

## Original Research Article

# Determination of essential oil from *Scutellaria baicalensis* Georgi (Lamiaceae) by GC-MS, and assessment of its insecticidal properties

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Sent for review: 10 May 2018

Revised accepted: 26 May 2022

### Abstract

**Purpose:** To isolate essential oil from the aerial parts of *Scutellaria baicalensis* using GS-MS, and to assess its insect-killing effect against booklice (*Liposcelis bostrychophila*).

**Methods:** The oil from the aerial parts of *S. baicalensis* was hydro-distilled and subjected to gas chromatographic and mass spectroscopic (GC-MS) techniques. The components of the oil with insect-killing potentials were fractionated through bioactivity-guided isolation, and the isolated oil and its components were screened for their toxic effects on booklice.

**Results:** A total of 31 components were obtained, with eugenol as the most abundant. When used to fumigate *S. baicalensis*, the oil and two of its constituents (myristicin and eugenol) exerted appreciable toxic effects. Moreover, the oil and its isolates manifested contact toxicity against booklice.

**Conclusion:** These findings indicate the insecticidal benefits of the essential oil and its components against *Liposcelis bostrychophila*.

**Keywords:** *S. baicalensis*, Essential oil, Booklice, Toxicity, *Liposcelis bostrychophila*

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## INTRODUCTION

The Chinese Baikal skullcap (*S. baicalensis*) is a perennial herbaceous plant that reaches up to 120 cm in height. The plant grows in many provinces in China, and is also found in Siberia, Japan and Korea [1]. In China, *S. baicalensis* is used as a medicinal herb for treating hematological and cardiovascular ailments [2]. Studies have demonstrated that *S. baicalensis* which is rich in essential oil, flavonoids and terpenes, exerts antioxidant and anti-

carcinogenic properties [3]. Several investigations have been carried out on the analysis of essential oil from *S. baicalensis* [4 - 6]. However, not much is known about the possible insecticidal property of essential oil from this medicinal plant. The aim of this study was to investigate the components of the essential oil isolated from the aerial portion of *S. baicalensis*, evaluate the insect-killing potential of the oil against *Liposcelis bostrychophila*, and use the technique of GC-MS to identify its bioactive composition.

## EXPERIMENTAL

### Plant sample and oil extraction

A sample of the aerial portions of the plant containing the flowers was harvested from a garden in Tangshan City in September, 2017. Identification of the sample was carried out by a competent taxonomist, and a voucher specimen (no. 001-tssf-01657) was kept for future reference at herbarium of Tangshan Normal University, China. Following slicing into smaller sections, the sample was hydro-distilled for about six hours using a modification of the Clevenger-like appliance. The resultant distillate was subjected to oil extraction using *n*-hexane, and the oil was dehydrated with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Its density was determined, after which it was kept refrigerated at 4 °C in sealed vials prior to use.

### Analysis of the essential oil from the aerial parts of *S. baicalensis*

A Hewlett–Packard 5800 series was used for GC analysis of the oil. The equipment was fitted with a 30 m × 0.25 mm capillary column of film thickness 0.25 μm, and the GC was carried at an initial temperature of 60 ° for 10 min, which was ramped up to 280 °C at 2 °C/min. The injector and detector temperatures were of 270 °C and 300 °C, respectively. Nitrogen served as carrier gas (flow rate, 1 mL/min). Dual FID detector was used at split ratio of 1:30. The sample injection volume was 0.5 μL. The oil was also subjected to GC-MS analysis using an Agilent 6890N gas chromatograph linked to an Agilent 5973N mass selective detector. A 30 m × 0.25 mm × 0.25 μm HP-5MS capillary column was used, at ionization energy of 70 eV. The carrier gas was helium (flow rate, 1.0 mL/min). The temperature of the oven was increased from 60 to 180 °C at ramp rate of 10 °C/min, maintained at 180 °C for 1 min, and thereafter increased 280 °C at the rate of 20 °C/min. It was maintained at 280 °C for 15 min. Injector temperature was kept at 270 °C. Sample injection was with a split ratio of 1:10, and the scan covered the range of 20 to 550 m/z at 2 scans/s. Most of the components of the essential oil were identified by comparison of retention times with those of standards, or published data. Retention indices were determined using retention times of *n*-alkanes (C<sub>8</sub>–C<sub>24</sub>) under the same chromatographic conditions. In addition, the NIST 05 and Wiley 275 library data of the peaks were compared with published data [7]. The relative amounts of the

components were obtained based on peak areas without correction factors.

