SUPPLEMENTARY MATERIAL

Composition of the essential oils of three Uzbek *Scutellaria* species (Lamiaceae) and their antioxidant activities

Nilufar Zokirjonovna Mamadalieva^{*a}, Farukh Sharopov^b, Prabodh Satyal^c, Shahnoz Sadikovna Azimova^a, and Michael Wink^b

^aInstitute of the Chemistry of Plant Substances, Mirzo Ulugbek Str 77, Tashkent 100170, Uzbekistan

^bHeidelberg University, Institute of Pharmacy and Molecular Biotechnology, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

^cDepartment of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

The chemical composition of the essential oils obtained from aerial parts of *Scutellaria immaculata* Nevski ex Juz., *S. ramosissima* M. Pop. and *S. schachristanica* Juz. (Lamiaceae) growing wild in Uzbekistan, were analyzed by GC and GC-MS. The main constituent of the essential oils from *S. immaculata* were acetophenone (30.39%), eugenol (20.61%), thymol (10.04%) and linalool (6.92%), whereas that of *S. schachristanica* produced acetophenone (34.74%), linalool (26.98%), eugenol (20.67%). The *S. ramosissima* oil is dominated by germacrene D (23.96%), β -caryophyllene (11.09%), linalool (9.63%) and hexadecanoic acid (8.34%). The essential oils of *Scutellaria* species exhibited weaker antioxidant effects in DPPH, ABTS and FRAP assays. In FRAP assay, only eugenol exhibited a substantial reducing power $IC_{50}=2476.92\pm15.8$ (mM Fe (II)/g).

Keywords: Flora of Uzbekistan, *Scutellaria*, GC-MS, essential oils, antioxidant activity

Experimental

Plant material. The aerial parts (stems, leaves, flowers) of *S. immaculata*, *S. ramosissima* and *S. schachristanica* employed in this investigation were collected in the flowering stage (May-July 2014) from Namangan, Tashkent and Jizzakh regions of Uzbekistan, respectively and dried in shadow. The authenticated voucher specimens were kept in the Department of Herbal Plants (Institute of the Chemistry of Plant Substances, Uzbekistan) [*S. immaculata* (accession no. 20088045), *S. ramosissima* (accession no. 20088052) and *S. schachristanica* (accession no. 20141424)].

Isolation of essential oils. The air-dried aerial parts (moisture content was 10-12% w.b.) of the *S. immaculata*, *S. ramosissima* and *S. schachristanica* (200 g each) were hydrodistilled for 2 h, using a Clevenger-type apparatus (yields of 0.2%, 0.12% and 0.09%, respectively). As only small amounts of essential oil were present, the oils were trapped in dichloromethane, which were dried over anhydrous sodium sulphate and left over night in a hood to remove the dichloromethane solvent till the weights were fixed stored at -4°C until use.

Gas chromatography – mass spectrometry (GC-MS) analysis. The essential oils were analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the EI mode [(electron energy = 70eV), scan range = 3.0 scans/sec], and GC-MS solution software. GC-MS analyses were performed on ZB-5 (Phenomenex, USA) fused silica capillary columns (30 m × 0.32 mm (i.d.), film thickness: 0.25 µm). The carrier gas was helium with a column head pressure 80 psi and flow rate of 1.37 ml/min. Injector temperature was 250°C and the ion source temperature was 200°C, increase in temperature rate 2°C/min to 260°C. The GC oven temperature program was programmed for 50°C initial temperature, increase in the rate 2°C/min to 260°C. A 5% w/v solution of the sample in CH₂Cl₂ was prepared and 0.1 µL was injected in splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes (Kovats RI), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams 2007) and stored in the MS library (NIST 11, WILEY 10, FFNSC version 1.2). The values provided are averages from three analyses. Relative standard deviations from these replicate injections were all small (less than 2%).

Antioxidant activity. The antioxidant activity of the essential oils was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) and ferric reducing antioxidant power (FRAP) assays. DPPH, ABTS and FRAP assays were analyzed as described earlier by us (Mamadalieva et al. 2011; Sharopov et al. 2015). All

experiments were carried out three times unless mentioned in the procedure. Continuous variables were presented as mean $\pm$ SD. The IC₅₀ was determined as the drug concentration that resulted in a 50% reduction or inhibition of the biological activity. Statistical significance was assessed using a one way analysis of variance (ANOVA) followed by a multiple comparison test (Tukey's post hoc test), where $p < 0.05$ was considered significant.

