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Original Research Article

Chemical composition of Dipsacus asper Wallich ex Candolle (Dipsacaceae) essential oil and its activity against mosquito larvae of Aedes aegypti and Culex pipiens pallens

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Abstract

Purpose: *To evaluate the larvicidal activity of the essential oil of Dipsacus asper Wallich ex Candolle (Dipsacaceae) roots against the larvae of Aedes aegypti L. and Culex pipiens pallens Coquillett.*
Methods: *Essential oil was extracted from D. asper roots by hydrodistillation and analyzed for its composition by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The oil was evaluated for larvicidal activity, using World Health Organization (WHO) procedures, against the fourth larvae of A. aegypti and C. pipiens pallens within 24 h, and activity was recorded for various concentrations of the oil ranging from 12.5 – 200.0 µg/mL.*
Results: *A total of 34 components of the essential oil of D. asper were identified. The major compounds of the essential oil were caryophyllene oxide (13.29 %), caryophyllene (9.14 %), cubebene (7.87 %), β-gurjunene (6.43 %), carvone (5.38 %), 1,8-cineole (5.29 %), and calamenene (5.05 %). The oil exhibited larvicidal activity against A. aegypti and C. pipiens pallens at median lethal concentrations (LC_{50}) of 56.29 µg/mL and 47.49 µg/mL, respectively.*
Conclusion: *The essential oil of D. asper roots has potentials for use in the control of A. aegypti and C. pipiens pallens and may be useful in the search for new, safer and more effective natural larvicides.*

Keywords: *Dipsacus asper, Aedes aegypti, Culex pipiens pallens, Essential oil, Larvicidal activity, Caryophyllene, Cubebene, β-Gurjunene, Carvone*

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INTRODUCTION

The mosquitoes, *Aedes aegypti* L. and *Culex pipiens pallens* Coquillett (Diptera: Culicidae) are two worldwide insects causing dreadful nuisance and transmitting many dangerous diseases [1,2]. In China, *A. aegypti* is considered one of the major vectors for the transmission of dengue fever (common clinical characteristics as fever, headache, chilly, joint pain) and *C. pipiens pallens* is the primary vector of wuchereriasis and epidemic encephalitis B (clinical symptoms included high fever, nausea and vomiting, altered consciousness) [1,2]. Currently, synthetic insecticides and insect growth regulators are widely applied to control larval mosquitoes [3]. However, repeated and injudicious application of these synthetic insecticides have caused disrupt of the natural biological control systems and sometimes resulted in the widespread development of resistance as well as undesirable effects on non-target organisms, toxic residues in food, workers' safety, and high cost of procurement [4]. Essential oils and their

constituents have been suggested as alternative sources for conventional mosquito larvicides [5-7]. During the present author's mass screening program for new agrochemicals from wild plants and Chinese medicinal herbs, the essential oil of *Dipsacus asper* Wallich ex Candolle (Family: Dipsacaceae) roots, was found to possess larvicidal activity against the larvae of *A. aegypti* and *C. pipiens pallens*.

Himalayan teasel (*D. asper*) is a perennial herb distributed widely in the southwest of China (Chongqing, Guangdong, Guangxi, Guizhou, Hubei, Sichuan, Xizang, and Yunnan province) and India as well as Myanmar [8]. The roots of *D. asper* has been used in traditional Chinese medicine for hundreds of years as an antiosteoporosis, tonic and antiaging agent for the therapy of low back pain, traumatic hematoma, threatened abortion and bone fractures [9,10]. In the previous studies, dozens of chemical constituents, including triterpenoids, triterpene saponins, iridoids, iridoid glucosides, lignans, phenolics, and alkaloids, have been identified from the roots of *D. asper* [9-15]. However, a literature survey has shown that there is no report on chemical composition of essential oil of *D. asper* and larvicidal activity of *D. asper* essential oil against mosquitoes. Hence, the objective of the present study was to investigate the chemical constituents and larvicidal activity of the essential oil of the plant against two species of mosquitoes.

