SUPPLEMENTARY MATERIAL

Volatiles composition and antioxidant activity *Inula oculus-christi* L. from Serbia

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The chemical composition of the essential oil and the volatiles obtained by static headspace (HS) of *Inula oculus-christi* L. is presented. The GC-MS analysis of the hydrodistilled oil resulted in the identification of 90 components, representing 92.7% of the oil. The most abundant compounds were: caryophyllene oxide (9.8%), *trans*-longipinocarveol (9.2%), eucalyptol (7.3%) and intermedeol (6.2%). The major constituent of *I. oculus-christi* L. HS volatiles was eucalyptol (87.4 %). The antioxidant activity was evaluated by four different methods: 2,2-diphenyl-1-picryl-hydrazylhydrate free radical assay (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method, total reducing power (TRP), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC). Total phenolic content in (TPC) examined oil was 177.95 μg GAE/mg oil. Radical scavenging potential of the oil was promising RSC-DPPH was 57.4% and RSC-ABTS was 82.7%.

Keywords: *Inula oculus-christi* L., essential oil, headspace volatiles, chemical composition; GC-MS, antioxidant activity

Plant material and sample preparation: The plant material of collected species *Inula oculus-christi* L. was identified according to Gajić (1975). The samples of plant *Inula oculus-christi* L. were collected at full blooming state on the dry grasslands places of the village Kravlje near Nis city, Serbia. A voucher specimen (13225) was deposited in the "Herbarium collection of the Faculty of Science and Mathematics, University of Niš". The air-dried aerial parts of plant materials (500 g) were powdered and submitted for 2 h to hydrodistillation using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and after filtration, stored at +4 °C prior to GC and GC/MS analysis.

Gas Chromatography and Gas Chromatography-Mass Spectrometry Analysis:

Samples were analyzed on a 7890/7000B GC-MS/MS triple quadrupole system (Agilent Technologies, USA, equipped with a Combi PAL auto sampler) operating in MS1 scan mode, on Agilent HP-5 MS column (30 m×0.25 mm i.d.×0.25 μm film thickness). The GC conditions were: He as carrier gas at a flow rate of 1.0 mL/min, GC oven temperature was kept at 45°C for 2.25 min and programmed to 290°C at a rate of 4°C/min, split ratio was adjusted at 40:1, injection volume 1 μL. Post run: back flash for 1.89 min, at 280°C, with helium pressure of 50 psi. The injector temperature was set at 230°C. Ionization mode was electronic impact at 70 eV. Mass range was set from 40 to 440 Da.

For GC/FID analysis, the same GC parameters were applied as described for GC/MS. FID detector temperature was 300°C. The percentage amounts of the separated compounds were calculated from the GC peak areas using the normalization method without correction factors.

For **static headspace experiment**, 500 mg of milled dry plant material was put into a 20 mL HS vial and soaked with 2 mL of distilled water. The sample was heated at 80°C for 20 minutes. 500 μL of generated vapor was drawn out from the vial using a gas-tight syringe (90°C) and injected directly in the chromatographic column.

Identification of Components:

Oil constituents were identified by comparison of their linear retention indices (relative to C_8-C_{20} and C_{21}-C_{44} alkanes (Van den Dool and Kratz 1963, Adams 2007) on the HP-5MS column) with literature values and their MS with those of authentic standards, as well as those from Wiley 6, NIST11, Agilent Mass Hunter Workstation B.06.00 software (Stein 1990) and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils by the application of the AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA-NIST, 2011).

Antioxidant activity assays: Total phenolic content (TPC) and antioxidant activity of the examined essential oil was determined according to the DPPH, ABTS, CUPRAC, FRAP and TRP methods previously described (Dimitrijevic et al. 2015). All determinations were performed in triplicate.

