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Original Research Article

Chemical Composition of *Salvia plebeian* R.Br. Essential Oil and its Larvicidal Activity against *Aedes aegypti* L

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Abstract

Purpose: *To evaluate the larvicidal activity of the essential oil of Salvia plebeian R.Br. (Labiatae) aerial parts against the larvae of Aedes aegypti L.*
Methods: *The essential oil of S. plebeian aerial parts was obtained by hydro-distillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The essential oil was evaluated for larvicidal activity using World Health Organization (WHO) procedures, against the fourth larvae of A. aegypti within 24 h, and activity was recorded for various concentrations of the ranging from 12.5 – 200.0 µg/mL.*
Results: *A total of 33 components of the essential oil of S. plebeian were identified. The major compounds of the essential oil were caryophyllene oxide (15.54 %), γ-eudesmol (14.03 %), τ-cadinol (10.21 %), calamenene (9.63 %), copaene (5.70 %), γ-cadinene (5.30 %), cadalene (5.28 %), α-muurolene (5.19 %), ledol (5.14 %) and α-cadinol (5.08 %). The essential oil exhibited larvicidal activity against A. aegypti at a median lethal concentration (LC_{50}) of 46.26 µg/mL.*
Conclusion: *The findings indicate that the essential oil of S. plebeian aerial parts has potentials for use in the control of A. aegypti larvae and may be useful in the search for newer, safer and more effective natural compounds as larvicides.*

Keywords: *Salvia plebeian, Essential oil, Larvicidal activity, Aedes aegypti, Caryophyllene oxide, γ-Eudesmol, τ-Cadinol, Calamenene*

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INTRODUCTION

Dengue fever is transmitted by mosquitoes [1]. There are not yet any vaccines to prevent infection with dengue virus. Thus, the prevention of dengue fever is mainly dependent on control of the mosquitoes. Currently, synthetic insecticides and insect growth regulators are widely used to control larval mosquitoes. However the adverse effects of usage of synthetic larvicides have received wide public concern, like insecticide resistance, environmental pollution, toxic hazards to human and other organisms. Fortunately, natural products derived from plants including essential oils can provide an alternate to synthetic chemical insecticides [2]. Many essential oils and their constituents can exert toxic activity against mosquito species [3-10]. During the present author's mass screening program for new agrochemicals from wild plants, the essential oil of *Salvia plebeian* R.Br. (Family: Labiatae) aerial parts, was found to possess larvicidal activity against the yellow fever mosquito, *Aedes aegypti* L.

Sage weed (*S. plebeian*) is a biennial herb and distributed widely in China (in every province except Gansu, Qinghai, Xinjiang, and Tibet), Afghanistan, India, Indonesia, Japan, Korea, Malaysia, Myanmar, Russia, Thailand, Vietnam, and Australia [11]. This plant has been used in traditional medicines for the treatment of hepatitis, cough, inflammation, and haemorrhoids [11]. Previous phytochemical studies on *S. plebeia* reported that it contained flavonoids, diterpenoids, triterpenoids, lignans, and caffeic acid derivatives [12-17]. The chemical composition of the essential oil of *S. plebeia* has been determined in previous studies [18,19]. The essential oil of *S. plebeia* exhibited toxicity and repellency against pulse beetles *Callosobruchus chinensis* and *C. maculatus* [20]. However, a literature survey has shown that there is no report on larvicidal activity of *S. plebeian* 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 yellow fever mosquito.

EXPERIMENTAL

Plant collection and identification

Fresh aerial parts at flowering stage of S. plebeian (10 kg) were harvested in July 2013 from Changzhou city (31.59 ^{o}N, 119.18 ^{o}E), Jiangsu province, PR China. The herb was identified by Dr. Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (no. ENTCAU-Labiatae-Hamacao-10022) was deposited at the herbarium of Department of Entomology, China Agricultural University.