EXPERIMENTAL

Plant collection and identification

Dried roots of *D. asper* **(10 kg,** harvested from Hubei Province, China) were purchased from Anguo Herb Market (Anguo, Hebei Province). The species was identified by Dr. Liu, Q.R., College of Life Sciences, Beijing Normal University, Beijing 100875 and a voucher specimen of *D. asper* (Dipsacaceae-Chuanxuduan-Hubei-07) was deposited at the museum of Department of Entomology, China Agricultural University.

Oil extraction

The sample was chopped to small pieces and immersed in water at a ratio of 1:4 (w/v) for 1 h, and subjected to hydro distillation using a modified Clevenger-type apparatus for 6 h. The essential oil was extracted from the distillate with *n*-hexane and then dried over anhydrous Na_2SO_4. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 35 °C and the pure oil was kept in a refrigerator (4 °C) pending subsequent experiments.

Analysis of the essential oil

Gas chromatographic analysis 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), operated at a flow rate of 1 mL min^{-1}. Column temperature was initially 60 °C for 1 min, then gradually increased to 180 °C at 10 °C min^{-1}, and finally increased to 280 °C at 20 °C min^{-1}. The components of the essential oil were separated and identified by gas chromatography–mass spectrometry (GC - MS). (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 maintained at 60 °C for 1 min and increased 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 kept there for 15 min. The injector temperature was maintained at 270 °C. The essential oil was diluted 100:1 (v/v) with acetone and the diluted samples (1 µL) were injected automatically in splitless mode. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. Spectra were detected over the scan range 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography and comparison of their retention indices with those published in the literature or with those of authentic compounds available in our laboratories. Retention index was measured in relation to a homologous series of *n*-alkanes (C_8 – C_{24}) under the same operating conditions. Further identification was taken by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [16]. Relative contents of the oil components were calculated based on GC peak areas without applying correction factors.

Insect cultures and rearing conditions

Mosquito eggs of *A. aegypti* and egg masses of *C. pipiens pallens* utilized in bioassays were obtained from a laboratory colony maintained in the Department of Vector Biology and Control, Institute for Infectious Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The eggs of *A. aegypti* and egg rafts of *C. pipiens pallens* were collected from Nanjing, Jiangsu province, China in 1997. The adults were maintained in a cage (60 × 30 × 30

cm) at 28 – 30 °C and 75 – 85 % RH. The female adults were fed with rat blood every alternate day whereas the males were fed with 10 % glucose solution soaked on cotton pad, which were hung in the middle of the cage. A beaker with strips of moistened filter paper was kept in the cage for oviposition of *A. aegypti*. The eggs laid on paper strips were kept wet for 24 h and then dehydrated (air-dried) at room temperature. The dehydrated eggs were put into plastic tray containing tap water in our laboratory at 26 – 28 °C and natural summer photoperiod (L14:D10) for hatching and yeast pellets provided as food for the emerging larvae. However, *C. pipiens pallens* deposited in tap water and the egg masses were transferred to a white porcelain basin containing tap water for hatching. Larvae were daily observed until they reached the fourth instar, when they were employed for bioassays (within 12 h).

Larvicidal bioassay

Range-finding studies were performed to determine the appropriate testing concentrations. Concentrations of 200, 100, 50, 25, and 12.5 µg/mL of essential oil were assessed. The larval mortality bioassays were carried out according to the test method of larval susceptibility as recommend by WHO [17]. Twenty larvae were put in a glass beaker with 250 mL of aqueous suspension of tested material at various concentrations. Five replicates were run simultaneously per concentration and with each experiment, a set of control and untreated sets of larvae in tap water, were also run for comparison. Commercial rotenone (purchased from Aladdin Industrial Inc., Shanghai, China) was utilized as a positive control. The assays were placed in a growth chamber (L16:D9, 26 – 28 ^{o}C, 78 – 80 % RH). Mortality was detected after 24 h of exposure.

