Tropical Journal of Pharmaceutical Research February 2014; 13 (2): 275-280
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i2.17

Original Research Article

Gas Chromatography-Mass Spectrometric Analysis of Essential Oil of Aerial Parts of *Glycosmis parviflora* (Sims) Little (Rutaceae)

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Received: 17 January 2013 *Revised accepted: 6 January 2014*

Abstract

Purpose: *To investigate the chemical composition and toxicity of the essential oil of Glycosmis parviflora (Sims) Little aerial parts against root-knot nematode and two grain storage insects (maize weevil and red flour beetle).*
Methods: *Steam distillation of G. parviflora was carried out using Clavenger apparatus in order to extract its volatile oil. Gas chromatography/mass spectrometric (GC/MS) analysis (HP-5MS column) of the essential oil was performed and the toxicity of the oil determined by contact test.*
Results: *A total of 37 components comprising 98.7 % of the essential oil were identified, of which (Z)-caryophyllene (20.6 %), methyl isoeugenol (11.1 %), (Z)-β-ocimene (8.9 %), α-cubebene (6.4 %), nerolidol (5.4 %), aromandendrene (4.9 %) and γ-pyronene (4.7 %) were found to be the major components. The essential oil possessed strong nematicidal activity against M. incognita with an LC_{50} value of 92.84 µg/ml. The essential oil of G. parviflora exhibited strong contact toxicity against S. zeamais and T. castaneum adults with LD_{50} values of 41.7 and 22.6 µg/adult, respectively.*
Conclusion: *The study indicates that the essential oil of G. parviflora aerial parts has a potential for development into a natural insecticide/nematicide for control of nematodes and grain storage insects.*

Keywords: *Glycosmis parviflora, Essential oil, Meloidogyne incognita, Sitophilus zeamais, Tribolium castaneum, Contact toxicity*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Glycosmis represents a rather clear-cut genus within the tribe Clauseneae of the Aurantioideae subfamily of the Rutaceae comprising about 40 species and about 12 species was found in China [1]. Its range of distribution is centered in south and southeastern Asia and extends to south China and Taiwan as well as to New Guinea and north Australia. *Glycosmis parviflora* (Sims) Little (syn.: *G. citrifolia* (Willd.) Lindley) is a shrub or tree, mainly distributed in Southeastern China: Fujian, Guangdong, Guangxi, Guizhou, Hainan, Taiwan, and Yunnan Province and Japan, Myanmar, and Vietnam [1]. Its roots and stem bark have been used as a folk medicine in the treatment of skin itch, scabies, boils and skin ulcers [2]. Phytochemical analyses of *G. parviflora* led to the isolation of a number of different carbazole, acridone, and quinoline alkaloids, quinazolines and sulfur-containing amides as well as coumarins, flavonoids [2-7].

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *G. parviflora* aerial parts was found to possess insecticidal and nematicidal toxicities against root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and two grain storage insects (maize weevils, *Sitophilus zeamais* Motsch and red flour beetles, *Tribolium castaneum* Herbst). However, literature survey indicated no report on the volatile constituents and nematicidal/insecticidal activity of *G. parviflora*; thus, we decided to investigate the chemical constituents and nematicidal/ insecticidal activities of the essential oil of *G. parviflora* against .

EXPERIMENTAL

Plant collection and identification

Fresh aerial parts (10 kg) of *G. parviflora* were harvested from the suburb of Nanning City (22.8°N, 108.3°E, Guangxi Zhuang Autonomous Region, China) in August 2010. The plant sample was identified by Dr. Liu, (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen (no. CMH-Xiaohuashanxiaoju-Guangxi-2010-08) was deposited in the Department of Entomology, China Agricultural University (Beijing 100193, China).

Extraction and purification of essential oil
To obtain the volatile oil, the air-dried sample was first ground to a powder using a grinding mill (Retsch Mühle, Germany), then soaked in water at a ratio of 1:4 (w/v) for 1 h, prior to hydrodistillation using a round bottom flask over a period of 6 h. Separation of the essential oil from the aqueous layer was done in a separatory funnel, using the non-polar solvent, *n*-hexane. The hexane layer was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-124, Switzerland). The residual oil was dried over anhydrous Na_2SO_4 and kept in a refrigerator (4 °C) for subsequent experiments.