References

- Adams RP. 2007. Identification of Essential Oil Components by Gas Chromatography /Mass Spectrometry. 4th edn., Allured Publishing Corporation, Carol Stream, IL, p. 804.
- Mamadalieva NZ, Herrmann F, El-Readi MZ, Tahrani A, Hamoud R, Egamberdieva D, Azimova SS, Wink M. 2011. Flavonoids in *Scutellaria immaculata* and *S. ramosissima* and their biological activities. J Pharm Pharmacol. 63:1346–1357.
- Sharopov F S, Wink M, Setzer WN. 2015. Radical scavenging and antioxidant activities of essential oil components-an experimental and computational investigation. Nat Prod Comm. 10: 153–156.

Table S1. Qualitative and quantitative composition (% v/v) of volatile compounds from Uzbek *Scutellaria* species

Compound	KI ^a	KI ^b	Composition, %			Method of identification
			<i>S. imma- culata</i>	<i>S. ramosi- ssima</i>	<i>S. schachri- stanica</i>	
Prenol	774	765	--	--	0.05	MS, RI
Hexanal	798	797	--	0.39	--	MS, RI, AU
3-Methylcyclo pentanone	837	832	--	--	0.13	MS, RI
Pent-3-en-1-ol	845	849	--	--	0.17	MS, RI
E-Hexen-1-ol	860	863	0.26	--	--	MS, RI
2-Heptanone	887	889	0.11	--	0.07	MS, RI
Styrene	892	893	0.23	--	--	MS, RI
Isobutyl isobutyrate	912	908	--	--	0.07	MS, RI
Benzaldehyde	960	952	2.49	--	0.13	MS, RI, AU
1-Octen-3-one	975	972	1.02	--	--	MS, RI, AU
1-Octen-3-ol	978	972	2.89	2.08	3.73	MS, RI, AU
Octan-3-one	983	979	0.65	--	0.55	MS, RI, AU
Acetylcyclohexane	985		--	--	0.23	MS, RI
3-Octanol	996	994	0.43	--	0.27	MS, RI, AU
1-Acetylcyclohexene	1025	1023	--	--	0.25	MS, RI
Limonene	1028	1024	--	1.14	--	MS, RI, AU
1,8-Cineole	1032	1026	2.25	--	--	MS, RI, AU
(Z)- β -Ocimene	1038	1032	--	0.47	--	MS, RI, AU
Benzeneacetaldehyde	1042	1036	0.21	--	--	MS, RI
Acetophenone	1058	1059	30.39	4.67	34.74	MS, RI
Octanol	1069	1063	--	--	1.56	MS, RI, AU
o-Guaiacol	1083	1087	--	--	1.19	MS, RI
Linalool oxide (Furanoid)	1086	1084	--	--	0.05	MS, RI

Linalool	1099	1095	6.92	9.63	26.98	MS, RI, AU
Phenylethyl alcohol	1111	1106	1.99	--	0.55	MS, RI
β -Cyclocitral	1123	1222	--	0.25	--	MS, RI
Camphor	1147	1141	0.76	--	--	MS, RI, AU
2-(1Z)-Propenylphenol	1152		--	--	0.09	MS, RI
n-Nonanol	1171	1165	0.37	--	--	MS, RI
Borneol	1172	1165	0.74	--	--	MS, RI, AU
Isopinocampnone	1176	1172	0.40	--	--	MS, RI
Terpinen-4-ol	1180	1177	--	0.06	--	MS, RI, AU
2-Methoxy- <i>p</i> -cresol	1186	1188	0.29	--	1.89	MS, RI
α -Terpineol	1189	1182	--	1.39	--	MS, RI, AU
γ -Terpineol	1194	1199	1.05	--	3.57	MS, RI, AU
Decanal	1206	1201	--	0.70	--	MS, RI
cis-Piperitone epoxide	1249	1257	--	--	0.12	MS, RI
<i>p</i> -Anisaldehyde	1254	1247	--	--	0.05	MS, RI, AU
Piperitone	1254	1249	--	--	0.10	MS, RI, AU
Thymol	1289	1289	10.04	3.01	0.30	MS, RI, AU
Carvacrol	1297	1298	1.62	--	--	MS, RI, AU
4-Vinyl guaiacol	1308	1315	2.50	2.42	1.64	MS, RI
Eugenol	1350	1356	20.61	5.29	20.67	MS, RI, AU
α -Cubebene	1352	1345	--	0.46	--	MS, RI
trans-Benzalacetone	1356	1330	1.91	--	--	MS, RI
Nonalactone	1358	1358	0.15	--	--	MS, RI
β -Bourbonene	1386	1387	--	1.08	--	MS, RI
Vanillin	1392	1393	0.19	--	--	MS, RI, AU
α -Caryophyllene	1403	1408	1.46	--	--	MS, RI
β -Caryophyllene	1416	1417	1.56	11.09	0.10	MS, RI, AU
Aromadendrene	1438	1439	--	0.80	--	MS, RI, AU
Geranyl acetone	1446	1453	0.19	--	0.04	MS, RI
α -Humulene	1455	1452	0.38	2.03	--	MS, RI, AU

