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Table: 1, Figs : 4

Chemical composition and allelopathic activity of essential oils from *Geranium wilfordii* Maxim.

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ABSTRACT

Essential oils from fresh roots and leaves of *G. wilfordii* were analysed by Gas chromatography coupled to mass spectrometry (GC-MS). A total of 43 and 33 compounds were identified from roots and leaves, respectively. Germacrone was the most abundant compound in oils of both root (45.27%) and leaf (20.22%). Besides, cyclohexanone (24.14%) and *β*-eudesmol (4.18%) were the main components of root oil, while ledol (18.82%) and *β*-elemenone (14.65%) were the major compounds in leaf oil. The allelopathic potential of these oils was tested on seed germination of 9-plants [*Brassica campestris* L. ssp. *chinensis* Makino var. *communis* Tsen et Lee, *Brassica rapa pekinensis*, *Brassica campestris* L., *Cucumis sativus* L., *Scrophularia ningpoensis* Hemsl., *Eruca sativa* Mill., *Lolium perenne* L., *Clitoria ternatea* L. and *Portulaca oleracea* L]. The seeds of *Brassica campestris* were the most susceptible to both oils of root and leaf, with RI values lower than -0.93. Furthermore, root oil led to RI values -0.97 for the root length of *B. campestris* and -0.88 for the shoot length of *Lolium perenne*. The seedling growth of *B. campestris* L. and *Clitoria ternatea* L. exposed to leaf oil showed RI values of about -1. These results suggested that essential oils of *G. wilfordii* can be used as plant growth regulators.

Keywords: Allelopathic activity, *Brassica campestris* L. spp. *chinensis*, *Brassica campestris* L., *Brassica rapa pekinensis*, chemical composition, *Clitoria ternatea* L., *Cucumis sativus* L., *Eruca sativa* Mill., essential oil, GCMS, *Geranium wilfordii* Maxim., germination, leaf, *Lolium perenne* L., *Portulaca oleracea* L., root, *Scrophularia ningpoensis* Hemsl., seed, seedling growth.

INTRODUCTION

Essential oils (EOs) are mixtures of volatile compounds (VOC) in aromatic plants with strong odours (3). These VOC have wider use in fields of food, cosmetic, pharmacy and agriculture because of their antioxidant, antiseptic, virucidal, bactericidal and fungicidal activities (1,2,4,12,23,32,43). China has prepared 266 kinds of Patented Chinese medicines from the essential oils of peppermint oil and *Eucalyptus* oil (30). Some essential oils exert phytotoxic effects on some plants due to allelopathy (9,35,37), revealing the potential use of essential oil as bio-herbicides or plant growth regulators.

Geranium wilfordii Maxim., (Family *Geraniaceae*) is perennial medicinal herb in China, Korea, Japan, Manchuria and Taiwan (5,8). In China, since the Ming dynasty,

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G. wilfordii has been used to treat rheumatic pains, invigorate blood circulation, clear heat and remove toxicity (16). It also possesses pharmacological properties [antiviral activity, blood coagulation, anthelmintic action and diuretic action] and is used in clinical medicine against herpes keratitis and intestinal infections (28).

Figure 1. *Geranium wilfordii* Maxim plant.

Owing to its broad medicinal value, the *G. wilfordii* chemical constituents have been studied since past many years. Its main components are tannins, flavonoids and organic acids (13,14,21) which are responsible for its biological activities. Although, few studies have reported its allelopathic activity, but there is lack of information about its chemical composition and allelopathic potential of its essential oil. Therefore, this study aimed to determine the composition and the phytotoxic effects of essential oils of *G. wilfordii* on seeds germination and seedling growth of test crops. This is the first report about the chemical composition and allelopathic activity of essential oils of *G. wilfordii* and its use as bio-herbicides or plant growth regulators.

MATERIALS AND METHODS

Instruments and plant materials

The study was done in Laboratory of Plant Science College, Jilin University, Changchun city, Jilin Province, Northeast China [N 43°88', E 125°35']. The mean height above sea level: 219 m and annual rainfall 580 mm. The maximum and minimum temp: 29°C and -32°C]. The entire plant of *Geranium wilfordii* was collected from Changbai Mountain，Jilin province in July 2015. The fresh leaves and roots of *G. wilfordii* were separated and then their volatile oils were extracted. The seeds of 9-test plants [*Brassica campestris* L.ssp. *chinensis* Makino var. *communis* Tsen et Lee, *Brassica* rapa *pekinensis*, *Brassica campestris* L., *Cucumis sativus* Linn, *Scrophularia ningpoensis* Hemsl., *Eruca sativ* Mill., *Lolium perenne* L., *Clitoria ternatea* L. and *Portulaca oleracea* L. were purchased from Flower Goddess Ltd., Beijing.