Analysis of the essential oil

Capillary gas chromatography was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector and fused silica capillary column HP-5 (5 % diphenyl and 95 % dimethylpolysyloxane, 30 m × 0.25 mm, 0.25 µm film thickness); injector and detector temperatures were 270 °C and 300 °C, respectively**.** The components of the essential oil were separated by the GC and identified by mass spectrometry (GC–MS) using Agilent 6890N gas chromatography coupled to Agilent 5973N mass selective detector. 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 where it was held for 1 min, and then ramped at 20 °C min^{-1} to 280 °C and held there for 15 min. The sample (1 µL, diluted 1:100 in acetone) was 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 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 (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 [8]. Relative percentages of the oil components were calculated based on GC peak areas without using correction factors.

Rearing of nematode and insects

Maize weevils, *S. zeamais* and red flour beetles, *T. castaneum* were obtained from our laboratory cultures maintained in the dark in incubators at 29 - 30°C and 70 – 80 % R.H. *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w/w) while *S. zeamais* were reared on whole wheat at 12 – 13 % moisture content [using Kett's grain moisture tester (Model PB-1D2, Kett Electric Laboratory, Japan)] in glass jars (diameter 85 mm, height 130 mm). Unsexed adult weevils/beetles used in all the experiments were one week old. All containers housing insects and the petri dishes used in experiments were made insect-escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK).

Second stage juveniles of the root-knot nematode, *M. incognita* were obtained from a pure culture that was previously initiated by egg masses and propagated on tomatoes (*Solanum lycopersicum*) in the glasshouse [9]. Egg masses were manually picked using sterilized forceps from heavily infected roots (40 days after incubation). These egg masses were washed in distilled water, placed in a 750 µm (15- mesh) sieves (8 cm in diameter) containing crossed layers of tissue paper in Petri-dishes with water just deep enough to contact the egg masses and the dishes were incubated at 25 - 26 °C to obtain freshly hatched second stage juveniles [9]. Only the second stage juveniles collected within 48 h were used.

Contact toxicity test

The contact toxicity of the essential oil against *S. zeamais* and *T. castaneum* was measured as described by Liu and Ho [10]. Range-finding studies were run to determine the appropriate testing concentrations of the oil of *G. parviflora* aerial parts. A serial dilution of the oil (15.0, 12.0, 9.0, 6.5, and 5.0 %.) was prepared in *n*-hexane. Aliquots of 0.5 µL per insect were topically applied dorsally to the thorax of the weevils/beetles, using a Burkard Arnold microapplicator (Burkard Scientific Supply, Rickmansworth, England). Controls were determined using 0.5 µL *n*-hexane per insect. Ten insects were used for each concentration and control, and the experiment was replicated six times. Both the treated and control weevils/beetles were then transferred to glass vials (10 insects/vial) with culture media [wheat flour mixed with yeast (10:1, w/w)] and kept in incubators at 29 - 30°C and 70 – 80 % relative humidity. Mortality was observed after 24 h. The insects were considered dead if appendages did not move when probed with a camel brush. The observed mortality data were corrected for control mortality using Abbott's formula.

Nematicidal toxicity bioassay

The nematicidal activity of the essential oil against the root-knot nematodes was measured as described by Li *et al*. [9]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (five concentrations, dissolved first in 10 µL ethanol) was prepared in H_2O solution with 2% DMSO. Aliquots of H_2O (20 µL) containing ca. 100 juveniles were transferred to vials to which 980 µL of the solution containing ethanol extract was added. The vials were kept on a hood at 25 °C. The inactive juveniles were counted every 24 h for 72 h. After the last count, 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 well as a 2% DMSO in H_2O solution containing 10 µL ethanol as negative controls. The experiments were repeated three times. Carbofuran (commerical nematicide) was purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

Statistical analysis

The results from all replicates in contact toxicity and nematicidal activity were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LD_{50} and LC_{50} values, respectively [11]. Samples for which the 95 % fiducial limits did not overlap were considered no significantly different.