Statistical analysis

Percent mortality was corrected for control mortality using Abbott's formula [18]. Results from all replicates for the pure compounds/oil were subjected to probit analysis using PriProbit Program V1.6.3 (http://ars.usda.gov/Services/docs.htm?docid =11284) to estimate LC_{50} values and their 95 % confidence intervals [19]. Samples for which the 95 % fiducial limits did not overlap were regarded as significantly different.

RESULTS

The yield of essential oil from 10 kg of dried *D. asper* roots was 8.5 ml while its density was 0.87 g/ml. A total of 34 components of the essential oil of *D. asper* roots were identified (Table 1). The principal compounds of the essential oil were caryophyllene oxide (13.29 %), caryophyllene (9.14 %), cubebene (7.87 %), β-gurjunene (6.43 %), carvone (5.38 %), 1,8-cineole (5.29 %) and calamenene (5.05 %) (Table 1). Sesquiterpenoids represented 18 of the 34 constituents, corresponding to 62.80 % of the essential oil of *D. asper* roots while 13 of the 34 compounds were monoterpenoids, corresponding only to 32.81 % of the whole essential oil.

The essential oil of *D. asper* exhibited larvicidal activity against the 4th instar larvae of *A. aegypti* and *C. pipiens pallens* with LC_{50} values of 56.29 µg/mL and 47.59 µg/mL, respectively.

DISCUSSION

The main constituents of *D. asper* essential oil were caryophyllene oxide, caryophyllene, cubebene, β-gurjunene, carvone, 1,8-cineole, and calamenene. This is the first time to report chemical composition of *D. asper* roots essential oil. The results are quite different from the essential oils of other Chinese species in *Dipsacus* [20,21]. For example, the essential oil of *D. asperoides* roots mainly contained carvotanaceton (8.54 %), 2,4,6-tri-t-butyl-phenol (5.46 %), 3-enthyl-5-methyl-phenol (4.15 %) and 4-methyl-phenol (3.98) and the essential oil of *D. asperoides* had remarked effect against *Staphyloccus aureus* [20]. However, the main constituents of the essential oil of the aerial parts of *D. japonicus* at the flowering stage were linalool (11.78 %), *trans*-geraniol (8.58 %), 1,8-cineole (7.91 %), β-caryophyllene (5.58 %), α-terpineol (5.32 %), β-selinene (5.15 %), and spathulenol (5.04 %) and the essential oil of *D. japonicus* exhibited contact and fumigant toxicity against two grain storage insects (*Sitophilus zeamais* and *Tribolium castaneum*) [21].

The essential oil of *D. asper* roots possessed strong larvicidal activity against the 4th instar larvae of *A. aegypti* and *C. pipiens pallens*. The commercial insecticide, rotenone showed larvicidal activity against the two species of mosquitoes with LC_{50} values of 3.75 µg/mL and 1.88 µg/mL, respectively.

However, compared with the other essential oils/extracts in the literature, the essential oil of *D. asper* exhibited the same level of or stronger larvicidal activity against *A. aegypti* larvae, e.g., essential oil of *Eucalyptus urophylla* (LC_{50} = 95.5 µg/mL) [22]; essential oils from four Guarea species (*G. scabra* leaves, LC_{50} = 98.6 µg/mL; *G. silvatica* leaves, LC_{50} = 117.9 µg/ml and *G.*

