranone	1679	3.7
30	**Isovaleric acid**	875	**6.5**
31	**3-Methylvaleric acid**	902	**7.3**
32	Valeric acid	912	0.5
33	Eugenol	1356	**0.3**
	Others		**14.6**
	Total identified		***98.3***

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

Table 2: Nematicidal toxicity of *Valeriana amurensis* essential oil and its major constituents against *Heterodera avenae*

Treatment	Concentration (μg/mL)	LC_{50} (μg/mL)	95% Fiducial limits	Slope ± SD	Chi-square value
Essential oil	25-1600	311.6	284.4-339.1	3.3 ± 0.2	11.4
Bornyl acetate	25-1600	>1600	-	-	-
Caryophyllene	25-1600	>1600	-	-	-
Isovaleric acid	25-1600	213.2	192.5-234.6	3.8 ± 0.2	12.8
3-Methylvaleric acid	25-1600	683.8	618.3-731.7	3.6 ± 0.3	11.9
Patchoulol	25-1600	>1600	-	-	-
Fosthiazate	10-640	84.7	77.8-91.3	2.3 ± 0.2	8.5

In the previous report, isovaleric acid and 3-methylvaleric acid exhibited contact and fumigant toxicities against the booklice, *Liposcelis bostrychophila* [22]. However, this is the first time to report the nematicidal activity of isovaleric acid and 3-methylvaleric acid. However, considering the currently used nematicides are synthetic nematicides, nematicidal activity of the essential oil of *V. amurensis* and its two major components, isovaleric acid and 3-methylvaleric acid is quite promising and has some promise as possible natural nematicides for the control of the cereal cyst nematodes. However, to develop a practical application for the essential oil and its major constituents as novel nematicides, further research into the safety of the essential oil and its constituents in humans is needed. Additional studies on the development of formulations are also necessary to improve efficacy and stability as well as to reduce cost.

CONCLUSION

The essential oil of *V. amurensis* roots and its two major constituents demonstrate some toxicity against cereal cyst nematodes but need to be further evaluated for safety in humans and to enhance their activity.

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