RESULTS

The essential oil yield of *G. parviflora* aerial parts was 0.64 % (v/w based on dry weight) and the density of the concentrated oil was 0.77 g/ml. A total of 37 components were identified in the essential oil, accounting for 97.3 % of the total oil (Table 1). The main components of the essential oil were (*Z*)-caryophyllene (20.6 %), methyl isoeugenol (11.1 %), (*Z*)-β-ocimene (8.9 %), α-cubebene (6.4 %) and nerolidol (5.4 %) followed by aromandendrene (4.9 %) and γ-pyronene (4.7 %) (Table 1). Monoterpenoids represented 10 of the 37 compounds, corresponding to 26.5 % of the whole oil while 20 of the 37 constituents were sesquiterpenoids (53.0 % of the crude essential oil).

The essential oil possessed strong contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 41.7 µg/adult and 22.6 µg/adult, respectively (Table 2). Moreover, the oil also exhibited strong nematicidal activity against *M. incognita* with an LC_{50} value of 92.84 µg/ml (Table 3).

DISCUSSION

The main components of the essential oil of *G. parviflora* aerial parts were (*Z*)-caryophyllene (20.6 %), methyl isoeugenol (11.1 %), (*Z*)-β-ocimene (8.9 %), α-cubebene (6.4 %) and nerolidol (5.4 %). Genus *Glycosmis* comprises about 40 species in the world and about 12 species was found in China [1]. However, Chemical composition of only one species [*G. pentaphylla* (Cor.)] essential oil has been measured so far. The essential oil of *G. pentaphylla* barks collected from India mainly contained 2-undecanone (58.1 %) and 2-tridecanone (23.4 %) [12]. However, the major components of essential oil of *G. pentaphylla* collected from Ha Tinh, Vietnam were β-pinene (27.4 %), limonene (42.4 %), and β-caryophyllene (3.5 %) while that harvested from Nghe An, Vietnam contained β-pinene (24.4 %), limonene (31.7 %), and β-caryophyllene (11.1 %) [13].

The essential oil of *G. parviflora* exhibited nematicidal activity against the root-knot nematode. Compared with a synthetic insecticide, carbofuran (LC_{50} = 72.29 µg/ml), the essential oil of *G. parviflora* exhibited the same

Table 1: Chemical constituents of essential oil of *Glycosmis parviflora*

Peak no.	*Compound*	*RI**	Peak area (%)
1	α-Pinene	939	2.2
2	β-Pinene	974	0.1
3	β-Myrcene	991	0.9
4	δ-3-Carene	1016	3.5
5	Limonene	1029	1.6
6	(*Z*)-β-Ocimene	1035	8.9
7	Linalool	1094	3.5
8	Allo-ocimene	1139	0.7
9	Camphor	1143	0.4
10	Dihydroedulan I	1293	0.4
11	4-Vinylguaiacol	1318	0.5
12	γ-Pyronene	1338	4.7
13	α-Cubebene	1345	6.4
14	Dehydro-ar-ionene	1355	0.7
15	β-Elemene	1389	0.7
16	Methyl Eugenol	1403	1.7
17	(*Z*)-Caryophyllene	1409	20.6
18	β-Caryophyllene	1420	0.5
19	**Aromandendrene**	**1436**	**4.9**
20	α-Caryophyllene	1454	0.6
21	Elixene	1456	0.1
22	γ-Muurolene	1473	2.6
23	β-Neoclovene	1475	0.9
24	Germacrene D	1485	0.5
25	β-Selinene	1490	1.1
26	**Methyl isoeugenol**	**1500**	**11.1**
27	Bicyclogermacrene	1513	0.9
28	δ-Cadinene	1523	0.5
29	Nerolidol	1563	5.4
30	Spathulenol	1572	3.7
31	Caryophyllene oxide	1583	0.5
32	Viridiflorol	1588	0.7
33	Isoaromadendrene epoxide	1594	0.6
34	Isoelemicin	1596	2.5
35	α-Cadinol	1654	0.8
36	Eudesma-4,11-dien-2-ol	1691	1.0
37	Phytol	2119	0.9
	Total identified		97.3
	Monoterpenoids		26.5
	Sesquiterpenoids		53.0
	Others		17.8

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

level of toxicity against *M. incognita*. Moreover, the essential oil of *G. parviflora* exhibited stronger nematicidal toxicity against the root-knot nematode, e.g. essential oil of *Kadsura heteroclita* [9] and *Chenopodium ambrosioides* [22]. The above findings indicated the essential oil of *G. parviflora* shows potential to be developed as a possible natural nematicide for control of the root-knot nematodes.