Table 1: Main compounds of the essential oil of *Dipsacus asper* roots

Peak	Compound	Retention index	(%)
	Monoterpenoids		***32.81***
1	α-Pinene	931	3.57
2	β-Pinene	974	0.68
3	β-Myrcene	991	0.61
4	Limonene	1029	0.45
5	**1,8-Cineole**	1032	**5.29**
6	Linalool	1094	4.18
7	Camphor	1146	1.45
8	Isoborneol	1158	2.28
9	Borneol	1174	0.21
10	4-Terpineol	1179	2.93
11	α-Terpineol	1189	3.32
12	**Carvone**	1238	**5.38**
13	Bornyl acetate	1287	2.46
	Sesquiterpenoids		***62.80***
14	α-Copaene	1374	2.81
15	**Caryophyllene**	1420	**9.14**
16	Aromandendrene	1426	1.19
17	**β-Gurjunene**	1431	**6.43**
18	α-Caryophyllene	1452	1.12
19	γ-Muurolene	1473	1.02
20	β-Selinene	1489	4.26
21	**Calamenene**	1517	**5.05**
22	**Cubebene**	1529	**7.87**
23	α-Calacorene	1546	0.41
24	Ledol	1567	2.23
25	Spathulenol	1578	1.05
26	**Caryophyllene oxide**	1583	**13.29**
27	α-Cadinol	1654	2.81
28	Eremophilene	1500	0.69
29	β-Eudesmol	1648	0.85
30	Cadalene	1674	1.57
31	β-Bisabolol	1673	1.01
	Others		***2.25***
32	3-Octanol	993	0.11
33	2-Undecanone	1303	0.35
34	Eugenol	1356	1.80
	Total identified		***97.87***

*RI = retention index

Table 2: Larvicidal activity of *Dipsacus asper* essential oil against fourth-instar larvae of *Aedes aegypti* and *Culex pipiens pallens*

Mosquito	Treatment	LC_{50} (μg/ml) (95% CL)	LC_{95} (μg/ml) (95% CL)	Slope ± SD	Chi-square value (χ^2)
Aedes aegypti	*Dipsacus asper*	56.29 (51.56 - 60.63)	88.93 (81.34 - 97.21)	6.05 ± 0.57	9.51
	Rotenone	3.75 (3.33-4.25)	9.64 (8.69-10.66)	3.22 ± 0.33	18.20
Culex pipiens pallens	*Dipsacus asper*	47.59 (42.86 - 51.83)	106.08 (97.34 - 115.21)	5.45 ± 0.52	9.51
	Rotenone	1.88 (1.63-2.01)	3.98 (3.61-4.23)	6.87 ± 0.61	15.08

convergens branches 145.1 μg/mL) [23] and leaf essential oil of *Cryptomeria japonica* (LC_{50} = 56.8 μg/mL) [24]. However, the essential oil of *D. asper* was less toxic than essential oils of *Salvia plebeian* aerial parts (LC_{50} = 46.26 μg/mL) [25], *Isodon japonicus* var. *glaucocalyx* aerial parts (LC_{50} = 40.82 μg/mL) [27] and *Illicium difengpi* stem bark (LC_{50} = 31.68 μg/mL) [26]. Considering that the currently used larvicides are synthetic insecticides, larvicidal activity of the

essential oil of *D. asper* roots is quite promising and it shows its potential for use in the control of *A. aegypti* and *C. pipiens pallens* larvae and could be useful in the search for new, safer and more effective natural compounds as larvicides.

For the actual use of *D. asper* essential oil as a novel larvicide or insecticide to be realized, further research is needed to establish their human safety and environmental safety. In traditional Chinese medicine, the plants are used as a tonic and antiaging agent for the therapy of low back pain, traumatic hematoma and threatened abortion and bone fractures [12,13]. It appears to be safe for human consumption. However, no experimental data on toxicity of the essential oil to human is available, to the best of our knowledge. Additionally, their larvicide modes of action have to be established, and formulations for improving larvicidal potency and stability need to be developed. Furthermore, field evaluation and further investigation of the effects of the essential oil on non-target organisms are necessary.

CONCLUSION

The essential oil of *D. asper* roots demonstrates some activity against *Aedes aegypti* and *Culex pipiens pallens* mosquito larva but needs to be further evaluated for safety in humans and to enhance its activity.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. ZLL conceived and designed the study. HYL and XCL performed the experiments and QZL analyzed the data. The manuscript was written by ZLL. All authors read and approved the final version of the manuscript.

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