### Purification and characterization of 4 components of the essential oil

The purification was carried out using SiO<sub>2</sub> column chromatography. Twelve fractions resulted from gradient elution with *n*-hexane: ethyl acetate (100:0 - 0:100, v/v). Fractions 4, 6, 8 and 9 were selected for preparative silica gel column chromatography (PTLC) on the basis of contact bioassay results. The PTLC produced eugenol, caryophyllene, myristin and caryophyllene oxide. Bruker Avance DRX 500 equipment was used for recording their <sup>1</sup>H and <sup>13</sup>C NMR spectra in CDCl<sub>3</sub> (solvent). The internal standard was TMS.

### Booklice sample and culture

Booklice (*L. bostrychophila*) colony was provided by the Academy of State Administration of Grain, Beijing, China. They were maintained on an artificial diet of whole meal wheat flour, milk powder, and brewer's yeast (compounded at a ratio of 5:1:1) at 28 - 30 °C and relative humidity of 70 - 80 %. The adult insects were used at 1 week of age.

### Determination of contact toxicity

Contact toxicity against the booklice was measured using a slight modification of the procedure of Zhao *et al* [8,9].

### Evaluation of fumigant toxicity

The fumigant toxicity of the essential oil/constituents against the booklice was determined as described by Zhao *et al* [8,9].

### Statistical analysis

The LC<sub>50</sub> values of the essential oil/compounds and their 95 % confidence intervals were estimated using PriProbit Program V1.6.3 [10]. Samples for which the 95 % fiducial limits did not overlap were regarded as significantly different.

## RESULTS

Table 1 shows that the essential oil level of the aerial parts of *S. baicalensis* was 0.09 % (v/w, based on fresh weight) while its density was 0.93 g/mL. A total of 31 components were identified, accounting for 97.64 % of the crude essential oil.

**Table 1:** Chemical composition (%) of the essential oil of *Scutellaria baicalensis*

Compound	Molecular formula	Retention time (min)	Retention index	Percent area
<b>Monoterpenoids</b>				<b>20.08</b>
<i>α</i> -Pinene	C <sub>10</sub> H <sub>16</sub>	4.17	939	1.12
<i>β</i> -Pinene	C <sub>10</sub> H <sub>16</sub>	4.82	974	4.11
Limonene	C <sub>10</sub> H <sub>16</sub>	5.31	1029	1.32
<i>β</i> -Phellandrene	C <sub>10</sub> H <sub>16</sub>	5.39	1030	1.79
1,8-Cineole	C <sub>10</sub> H <sub>18</sub> O	5.49	1032	0.67
( <i>Z</i> )- <i>β</i> -Ocimene	C <sub>10</sub> H <sub>16</sub>	5.66	1048	0.89
<i>γ</i> -Terpinene	C <sub>10</sub> H <sub>16</sub>	5.87	1060	0.92
Fenchone	C <sub>10</sub> H <sub>16</sub> O	6.08	1088	1.72
Linalool	C <sub>10</sub> H <sub>18</sub> O	6.24	1094	3.45
Camphor	C <sub>10</sub> H <sub>16</sub> O	6.83	1146	0.89
Borneol	C <sub>10</sub> H <sub>18</sub> O	7.13	1160	0.22
4-Terpineol	C <sub>10</sub> H <sub>18</sub> O	7.23	1175	0.77
<i>α</i> -Terpineol	C <sub>10</sub> H <sub>18</sub> O	7.36	1189	2.21
<b>Sesquiterpenoids</b>				<b>48.43</b>
Copaene	C <sub>15</sub> H <sub>24</sub>	9.37	1375	0.68
<i>β</i> -Elemene	C <sub>15</sub> H <sub>24</sub>	9.67	1391	2.19
<i>α</i> -Cedrene	C <sub>10</sub> H <sub>24</sub>	9.87	1409	1.43
<b>Caryophyllene</b>	C <sub>10</sub> H <sub>24</sub>	9.98	1420	<b>15.17</b>
<i>α</i> -Caryophyllene	C <sub>10</sub> H <sub>24</sub>	10.37	1454	1.37
<b>Germacrene D</b>	C <sub>10</sub> H <sub>24</sub>	10.59	1485	<b>5.44</b>
Valencene	C <sub>10</sub> H <sub>24</sub>	10.87	1493	1.26
Nerolidol	C <sub>15</sub> H <sub>26</sub> O	11.35	1564	0.64
Spathulenol	C <sub>15</sub> H <sub>24</sub> O	11.44	1572	4.24
<b>Caryophyllene oxide</b>	C <sub>15</sub> H <sub>24</sub> O	12.47	1583	<b>13.89</b>
<i>β</i> -Eudesmol	C <sub>15</sub> H <sub>26</sub> O	13.25	1649	1.27
Alloaromadendrene oxide	C <sub>15</sub> H <sub>24</sub> O	14.16	1681	3.39
<b>Phenylpropanoids</b>				<b>24.13</b>
<b>Eugenol</b>	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	9.04	1356	<b>18.39</b>
Methyleugenol	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	9.79	1403	2.16
( <i>E</i> )-Methylisoeugenol	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	10.92	1500	1.03
Myristicin	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>	11.09	1513	4.71
<b>Others</b>				<b>0.30</b>
Phenethyl alcohol	C <sub>8</sub> H <sub>10</sub> O	6.43	1116	0.16
<i>β</i> -Ionone	C <sub>13</sub> H <sub>20</sub> O	10.80	1485	0.14
<b>Total identified</b>				<b>97.64</b>