Extraction of essential oils

The extraction of essential oils from leaves (10 kg) and roots (8 kg) of *Geranium wilfordii* was done using the steam distillation method (7,10,18,41). The fresh leaves and roots of *G. wilfordii* were shredded and then the small pieces were soaked into distilled water overnight. Thereafter, the mixture was distilled for 3 h into a Clevenger-type apparatus. Each sample was extracted twice. After ethyl ether extraction and filtration, the obtained essential oils were dried over anhydrous sodium sulphate and stored at 4 °C for further study.

GCMS Chemical Analysis of essential oils

The composition of the essential oils was analyzed by GC-MS according to the program proposed by Wang, D.C. *et. al.* (11,31,33,34,39). The GC-MS (70eV) analysis was carried out on a Thermo TRAEE Gc Agilent 5975, equipped with HP-5 MS capillary column (30m × 0.25 mm, 0.25 μm film thickness). The temperature was programmed from 60 °C to 280 °C at 8 °C/min; held isothermal at 60 °C for 3 min and at 280 °C for 20 min. The temperatures of the injector was kept at 280 °C; sample injection volume, 1μL; split ratio was 30:1. The carrier gas was helium at a flow rate of 1 mL/min; MS source temperature at 230 °C; MS quadrapole temperature at 150 °C; interface temperature at 280 °C; mass scan, 20-450 amu (atomic mass units). The components of the essential oils were ascertained by using NIST (National Institute of Standards and Technology) MS Search database. The relative percentage of each constituent was obtained according to the peak-areas from the GC-MS total ion current (TIC) data.

Bioassay

The seeds of 9-test plants were immersed into sterilized water to discard the weak floating seeds. The allelopathic bioassay with essential oils extracted from *G. wilfordii* was done following Rial *et.al.* (22,26,38,40). The Petri dishes (9-cm dia) were lined with two layers of filter paper and 30 seeds of test weeds were placed equidistant per Petri dish. The essential oils of root and leaf were first dissolved in dimethyl sulfoxide (DMSO) and then mixed with distilled deionized water to get final concentration of 1.2 mg/mL. Five ml of emulsion was added to each Petri dish. Then the dishes were sealed with Parafilm. The treatments were replicated thrice. The negative control group consisted of 5 mL of distilled water. The positive group was set by using diphenylamine (1.2 mg/mL) mixed with water-DMSO. Then the Petri dishes were kept in growth chamber [12h light-dark cycle at 25°C]. The germination was recorded every 24 h, while the root and shoot lengths were recorded on 6^{th} day. As per the method of Yan *et al* (17,38). The response index (RI) was used to determine the allelopathic bioactivity of these essential oils and was calculated as under:

$$\text{If } T \geq C, \text{ then } RI = 1 - (C/T); \text{ if } T < C, \text{ then } RI = (T/C) - 1$$

Where RI: Response index, T: Treatment response, C: Control response, RI > 0: Stimulation and RI < 0: Inhibition.

C: Number of seeds germinated daily in the control, T: Number of seeds germinated daily in treated seeds treated with one oil emulsions, respectively.

Statistical analysis

The data were analysed by GraphPad Prism 6, IBM SPSS Statistics 19 and Microsoft Office Visio 2003. All experiments were done in triplicate and Data are presented as mean ± SD. Statistical significance was determined at $P < 0.05$ level.

RESULTS AND DISCUSSION

Chemical composition

The GC-MS technique was used to determine chemical composition of the essential oil (Fig 2). Two pale yellow colour essential oils were obtained by hydrodistillation. Oil yields were 0.15 % and 0.13 % (v/w) for leaves and roots of *Geranium wilfordii*, respectively. As shown in Table 1, 43 and 33 compounds were identified representing 91.44 % and 87.96 % oils in root and leaf, respectively. Germacrone was the most abundant component in oils of both root (45.27 %) and leaf (20.22 %). The main constituents of the root oil were: Germacrone, Cyclohexanone, 5-ethenyl-5-methyl-4-(1-methylethenyl)-2- (1-methylethyli-dene)*cis*- (24.139%), β-eudesmol (4.18%), β-guaiene (2.77%), elixene (1.54%) and γ-Elemene (1.45%), while, the leaf oil mainly composed of ledol (18.82%), β-elemenone (14.65%), α-bulnesene (7.90%) and spathulenol (7.48%).

