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CONSTITUENT COMPOSITION AND BIOLOGICAL ACTIVITY OF CO₂-EXTRACTS OF *Scabiosa isetensis* AND *S. ochroleuca*

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Scabiosa isetensis L. (Dipsacaceae) is a low (up to 45 cm) semi-bush that grows solitary or in loose groups among rocky steppe communities in the southeastern part of European Russia, Caucasus, southern Western Siberia, and northern Central Asia [1]. The chemistry of the plant is practically unstudied. Extracts of *S. isetensis* possessed high antifungal activity [2].

S. ochroleuca L. is a biennial or perennial bush (30–80 cm) indigenous to European Russia, Caucasus, Central Asia, Western and Eastern Siberia, southern Western Europe, Western China, and Mongolia [3].

Previously, glycosides of the flavonoids luteolin and quercetin [4–6], phenolic acids [6–8], triterpene glycosides [9], and the flavonoids luteolin, apigenin, quercetin, and kaempferol [2] were isolated from the MeOH extract of *S. ochroleuca*.

Herein, the composition, cytotoxicity, and antiradical activity of CO₂-extracts of *S. isetensis* and *S. ochroleuca* growing in Kazakhstan that were purified of waxes by treatment with EtOH are reported for the first time.

Aerial parts of *S. isetensis* and *S. ochroleuca* were collected in Karaganda Oblast, Republic of Kazakhstan, in the last third of July 2016 during full flowering in the vicinity of Kernei village, Bukhar-Zhyrau District (*S. ochroleuca*), and in the Ulytau foothills (*S. isetensis*).

Herbarium specimens are preserved in the herbarium of the Biology-Geography Faculty, Buketov Karaganda State University under codes 2013.07.20.06.04 (collection in Buiratau Mountains) for *S. isetensis* and 2010.06.11.01.14 (collection in Karkaraly Mountains) for *S. ochroleuca*.

CO₂-extracts were obtained at TOO Fitoaromat on a UUPE 51 apparatus from air-dried ground aerial plant parts at 69–76 atm and 18–21°C for 16–18 h [10]. The yield was 0.57% for *S. isetensis*; 0.85%, *S. ochroleuca*. Then, the extracts were treated with 10 times the volume of EtOH, cooled to –20°C, and filtered to remove waxy nonpolar precipitates. The filtrate was analyzed on a Clarus-SQ 8 gas chromatograph with a mass-spectrometric detector using a Restek Rxi[®]-1 ms capillary column (0.25 mm × 30 m × 0.25 μm), sample volume 1.0 μL, He carrier gas at 1 mL/min, flow division 1:25, column temperature 45°C (2 min) increasing at 1.5°C/min to 200°C and then at 15°C/min to 280°C where it was held for 10 min, vaporizer temperature 280°C, mass spectrometric detector at 240°C with EI+ = 70 eV and scan time 4–120 min over mass range *m/z* 39–500. The percent contents of constituents were calculated automatically using peak areas of the total-ion chromatogram. Constituents were identified using mass spectra and retention times and the NIST library. Constituent retention times were recalculated relative to saturated hydrocarbons.

Table 1 shows that the main volatile constituents of the CO₂-extract of *S. isetensis* included 1,8-cineole (29.1%), α-santonin (10.6), α-thujone (9.8), β-thujone (5.0), and an unidentified constituent that was presumably a steroid (16.4). The main constituents of the CO₂-extract of *S. ochroleuca* were α-santonin (21.8%), 1,8-cineole (14.9), *n*-hexadecanoic acid (5.6), campesterol (5.0), α-thujone (4.8), and a constituent that was presumably a steroid (7.3).

The cytotoxicities of the CO₂-extracts were determined by the literature method [11]. Three parallel tests were conducted with 20–40 larvae in each.

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TABLE 1. Constituent Composition of CO₂-Extracts of *S. isetensis* and *S. ochroleuca*, %

Constituent	RI	RI lit.	<i>S. isetensis</i>	<i>S. ochroleuca</i>
<i>o</i> -Cymene	1016	1022	1.6	1.0
1,8-Cineole	1024	1032 ± 2	29.1	14.9
<i>trans</i> -Sabinene hydrate	1063	1070 ± 5	0.5	–
<i>cis</i> -Sabinene hydrate	1095	1070 ± 8	0.5	0.4
α-Thujone	1100	1103 ± 2	9.8	4.8
β-Thujone	1111	1114 ± 2	5.0	2.5
Camphor	1135	1145 ± 2	0.4	–
Isothujol	1159	1168 ± 3	0.4	–
Terpinen-4-ol	1169	1177 ± 2	0.9	0.6
4-Terpenylacetate	1206	1301 ± 19	0.5	0.7
Isothujyl acetate	1284	1298 ± 7	0.9	0.7
3-Methyl-6-(1-methylethyl)-2-cyclohexen-1-ol acetate	1289	1303 ± N/A	0.5	–
α-Terpenylacetate	1340	1350 ± 3	0.8	0.7
<i>n</i>-Hexadecanoic acid	1969	1968 ± 7	–	5.6
Hexadecanoic acid ethyl ester	1992	1993 ± 3	1.2	–
Unident. 1	2088		0.4	1.5
Phytol	2105	2114 ± 5	0.8	1.9
(<i>Z,Z,Z</i>)-9,12,15-Octadecanoic acid	2136	2139 ± 20	–	2.9
Unident. 2	2147		–	1.4
Linoleic acid ethyl ester	2154	2162 ± 6	1.1	1.7
α-Santonin	2162	2117 ± N/A	10.6	21.8
Octadecanoic acid ethyl ester	2192	2195 ± 2	–	0.6
Unident. 3	2372		16.4	7.3
Unident. 5	2469		1.2	0.4
Unident. 6	2476		1.5	–
Dioctylphthalate	2532	2543 ± 3	0.9	0.8
Squalene	2809	2832 ± 13	1.6	1.5
α-Tocopherol	3113	3150 ± N/A	1.6	2.8
Campesterol	3222	3131 ± N/A	1.8	5.0
Unident. 7	3253		2.0	4.5

The experimental results found that the CO₂-extract of *S. isetensis* at concentrations of 10 and 5 mg/mL exhibited cytotoxicity. The lethality for larvae was 78–88%. It was nontoxic at a concentration of 1 mg/mL.

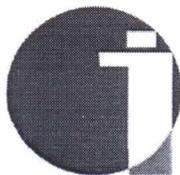
The CO₂-extract of *S. ochroleuca* at concentrations of 5 and 1 mg/mL did not exhibit cytotoxicity although it was cytotoxic at a concentration of 10 mg/mL. The lethality for larvae was 54%. The antiradical activity of the CO₂-extracts was established using the literature method [12, 13]. The experimental results showed that CO₂-extracts of *S. isetensis* and *S. ochroleuca* had low antiradical activity as compared with the reference drug butylhydroxyanisole.

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