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VOLATILE CONSTITUENTS OF *INULA OCULUS-CHRISTI* L. AERIAL PARTS

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Abstract

The chemical composition of the volatile extract from aerial parts of *Inula oculus-christi* L. was studied by GC-MS. Forty-six chromatographic peaks representing 95.0% of the total oil were detected, oxygenated terpenes (51.1%) being the dominating ones. The oil was characterized by relatively high concentration of fatty acids (16.7%) and aromatic compounds (14.8%) too. 1,8-Cineole (19.5%) and hexadecanoic acid (9.4%) were the principal constituents.

Key words: *Inula oculus-christi* L., 1,8-Cineole, p-Cymene-8-ol, p-Cymene-7-ol

Introduction. *Inula oculus-christi* L. is one of the representatives of the genus *Inula* (Asteraceae) occurring in Bulgaria. Our previous phytochemical investigations on this species afforded seven sesquiterpene lactones, three triterepene-3-O-palmitates, six flavones, two flavone glucosides, chlorogenic and 3,5-dicaffeoylquinic acids [1, 2]. The effect of different extracts of *I. oculus-christi* on normal (MDCK II and RPE1) and carcinoma cell lines (A549 and HepG2) [3] as well as their antioxidant capacity [2] have also been evaluated. Continuing our study on this species, here we report the volatile components of the plant growing in Bulgaria. The literature survey revealed only one publication on essential oil from *I. oculus-christi* of Iranian origin [4].

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Materials and methods. ***Plant material.*** Aerial parts of *I. oculus-christi* were collected in July 2016 in western Rhodope Mts (41°53′23.3″N 24°52′58.4″E). A voucher specimen (SOM - 1360) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

Isolation of essential oil. Air-dried plant material (5 g) was subjected to a micro distillation-extraction in a Likens-Nickerson apparatus for 3 hours using diethyl ether as a solvent. The essential oil dissolved in diethyl ether was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under N_2 flow, and the essential oil was stored at 4 °C before analysis. The oil yield was 0.5 mg/g.

Gas chromatography mass spectrometry (GC-MS). GC-MS analysis was performed on HP 6890 Plus instrument with MS detector 5973 of the same company which was operated in EI mode (70 eV), fitted with an HP 5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness), with helium as carrier gas. The injector temperature was 260 °C; column temperature was programmed from 60 °C (5 min isotherm) then to 230 °C at a rate of 4 °C/min and then held for 10 min at 230 °C. Quantitative data were obtained from TIC of each component. Retention indices (RI) of the oil components were calculated by using retention times of C_8-C_{30} n-alkanes under the same chromatographic conditions according to Van den Dool's method [5]. The individual components were identified by their MS, and RI referring to known compounds from the literature [6, 7] and also by comparison with those of NIST 98, WILEY and home-made MS databases.

Results and discussion. The volatile fraction from aerial parts of *I. oculus-christi* was examined by GC-MS. Forty-six chromatographic peaks, corresponding to components in concentration more than 0.3% were registered (Table 1). The results showed that the main classes of compounds were oxygen-containing monoterpenoids (30.9%) and sesquiterpenoids (22.7%), followed by fatty acids (16.7%) and aromatic compounds (14.8%). 1,8-Cineole (19.5%) was the principal constituent of this sample. Caryophyllene oxide (4.0%) was the main sesquiterpenoid. Cuminal (3.6%), p-cymene-8-ol (3.3%) and p-cymene-7-ol (3.7%) were aromatic compounds present in significant concentrations in the oil. The absence of monoterpene hydrocarbons and relatively low concentration of sesquiterpene hydrocarbons (2.5%) was characteristic feature for the studied extract. Large amount of fatty acids (16.7%) was found, of which hexadecanoic (palmitic) acid was the major one (9.4%).

The results of this study of Bulgarian (**Bg**) plant samples were compared with the literature data on the volatiles in *I. oculus-christi* from Iran (**Ir**) [4]. To the best of our knowledge, this report is the only one which describes essential oil components of *I. oculus-christi*. Thus, **Ir** similarly to **Bg** sample contained a great amount of fatty acids (19.3%), with domination of palmitic acid (13.6%). Aromatic compounds were found to be characteristic also for both **Ir**

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Volatile components from *Inula oculus-christi* aerial parts