Table 1. Chemical composition of essential oils from roots and leaves of *Geranium wilfordii*.

No.	Retention index	Compounds	Content (v/v %)	
			Root	Leave
1	993.322	Leaf alcohol	0.838	
2	998.806	3-Hexen-1-ol		0.389
3	1012.774	Leaf aldehyde	0.157	
4	1029.044	3-Furaldehyde	0.131	
5	1160.332	3-Cyclohexene-1-methanol, 2-hydroxy-ππ4-trimethyl-	0.091	
6	1173.615	5-trimethyl-2-furanmethanol	0.241	
7	1278.216	Linalool	0.899	
8	1276.014	l-Camphor		0.164
9	1279.886	Camphenol, 6-		1.422
10	1281.675	Terpinen-4-ol	0.130	
11	1289.339	Myrtenol		0.577
12	1291.056	2-Isopropenyl-5-methylhex-4-enal	0.166	
13	1293.411	Myrtenal		0.285
14	1298.877	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-		0.291
15	1305.062	(-)-alpha-Terpineol	0.531	
16	1307.601	alpha-Terpineol		0.371
17	1311.903	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-		0.418
18	1329.456	2-(4-Methylphenyl)propan-2-ol	0.097	
19	1340.771	Myrtenal	0.096	
20	1367.088	(1S)-(-)-pin-2-en-4-one	0.093	
21	1367.088	2-Pinen-4-one		0.393

No.	Retention index	Compounds	Content (v/v %)	
			Root	Leave
22	1445.920	Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-, [1S-(1π3aπ3bπ6	0.079	
23	1455.054	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	0.120	
24	1474.999	2-Pentene, 1-(pentyloxy)-, (E)-	0.115	
25	1488.432	Germacrene B	0.061	
26	1496.121	a-Guaiene		0.902
27	1499.054	γ-Elemene	1.446	0.248
28	1510.658	(-)-(E)-Lanceol	0.166	
29	1514.926	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	0.129	0.283
30	1527.214	Butanoic acid, 2-methyl-, phenylmethyl ester	0.094	
31	1537.509	Benzyl isovalerate	0.332	
32	1554.705	a-Gurjunene		0.356
33	1577.422	(+)-b-Selinene	0.472	0.307
34	1583.178	(-)-a-Selinene	0.298	
35	1586.042	a-Bulnesene	0.303	7.898
36	1608.534	(-)-b-Cadinene	0.335	1.349
37	1613.014	Bicyclo[10.1.0]tridec-1-ene	0.218	
38	1633.156	Eudesma-3,7(11)-diene	0.643	0.304
39	1664.242	Elixene	1.536	0.325
40	1710.887	(-)-T-Muurolol		0.281
41	1756.222	β-Elemenone		14.649
42	1759.971	Cyclohexanone, -ethenyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene)-, cis-	24.139	1.764
43	1763.654	Ledol		18.823
44	1769.051	b-Guaiene	2.769	0.246
45	1778.866	Spathulenol		7.475
46	1804.656	beta-Eudesmol	4.177	2.901
47	1817.373	Eudesm-7(11)-en-4-ol		3.098
48	1884.348	Germacrone	**45.274**	**20.222**
49	1907.694	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	0.745	
50	1912.162	πGurjunenepoxide-(2)		0.344
51	2010.187	Benzyl Benzoate	1.072	0.351
52	2087.509	Neocurdione		0.544
53	2276.277	Heneicosane	0.492	0.268
54	2288.864	Phytane(6CI)	0.267	
55	2299.649	Hentriacontane	0.227	
56	2373.337	Octacosane	0.133	0.273
57	2391.767	Pentacosane	0.308	
58	2452.009	Tetratetracontane	0.094	0.440
59	2492.445	4,8,12,16-Tetramethylheptadecan-4-olide	0.130	
60	2663.640	Heptacosane	0.582	
61	2861.588	Nonacosane	1.214	
		Total	**91.440**	**87.961**