Apocynin (acetovanillone)	1478	1480	0.38	--	--	MS, RI
Germacrene D	1478	1484	--	23.96	--	MS, RI, AU
Ar-Curcumene	1480	1483	0.35	--	--	MS, RI
α -Muurolene	1500	1500	--	0.58	--	MS, RI
γ -Cadinene	1515	1522	--	1.96	--	MS, RI
δ -Cadinene	1526	1537	--	1.53	--	MS, RI
Spathulenol	1580	1577	0.46	3.17	--	MS, RI, AU
Caryophyllene oxide	1581	1582	1.95	5.90	0.44	MS, RI, AU
Humulene epoxide II	1608	1608	0.30	--	--	MS, RI
10-epi- γ - Eudesmol	1621	1622	0.11	--	--	MS, RI
α -Cadinol	1654	1652	0.50	1.94	--	MS, RI
Phytone	1840	1838	0.81	--	0.22	MS, RI
Hexadecanoic acid	1958	1959	--	8.34	--	MS, RI
(Z)-9-Octadecanoic acid	2116	2097	--	1.92	--	MS, RI
Heptacosane	2700	2700	--	1.12	--	MS, RI
Nonacosane	2900	2900	--	1.65	--	MS, RI
<i>Monoterpene hydrocarbons</i>			0.76	1.86	0.00	
<i>Oxygenated monoterpenoids</i>			43.82	19.38	51.83	
<i>Sesquiterpene hydrocarbons</i>			3.75	43.49	0.10	
<i>Oxygenated sesquiterpenoids</i>			3.32	11.01	0.44	
<i>Benzenoid compounds</i>			40.58	7.09	40.28	
<i>Fatty acid and hydrocarbon derivatives</i>			5.73	16.2	6.47	
<i>Others</i>			0.96	0	0.83	
<i>Total identified</i>			98.92	99.03	99.95	
<i>Identified compounds</i>			38	29	30	

^a - Retention induces on ZB-5 fused silica capillary column; ^b - Reported Retention induces on DB-5 fused silica capillary column (Adams). The values provided are averages from three analyses. Relative standard deviations from these replicate injections were all small (less than 2%).

Table S2. Antioxidant activity of the essential oils and major compounds of *Scutellaria* species

Sample	DPPH	ABTS	FRAP
	IC ₅₀ , µg/ml	IC ₅₀ , µg/ml	mM FeSO ₄ /1 g sample
<i>S. immaculata</i>	31.5±1.8	37.8±0.9	720.19±4.8
<i>S. ramosissima</i>	82.8±3.1	93.6±0.8	837.23±3.2
<i>S. schachristanica</i>	57.6±2.7	66.6±1.2	779.64±8.6
Eugenol	0.50±0.007	1.20±0.01	2476.92±15.8
Thymol	0.50±0.008	1.71±0.01	974.09±22.5
Linalool	1914.40±12.5	8821.00±24.9	283.33±3.2
Ascorbic acid	0.30±0.005	7.20±0.22	3327.45±21.4

Figure S1. Free radical scavenging of *S. immaculata* essential oil by the DPPH assay ($IC_{50} = 31.5 \mu\text{g/ml}$)

Figure S2. Free radical scavenging of *S. immaculata* essential oil by the ABTS assay ($IC_{50} = 37.8 \mu\text{g/ml}$)