\*RI = retention factor

Table 1 shows that 31 compounds were identified from the oil of *Scutellaria baicalensis*. The components are shown in Table 1. Sesquiterpenoids occurred in 13 out of the 31 constituents, monoterpenoids were in 13 of the 31 constituents, while phenylpropanoids accounted for only 3 of the 31 compounds.

### Spectral characteristics

**Caryophyllene.** Colorless oil, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz)  $\delta$  (ppm): 0.99 (1H, *br. s.*, H-7) ; 1.01 (3H, *s.*, H-14), 1.03 (3H, *s.*, H-13), 1.23 (3H, *s.*, H-15), 1.36 - 1.39 (1H, *m.*, H-10), 1.44 (1H, *d.*, *J* = 2.8 Hz, H-6), 1.62 (1H, *br. s.*, H-3), 1.65 - 1.67 (1H, *m.*, H-6), 1.69 (1H, *br. s.*, H-3), 1.72-1.74 (1H, *m.*, H-5), 2.09-2.15 (2H, *m.*, H-7, H-11), 2.28 (1H, *dd.*, *J* = 3.6 and 8.4 Hz, H-10), 2.33-2.37 (1H, *m.*, H-11), 2.65 (1H, *d.*, *J* = 9.1 Hz, H-2), 2.90 (1H,

*dd.*, *J* = 4.1 and 10.7 Hz, H-9), 5.01 (1H, *s.*, H-12), 4.88 (1H, *s.*, H-12); <sup>13</sup>CNMR (CDCl<sub>3</sub>, 125MHz):  $\delta$  (ppm): 17.1 (C-15), 21.6 (C-13), 27.2 (C-6), 29.8 (C-11), 29.9 (C-14), 30.2 (C-10), 34.1 (C-4), 39.0 (C-7), 39.8 (C-3), 48.9 (C-2), 50.6 (C-5), 59.9 (C-8), 63.7 (C-9), 112.8 (C-12), 151.8 (C-1). The spectral data were consistent with the previous report [11].