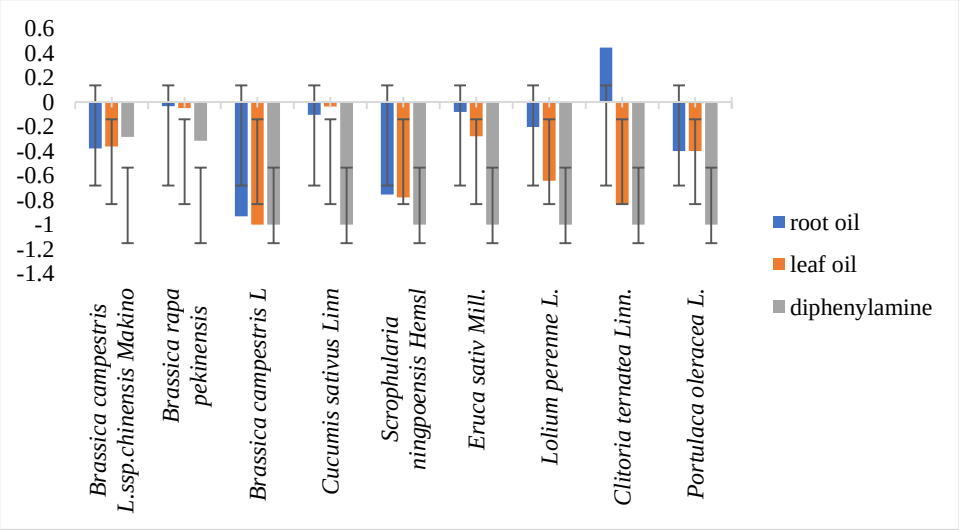

Figure 2. Effects of root and leaf oils of *Geranium wilfordii* and diphenylamine seeds germination of test crops on 6th day.

Allelopathic activity

Germacrone has broad activities (antibacterial, antitumoral and pesticidal effects) but also used to treat cough and asthma (15,19,27). The aerial parts of *Geranium macrorrhizum* L. contained high percentage of germacrone (49.7%) (6,25). The essential oils containing the germacrone were potent agent against *Bacillus subtilis* (25). This is the first report about the allelopathic activity of essential oil of *G. wilfordii* and its main component Germacrone.

The allelopathic effects of essential oils from roots and leaves of *G. wilfordii* were determined on seed germination and seedling growth of 9-test plants (Figure 2**)**. The RI values of the seeds treated with EOs and diphenylamine were compared with negative control. The diphenylamine significantly inhibited the seed germination and seedling growth of *Lolium perenne*, *Clitoria ternata*, *Portulaca oleracea* and *Amaranthus viridis* (24). In our experiment, the diphenylamine at 1.2 mg/ml concentration, drastically inhibited the seed germination of 9-test crops seeds as positive control (RI = -0.32, *B. rapa pekinensis*; RI = -0.28, *B. campestris* ssp chinensis makino var. *communis* RI = -1.0, for remaining test plant species). Root oil inhibited the seeds germination of all test plant species except *C. ternatea* with RI of -0.38 (*B. campestris* ssp chinensis makino var.*communis* Tsen et Lee), -0.03 (*Brassica rapa pekinensis*), -0.93 (*Brassica campestris*), -0.11 (*Cucumis sativus*), -0.76 (*Scrophularia ningroensis*), -0.08 (*Eruca sativa*), -0.20 (*Lolium perenne*), -0.44 (*Clitoria ternatea*) and -0.40 (*Portulaca oleracea*). Whereas, the leaf oil was very inhibitory to germination of all seeds (RI=-0.36, *Brassica campestris*

L.ssp. *chinensis* Makino var. *communis* Tsen et Lee; RI=-0.05, *Brassica* rapa *pekinensis*; RI=-1, *Brassica campestris* L.; RI=-0.04, *Cucumis sativus* Linn; RI=-0.78, *Scrophularia ningpoensis* Hemsl.; RI=-0.28, *Eruca sativ* Mill.; RI=-0.64, *Lolium perenne* L.; RI=-0.83, *Clitoria ternatea* Linn.; RI=-0.4, *Portulaca oleracea* L.). The results showed that *B. campestris* L. was most sensitive to oils of both root and leaf of *G. wilfordii*, followed by *Scrophularia ningpoensis* Hemsl. The lower RI value was in leaf oil treatment than root oil, reflects that leaf oil of *G. wilfordii* was more inhibitory to seeds germination.