Extraction and isolation of essential oil

The samples was cut to small pieces 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 ^{o}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 held at 60 ^{o}C for 1 min and ramped at 10 ^{o}C min^{-1} to 180 ^{o}C where it was held for 1 min, and then ramped at 20 ^{o}C min^{-1} to 280 ^{o}C and kept there for 15 min. The injector temperature was maintained at 270 ^{o}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 obtained 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 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 [21]. 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* 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 dehydrated eggs were placed on a plastic tray containing tap water to hatch and yeast pellets served as food for the emerging larvae. The eggs batches, collected daily, were kept wet for 24 h and then placed in distilled water in the laboratory at 24 - 26 °C and natural summer photoperiod for hatching. The newly emerged larvae were then isolated in groups of ten specimens in 100 mL tubes with mineral water and a small amount of dog food. Larvae were daily controlled until they reached the fourth instar stage, when they were utilized for bioassay (within 12 h).

Larvicidal bioassay

Range-finding studies were run to determine the appropriate testing concentrations. Concentrations of 200, 100, 50, 25, and 12.5 µg/mL of essential oil were tested. The essential oil was first diluted 100:1 (v/v) with dimethyl sulfoxide (DMSO) as a stock solution and DMSO concentration in the final test solution was less than 0.05 %. The larval mortality bioassay was carried out according to the test method for larval susceptibility proposed by the World Health Organization (WHO) [22]. Twenty larvae were placed in glass beaker with 250 mL of aqueous suspension of tested material at various concentrations. Five replicates per concentration were run simultaneously and with each experiment, a set of controls using 0.05 % DMSO and untreated sets of larvae in tap water, were also run for comparison. For comparison, commercial chlorpyrifos (purchased from National Center of Pesticide Standards, Tiexi District, Shenyang 110021 China) was used as positive control.

The toxicity of chlorpyrifos was determined at concentrations of 5, 2.5, 1.25, 0.6, and 0.3 µg/mL. The assay was carried out in a growth chamber (Ningbo Jiangnan Instrument Factory, Ningbo 315012, China. http://www.nb-jn.com) (L16:D9, 26 – 27 ^{o}C, 78-80 % relative humidity). Mortality was recorded after 24 h of exposure.

Statistical analysis

Percent mortality was corrected for control mortality using Abbott's formula [23]. 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 determine LC_{50} values and their 95 % confidence intervals [24]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The yield of essential oil from 5 kg of fresh *S. plebeian* aerial parts at flowering stage was 2.5 mL while its density was determined to be 0.83 g/mL. A total of 33 components of the essential oil of *S. plebeian* aerial parts were identified (Table 1). The principal compounds of the essential oil were caryophyllene oxide (15.54 %), γ-eudesmol (14.03 %), τ-cadinol (10.21 %), calamenene (9.63 %), copaene (5.70 %), γ-cadinene (5.30 %), cadalene (5.28 %), α-muurolene (5.19 %), ledol (5.14 %) and α-cadinol (5.08 %). Sesquiterpenoids represented 23 of the 33 constituents, corresponding to 95.13 % of the essential oil of *S. plebeian* aerial parts while 8 of the 33 compounds were monoterpenoids, corresponding only to 2.25 % of the whole essential oil.

The essential oil exhibited larvicidal activity against *A. aegypti* with a median lethal concentration (LC_{50}) of 46.26 µg/mL.

DISCUSSION

The main constituents of *S. plebeian* essential oil were caryophyllene oxide, γ-eudesmol, τ-cadinol, calamenene, copaene, γ-cadinene, cadalene, α-muurolene, ledol and α-cadinol. Its chemical composition was quite different from that reported in the previous studies [18,19].

For example, the major compounds in the essential oil of *S. plebeian* aerial parts collected from Rizhao City, Shandong province, China were enanthate octanyl (19.46 %), dodecyl caproate (11.32 %), 2-methyl octyl butyrate (9.81 %), and cinnamic acid (6.30 %) [18]. However, the essential oil of *S. plebeian* aerial parts harvested from Chongzuo city, Guangxi Zhuang Nationality Autonomous Region, China mainly contained β-eudesmol (22.55 %), γ-eudesmol (10.91 %), (-)-calamenene (7.40 %), agarospirol (5.41 %) and β-cadinene (4.78 %) [19]. The above results suggest that there were some variations in chemical composition of essential oil of *S. plebeian* aerial parts collected from different sites and at different collect times. Thus, essential oil standardization is needed because chemical composition of essential oil varies greatly with plant population.