**Caryophyllene oxide.** Colorless oil. <sup>1</sup>HNMR (CDCl<sub>3</sub>, 500MHz)  $\delta$  (ppm): 0.99 (1H, *br. s.*, H-7). 1.01 (3H, *s.*, H-14), 1.03 (3H, *s.*, H-13), 1.23 (3H, *s.*, H-15), 1.36-1.39 (1H, *m.*, H-10), 1.44 (1H, *d.*, *J* = 2.8 Hz, H-6), 1.62 (1H, *br. s.*, H-3), 1.65-1.67 (1H, *m.*, H-6), 1.69 (1H, *br. s.*, H-3), 1.72-1.74 (1H, *m.*, H-5), 2.09-2.15 (2H, *m.*, H-7, H-11), 2.28 (1H, *dd.*, *J* = 3.6 and 8.4 Hz, H-10), 2.33-2.37 (1H, *m.*, H-11), 2.64 (1H, *d.*, *J* = 9.1 Hz, H-2), 2.90 (1H, *dd.*, *J* = 4.1 and 10.7 Hz, H-9), 4.88 (1H, *s.*,

H-12), 5.00 (1H, s, H-12). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125MHz) δ: 17.0 (C-15), 21.5 (C-13), 27.1 (C-6), 29.8 (C-11), 29.9 (C-14), 30.1 (C-10), 33.9 (C-4), 39.2 (C-7), 39.8 (C-3), 48.9 (C-2), 50.7 (C-5), 59.9 (C-8), 63.7 (C-9), 112.8 (C-12), 151.7 (C-1). The spectra data were consistent with those reported earlier [12].

**Eugenol.** Colorless oil, <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500MHz) δ (ppm): 3.30 (2H, dt, J = 6.6, 1.5 Hz, H-7), 3.81 (3H, s, -OCH<sub>3</sub>), 5.06 (2H, m, H-9), 5.73 (1H, br.s, D<sub>2</sub>O exchangeable, -OH), 5.94 (1H, m, H-8), 6.65 (1H, d, J = 1.8 Hz, H-2), 6.66 (1H, dd, J = 8.4, 1.8 Hz, H-6), 6.83 (1H, d, J = 8.8 Hz, H-5). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 39.9 (C-7), 55.8 (C-10), 111.3 (C-9), 114.5 (C-2), 115.5 (C-5), 131.9 (C-1), 137.8 (C-8), 144.0 (C-4), 146.6 (C-3). The spectra data were consistent with those reported earlier [13].

**Myristicin.** Colorless oil, <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz): δ (ppm): 3.23 (2H, d, J = 6.7 Hz, H-3), 3.80 (3H, s, -OMe), 4.99 (1H, dd, J = 1.7, 8.0 Hz, H-1), 5.01 (1H, dd, J = 1.7, 17.1 Hz, H-1), 5.84 (1H, m, H-2), 5.86 (2H, s, O-CH<sub>2</sub>-O), 6.28 (1H, d, J = 1.4 Hz, H-2'), 6.33 (1H, d, J = 1.4 Hz, H-6'). <sup>13</sup>C-NMR (125Hz, CDCl<sub>3</sub>) δ (ppm): 33.8 (C-3), 51.1 (5'-OMe), 101.2 (O-CH<sub>2</sub>-O), 102.7 (C-2'), 104.8 (C-6'), 115.6 (C-1), 126.0 (C-1'), 137.3 (C-2), 137.7 (C-5'), 144.2 (C-3'), 144.6 (C-4'). These

spectra data were consistent with those reported earlier [14]. As shown in Table 2, the LC<sub>50</sub> of the anti-booklice contact toxicity of essential oil was 141.37 ug/cm<sup>2</sup>. Four components of the oil: myristate, caryophyllene, eugenol and caryophyllene oxide showed acute toxicity against the insect, with LC<sub>50</sub> values of 290.34 (caryophyllene), 104.32 (caryophyllene oxide), 85.75 (eugenol) and 21.13 (myristate) ug/cm<sup>2</sup>, respectively. The fumigant toxicity of the essential oil against the booklice was 607.35 ug/L. The LC<sub>50</sub> value of eugenol was 133.43 ug/L, and LC<sub>50</sub> of myristicin was 115.24 ug/L. Fumigant toxicity was not produced by caryophyllene and caryophyllene. These results are shown in Table 2.