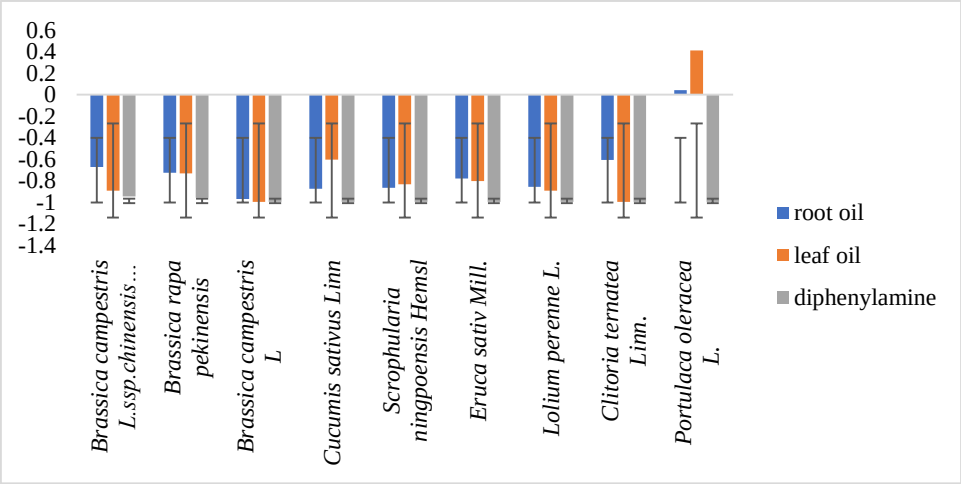

Figure 3. Effects of diphenylamine and essential oils from roots and leaves of *G. wilfordii* on root growth of test seeds.

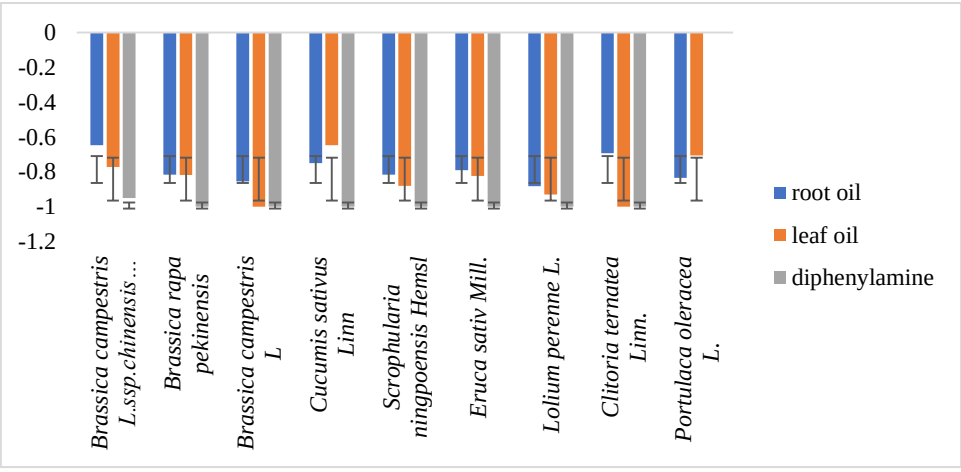

Figure 4. Effects of diphenylamine and essential oils from roots and leaves of *G. wilfordii* on shoot growth of test seeds.

The essential oils also inhibited the root and shoot length of test plants. The root oil of *G. wilfordii* was inhibitory to root growth of *B. campestris* (RI=-0.97) and shoot of *Lolium perenne* (RI= -0.88) (Figure 3 and 4). However, leaf oil was less inhibitory to both the root and shoot growth of *B. campestris* and *C. ternatea*. The inhibition rate of diphenylamine on the seedling growth of all seeds was > 95%. Thus the root and leaf oils were phytotoxic to seedling growth of tested seeds. However, the root and leaf oils promoted the root growth of *P. oleracea* L., and root oil stimulated the germination of *C. ternatea* L. We can hypothesize that it might be due to the synergistic role of Germacrone and other constituents in the functioning of essential oils with regard to the positive effects (24).

CONCLUSIONS

The root and leaf oils of *G. wilfordii* proved phytotoxic to the germination and seedling growth of the weed species assayed. Further, the phytotoxic activity of single compounds like germacrone in root and leaf oils needs to be investigated. Our work is the first relating to the chemical composition with the allelopathic potential of essential oils from *G. wilfordii*. Further research is needed to assess the use of these essential oils of *G. wilfordii* as bio-herbicides or plant growth regulators.

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