The essential oil of *S. plebeian* aerial parts possessed strong larvicidal activity against the 4th instar larvae of *A. aegypti*. The commercial insecticide, chlorpyrifos showed larvicidal activity against the mosquitoes with a LC_{50} value of 1.53 µg/mL, thus the essential oils of *S. plebeian* aerial parts was 30 times less toxic to *A. aegypti* larvae compared with chlorpyrifos. However, compared with the other essential oils/extracts in the literature, the essential oil of *S. plebeian* 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) [25]; essential oils from four Guarea species (*G. humaitensis* branches, LC_{50} = 48.6 µg/mL; *G. scabra* leaves, LC_{50} = 98.6 µg/mL; *G. silvatica* leaves, LC_{50} = 117.9 µg/mL and *G. convergens* branches 145.1 µg/mL) [26] and leaf essential oil

Table 1: Main compounds of the essential oil of aerial parts of *Salvia plebeian*

Peak	Compound	Retention index	(%)
	Monoterpenoids		***2.25***
1	β-Myrcene	991	0.11
2	1,8-Cineole	1031	0.18
3	*cis*-Linalool oxide	1075	0.19
4	Fenchone	1088	0.16
5	Linalool	1094	0.48
6	Camphor	1143	0.08
7	Borneol	1174	0.55
8	Bornyl acetate	1284	0.50
	Sesquiterpenoids		***95.13***
9	α-Cubebene	1345	2.88
10	Cyclosativene	1363	0.11
11	Ylangene	1370	0.24
12	**Copaene**	1378	**5.70**
13	β-Cubebene	1388	0.62
14	(Z)-Caryophyllene	1409	0.91
15	Caryophyllene	1420	0.88
16	Aromandendrene	1440	1.22
17	γ-Muurolene	1473	1.84
18	**α-Muurolene**	1502	**5.19**
19	**γ-Cadinene**	1512	**5.30**
20	**Calamenene**	1520	**9.63**
21	Cubenene	1532	0.88
22	α-Calacorene	1543	2.75
23	Elemol	1547	1.16
24	Germacrene B	1561	0.27
25	**Ledol**	1563	**5.14**
26	Spathulenol	1578	0.21
27	Caryophyllene oxide	1583	**15.54**
28	**γ-Eudesmol**	1631	**14.04**
29	**τ-Cadinol**	1642	**10.21**
30	**α-Cadinol**	1654	**5.08**
31	**Cadalene**	1675	**5.28**
	Others		***0.99***
32	Acetophenone	1066	0.21
33	Eugenol	1356	0.78
	Total identified		***98.37***

*RI = retention index

Table 2: Larvicidal activity of *Salvia plebeian* essential oil against fourth-instar larvae of *Aedes aegypti*

Treatment	LC_{50} (µg/mL) (95% CL)	LC_{95} (µg/mL) (95% CL)	Slope ± SD	Chi-square value (χ^2)
Salvia plebeian	46.26 (41.86 - 50.63)	131.56 (118.34 - 142.21)	5.01 ± 0.47	9.51
Chlorpyrifos	1.53 (1.36 - 1.75)	5.34 (4.78 - 5.88)	0.93 ± 0.04	4.24

of *Cryptomeria japonica* (LC_{50} = 56.8 µg/mL) [27].

Considering that the currently used larvicides are synthetic insecticides, larvicidal activity of the essential oil of *S. plebeian* aerial parts is quite promising and it shows its potential for use in the control of *A. aegypti* larvae and could be useful in the search for newer, safer and more effective natural compounds as larvicides.

For the actual use of *S. plebeian* 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 to treat hepatitis, cough, inflammation, and haemorrhoids [11] and appear to be safe for human consumption. However, no experimental data on its toxicity 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 *S. plebeian* demonstrates some activity against *Aedes aegypti* mosquito larva but needs to be further evaluated for safety in humans and to enhance its activity.

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