Table S1: Chemical composition (%) of *Inula oculus-christi* L. essential oil and headspace volatiles (HS)

RA	RI	Compound	Essential oil	HS	Id
778*	776	Hexanal	-	3.3	a,b
841	832	(*E*)-2-Hexenal	0.2	-	a,b
932	930	α-Pinene	tr	0.7	a,b,c
953	949	Thuja-2,4(10)-diene	tr	-	a,b
959	955	Benzaldehyde	tr	-	a,b
976	969	Sabinene	tr	-	a,b,c
980	973	β-Pinene	tr	0.5	a,b,c
978	975	1-Octen-3-ol	tr	-	a,b
985	982	6-Methyl-5-heptene-2-one	tr	-	a,b
988	987	dehydro-1,8-Cineole	tr	0.4	a,b
1003*	999	Octanal	tr	-	a,b
1005	1001	α-Phellandrene	tr	-	a,b
1009*	1007	2,4-(*E*,*E*)-Heptadienal	tr	-	a,b
1018	1014	α-Terpinene	tr	1.4	a,b
1022	1022	*o*-Cymene	0.3	1.1	a,b
1032	1028	**Eucalyptol**	**7.3**	**87.4**	**a,b,c**
1043*	1040	Benzeneacetaldehyde	0.5	-	a,b
1050	1045	β-*trans*-Ocimene	tr	-	a,b
1062	1057	γ-Terpinene	0.7	2.3	a,b
1068	1064	*cis*-Sabinene hydrate	tr	-	a,b
1074	1070	*cis*-Linalool oxide(furanoid)	tr	-	a,b
1084	1085	*trans*-Linalool oxide (furanoid)	0.3	-	a,b
1098	1097	Linalool	1.6	-	a,b,c
1100	1101	Nonanal	0.3	-	a,b
1112	1114	*trans*-Thujone	tr	-	a,b
1121	1119	*cis*-*p*-Menth-2-en-1-ol	tr	-	a,b
1140	1139	*cis*-Sabinol	2.4	-	a,b
1140	1143	*cis*-Verbenol	0.5	0.9	a,b
1152*	1154	Albene	0.3	-	a,b
1162	1157	(*E*)-2-Nonenal	tr	-	a,b
1162	1165	δ-Terpineol	0.5	-	a,b
1177	1176	Terpinen-4-ol	3.1	1.4	a,b,c
1183	1184	*p*-Cymen-8-ol	tr	-	a,b
1189	1189	α-Terpineol	3.8	0.6	a,b
1195	1196	Myrtenol	0.7	-	a,b
1201	1203	Decanal	tr	-	a,b
1205	1207	*trans*-Piperitol	tr	-	a,b
1255	1253	Geraniol	0.5	-	a,b
1290	1292	Thymol	0.5	-	a,b