The essential oil of *G. parviflora* exhibited contact toxicity against *S. zeamais* and *T. castaneum* adults. When compared with the positive control (pyrethrum extract, LD_{50} = 4.3 µg/adult and 0.4 µg/adult, respectively)[14], the essential oil of *G. parviflora* demonstrated 10 and 63 times less acute toxicity against *S. zeamais* and *T. castaneum* adults. However, compared with the other essential oils in the literature, the essential oil of *G. parviflora* exhibited stronger or the same level of contact toxicity against *S. zeamais* and *T. castaneum* adults. Examples are essential oils of *K. heteroclita* [9], *Illicium fragesii* [15], *I. simonsii* [16], *I. pachyphyllum* [17], *Caryopteris incana* [18], *Artemisia capillaries*, *A. mongolica* [19], *A. giraldii*, *A. subdigitata* [20] and *Murraya exotica* [21].

Among the main constituents of the essential oil of *G. parviflora*, methyl isoeugenol, (*Z*)-β-ocimene and nerolidol have been demonstrated to exhibit insecticidal and acaricidal activities against several insect pests/mites, e.g. house fly *Musca domestica* [23], American house dust mite (*Dermatophagoides farinae*), European house dust mite (*D. pteronyssinus*) and adult mould mites (*Tyrophagus putrescentiae*) [24], two-spotted spider mite (*Tetranychus urticae*) [25], and the yellow fever mosquito, *Aedes aegypti* larvae [26]. The isolation and identification of the bioactive compounds in the essential oil of *G. parviflora* are of utmost importance so that their potential application in controlling stored-product pests and nematodes can be fully exploited. The above findings suggest that the essential oil of *G. parviflora* can play an important role in stored

Table 3: Contact toxicity of essential oil of *Glycosmis parviflora* against *Sitophilus zeamais* (SZ) and *Tribolium castaneum* (TC) adults

INSECT	*Treatment*	LD_{50} (µg/adult)	95% fiducial limits	Slope ± SE	Chi square (χ^2)
SZ	Essential oil	41.7	37.03-48.5	4.2 ± 0.5	33.6
	Pyrethrum extract*	4.3	3.9-4.7	-	-
TC	Essential oil	22.6	20.1-25.1	4.1 ± 0.6	11.2
	Pyrethrum extract*	0.4	0.3-0.4	-	-

** Liu et al [19]*

Table 2: Nematicidal activity of essential oil of *Glycosmis parviflora* against *Meloidogyne incognita* juvenile

Treatment	LD_{50} (µg/mL)	95% fiducial limits	Slope ± SE	Chi square (χ^2)
Essential oil	92.84	83.73 - 101.59	4.56 ± 0.42	11.69
Carbofuran	72.29	37.86 - 117.97	6.23 ± 0.51	13.57

grain protection and nematode control and reduce the need for the same, and also the risks associated with of synthetic insecticides/ nematicides. However, for the practical application of the essential oil as a novel insecticide/nematicide, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

CONCLUSION

The essential oil of *G. parviflora* aerial parts demonstrated some activity against maize weevils and red flour beetles as well as root-knot nematodes but needs to be further evaluated for safety in humans and to enhance its activity.

ACKNOWLEDGEMENT

This work was funded by the Hi-Tech Research and Development of China 2011AA10A202 and National New-Drug Innovation Project 2009ZX09501-014. The authors thank Dr. QR Liu of the College of Life Sciences, Beijing Normal University, Beijing 100875, for the identification of the plant.

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