## DISCUSSION

The major components of the essential oil from the aerial parts of *S. baicalensis* were myristicin, eugenol, caryophyllene, caryophyllene oxide, germacrene D, spathulenol, and β-pinene. These are different from the components identified in previous reports [4 - 6, 15]. For example, acetophenone, germacrene D, caryophyllene and allyl alcohol were the major components of the essential oil from the aerial parts of *S. baicalensis* grown in Qinling Mountains [15].

**Table 2:** Fumigant and contact toxicities of essential oil from *Scutellaria baicalensis* and its main components against adult booklice

Toxicity	Treatment	LC <sub>50</sub> (95 % FL*)	Slope ± SD	Chi-square (χ <sup>2</sup> )
Contact (μg/cm <sup>2</sup> )	Essential oil	141.37 (129.46-152.73)	7.79 ± 0.77	11.27
	Pyrethrum extract	20.49 (18.86-22.12)	5.88 ± 0.55	10.89
	Myristicin	21.13 (19.54-23.54)	7.18 ± 0.64	8.56
	Eugenol	85.79 (78.34-92.48)	7.51 ± 0.72	9.08
	Caryophyllene oxide	104.32 (95.24-112.43)	6.44 ± 0.63	12.27
	Caryophyllene	290.34 (254.45-292.14)	6.47 ± 0.63	10.54
Fumigant (μg/L)	Essential oil	607.35 (557.64-655.27)	6.26 ± 0.59	10.89
	Dichlorvos	1.63 (1.59-1.71)	6.21 ± 0.61	6.77
	Myristicin	115.24 (107.09-128.16)	5.79 ± 0.55	10.34
	Eugenol	133.43 (121.34-144.76)	6.43 ± 0.62	9.47
	Caryophyllene oxide Caryophyllene	> 10,000 > 10,000	- -	- -

However, essential oil obtained from the stem of *S. baicalensis* had mainly diphenylamine, 2,2-methylenebis(6-tert-butyl-4-methylphenol)], bornyl acetate,  $\beta$ -caryophyllene, germacrene D and 1-octen-3-ol [4]. In contrast, the root oils from *S. baicalensis* roots grown in Japan contained palmitic acid, acetophenone, (*E*)-4-phenyl-3-buten-2-one, oleic acid and 1-phenyl-1,3-butanedione [5]. Thus, the components of the essential oils of *S. baicalensis* vary as a function of the plant part employed, geographical location and growth conditions. Consequently, prior to their application for industrial production of oils, the plants should be standardized.

The acute toxicity of the essential oil from *S. baicalensis* against the booklice was much higher than those reported for other essential oils [14-16]. The contact toxicities of eugenol, caryophyllene oxide and myristicin against the booklice were superior to that of the unfractionated oil, with myristicin producing toxicity level comparable with that of the pyrethrum extract. These results suggest that the contact toxicity produced by the oil was due to its contents of eugenol, myristicin and caryophyllene oxide. The essential oil was 373 times less toxic against the booklice than dichlorvos. However, comparison with published fumigant toxicity data for other essential oils using the same assay revealed that they were less toxic against the booklice than the essential oil from the aerial parts of *S. baicalensis* [17-19]. Moreover, the oil was less toxic than the essential oils from *A. chinense* [20]. Earlier investigations have reported strong fumigant effects of myristicin against a variety of insects [21 - 24]. Thus, since the available commercial fumigants (e.g. phosphine) are extremely toxic to man, the fumigant activities of the essential oil from *S. baicalensis* may provide safer and more natural alternatives.

## CONCLUSION

The results obtained in this study have demonstrated strong anti-booklice contact and fumigant toxicities of the essential oil isolated from the aerial parts of *S. baicalensis*. Thus, the oil and its components may be developed as insect-control agents for application on stored grains. However, further studies are needed to optimize their activities, and also to assess their safety to humans.

## DECLARATIONS

### Acknowledgement

This work was funded by Tangshan Science and Technology Bureau, Hebei Province, China

(Grant no. 13130246z); and Hebei Provincial Department of Human Resources and Social Security, Hebei High-level Talent Funding Project, Study on Green Control Technology of Chinese Herbal Medicine Extract on Wheat Major Pests (Grant no.201803042). We thank Dr. Ling-Ling Shi from Beijing Forestry University, China, for the identification of the investigated plant.

### 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.

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