1326	1325	Silphiperfol-5-ene	0.3	-	a,b
1334	1333	Presilphiperfol-7-ene	0.5	-	a,b
1345	1344	7-*epi*-Silphiperfol-5-ene	1.0	-	a,b
1358	1357	Silphiperfol-4,7(14)-diene	0.3	-	a,b
1377	1376	Silphiperfol-6-ene	1.3	-	a,b
1384*	1384	*trans*-β-Damascenone	0.3	-	a,b
1384	1386	β-Bourbonene	0.6	-	a,b
1405	1404	Italicene	0.3	-	a,b
1424	1421	2,5-dimethoxy-*p*-Cymene	3.6	-	a,b
1418	1421	*trans*-Caryophyllene	-	tr	a,b,c
1430	1430	β-Copaene	tr	-	a,b
1455*	1451	Geranylacetone	0.3	-	a,b
1455	1454	α-Humulene	0. 5	-	a,b
1477	1477	γ-Muurolene	0.4	-	a,b
1483	1482	α-Curcumene (ar)	3.8	-	a,b
1493	1496	*epi*-Cubebol	0.5	-	a,b
1500	1500	α-Muurolene	0.6	-	a,b
1515*	1511	Nerylisobutyrate	0.3	-	a,b
1513	1515	γ-Cadinene	1.3	-	a,b
1518	1518	endo-1-Bourbonanol	0.7	-	a,b
1522	1524	δ-Cadinene	3.4	-	a,b
1542*	1540	Selina-3,7(11)-diene	1.3	-	a,b
1542	1544	α-Calacorene	0.2	-	a,b
1549	1551	Elemol	0.6	-	a,b
1551*	1554	7-*epi*-*cis*-Sesquisabinene hydrate	0.2	-	a,b
1562	1560	Longicamphenylone	0.3	-	a,b
1564	1564	β-Calacorene	tr	-	a,b
1570	1570	*cis*-3-Hexenyl benzoate	tr	-	a,b
1575	1576	Prenopsan-8-ol	1.0	-	a,b
1576	1580	Spathulenol	0.4	-	a,b
1582	1585	**(-)-Caryophyllene oxide**	**9.8**	**-**	**a,b,c**
1600*	1601	Cyperol	0.6	-	a,b
1610	1611	Humulenepoxide	1.0	-	a,b
1618	1617	***trans*-Longipinocarveol**	**9.2**	**-**	a,b
1630	1630	Muurola-4,10(14)-dien-1β-ol	1.5	-	a,b
1640	1643	T-Cadinol	3.5	-	a,b
1644	1648	δ-Cadinol	0.3	-	a,b
1649	1653	β-Eudesmol	0.9	-	a,b
1652	1657	α-Cadinol	2.0	-	a,b
1665	1663	**Intermedeol**	**6.2**	**-**	a,b
1676	1675	Guaia-3,10(14)-dien-11-ol	0.6	-	a,b
1685	1684	α-Bisabolol	0.6	-	a,b

1685	1690	Eudesma-4(15),7-dien-1β-ol	1.0	-	a,b
1695	1694	Cyperotundone	1.1	-	a,b
1715*	1711	Pentadecanal	0.3	-	a,b
1718	1722	(*Z*,*E*)-2,6-Farnesol	0.8	-	a,b
1762	1758	β-Acoradienol	1.1	-	a,b
1789	1789	β-Bisabolenol	0.8	-	a,b
1843*	1842	Hexahydrofarnesyl acetone	1.8	-	a,b
2114	2114	Phytol	0.4	-	a,b
2300	2295	Tricosane	0.3	-	a,b
2400	2395	Tetracosane	tr	-	a,b
2500	2494	Pentacosane	1.7	-	a,b
2700	2694	Heptacosane	0.2	-	a,b
		Total	**92.7**	**100**	
		Monoterpenoids	**26.4**	**96.7**	
		Hydrocarbons (M)	1.3	6.0	
		Oxygenated (MO)	25.1	90.7	
		Sesquiterpenoids	**61.8**		
		Hydrocarbons (S)	15.3		
		Oxygenated (SO)	46.5		
		Diterpene	**0.4**		
		Hydrocarbons (D)	0.4		
		Others (O)	**4.1**	**3.3**	

Compounds are listed in order of elution on a HP-5MS column; RA: Adams retention indices; RI: Experimental retention indices relative to C_8-C_{32} n-alkanes; (*): NIST Chemistry Web Book Retention indices; tr: traces (<0.1%); -: not detected; a: constituent identified by mass spectral comparasion; b: constituent identified by retention index matching; c: constituent identity confirmed by co-injection of an authentic sample.

References

Adams RP. 2007. Identification of essential oil components by gas chromatography-mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois.

Gajić M. 1975. *Inula* L. In: Josifović M (Ed). Flora SR Srbije VII [Flora of SR Serbia VII]. Serbian Academy of Sciences and Arts, Belgrade, Serbia; p. 46-59.

Dimitrijevic M, Stankov Jovanovic V, Cvetkovic J, Mihajilov-Krstev T, Stojanovic G, Mitic V. 2015. Screening of antioxidant, antimicrobial and antiradical activities of twelve selected Serbian wild mushrooms. Anal Method. 7:4181-4191.

National Institute of Standards and Technology 2005. NIST Chemistry WebBook, Nist Standard Reference database Website. http://webbook.nist.gov/chemistry/.

Stein SE. 1990. National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02.

Van den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A. 11:463-471.