No	RI_E	RI_L	Compound	%
1	1026	1031	1,8-Cineole	19.5
2	1140	1137	*trans*-Sabinol	6.3
3	1174	1177	Terpinene-4-ol	1.5
4	1179	1186	p-Cymene-8-ol	3.3
5	1186	1191	$\alpha-$Terpineol	1.0
6	1239	1241	Cuminal	3.6
7	1267	1267	Nonanoic(Pelargic)acid	1.5
8	1289	1292	Thymol	0.7
9	1289	1289	*trans* Sabinyl acetate	0.8
10	1293	1289	p-Cymene-7-ol	3.7
11	1297	1302	Carvacrol	0.3
12	1325	1325	p-Mentha-1,4-dien-7-ol	1.8
13	1332	–	Unidentified	0.9
14	1349	1356	Thymol acetate	0.7
15	1356	1359	Eugenol	0.7
16	1370	1367	Carvacrol acetate	0.9
17	1375	–	Unidentified	1.5
18	1387	1387	β-Bourbonene	0.6
19	1417	1422	β-Caryophyllene	0.3
20	1421	1423	*p*-Cymene-7-ol-acetate	0.9
21	1487	1488	β-Ionone	0.9
22	1488	1488	β-Ionone epoxide	0.5
23	1514	1514	Ionol	1.0
24	1516	1514	Cubebol	0.9
25	1517	1516	γ-Cadinene	1.3
26	1518	1518	*endo*-1-Bourbonanol	3.4
27	1521	1524	Calamenene	0.3
28	1535	1528	Dihydroactinidiolide	1.5
29	1565	1565	Dodecanoic (Lauric) acid	1.2
30	1582	1586	Caryophyllene oxide	4.0
31	1600	1599	Guaiol	1.0
32	1607	1610	β-Oplopenone	1.0
33	1621	–	$C_{15}H_{24}O$ (MW 220)	0.7

Table 1

Continued

No	RI_E	RI_L	Compound	%
34	1638	1643	*T*-Cadinol	1.3
35	1644	1644	*T*-Muurolol	1.0
36	1648	1649	δ-Cadinol	0.8
37	1659	1658	α-Cadinol	2.7
38	1675	1677	Cadalene	1.7
39	1685	1686	Kobusone	1.0
40	1688	1689	*cis*-14-*nor*-Muurol-5-en-4-one	0.7
41	1772	1774	Tetradecanoic (Myristic) acid	4.1
42	1835	1846	6,10,14-Trimethyl-2-pentadecanone	2.3
43	1872	1869	Pentadecanoic acid	0.5
44	1959	1965	Hexadecanoic (Palmitic) acid	9.4
45	2500	2500	Pentacosane	0.6
46	2700	2700	Heptacosane	0.7
			Total	**95.0**
			Oxygenated monoterpenes	30.9
			Sesquiterpene hydrocarbons	2.5
			Oxygenated sesquiterpenes	20.2
			Alkanes	1.3
			Aromatic compounds	14.8
			Fatty acids	16.7
			Other	6.2
			Unidentified	2.4

RI_E – Experimentally determined retention indices to C_8–C_{30} *n*-alkanes on HP-5MS column; RI_L – Literature retention indices [5, 6, 15]

and **Bg** oils**,** but they differed in the main aromatic constituents. Thus, dill apiole (11.4%) and methyl eugenol (9.6%) were the main constituents in **Ir**, while p-cymene derivatives (cuminal, p-cymene-8-ol and p-cymene-7-ol) characterized the **Bg** sample. The **Ir** oil contained almost equal amounts of alkanes, aromatic compounds and fatty acids (approximately 20% each), followed by oxygenated sesquiterpenes. Viridiflorol (5.7%), copaborneol (5.6%) and caryophyllene oxide (2.1%) were the main sesquiterpenoids in **Ir**. In **Bg** oil the first two components were not found, but caryophyllene oxide was the main sesquiterpenoid (4.0%), fol-

lowed by endo-1-bourbonanol (3.4%) and α-cadinol (2.7%). The **Ir** and **Bg** oils differed significantly in the content of monoterpenoids. This class of compounds in **Bg** sample is a principal one (30.9%), while in **Ir** it is about 1%. 1,8-Cineole is present in both oils, but in different amounts. It is 0.1% in **Ir** sample, while in **Bg** one it was the major constituent (19.5%). The studied to date essential oils from *Inula* species showed that this component is not characteristic for the genus. It has been identified in some *Inula* oils, but in the most cases this compound was detected in very low concentration (up to 0.2%) with exception of *I. macrocephalla* where it reached 2.5% [8].

p-Cymene is a widely distributed component in various essential oils, including oils of *Inula* species. This compound is a major constituent and reached 53.8% in the Spanish specimen of *I. crithmoides* [9]. It was not found in *I. oculus-christi*, but its 7-keto, 7-hydroxy, 7-acetoxy and 8-hydroxy derivatives accounted 11.5% altogether. Dihydroactinidiolide is a component of essential oils from green tea [10], *Actinidia macrosperma* [11], *Prunus armeniaca* L. [12] and in other species. Before its establishment in the present study, in the genus *Inula* it was found only in *I. Britannica* [13]. β-Ionone, its epoxide and β-ionol are new components for genus *Inula*. The simultaneous presence of these compounds and dihydroactinidiolide is probably a result of the degradation of β-carotene [14]. This degradation process caused by enzymes or various physical and chemical conditions is still under study. β-Ionone, its derivatives and dihydroactinidiolide have also been found in essential oil of some other species of Asteraceae family, for example *Calendula officinalis* L. [15], *Petiveria alliacea* L. [16], etc.

The chemical composition of the volatile extracts of Iranian and Bulgarian *I. oculus-christi* shows more differences than similarities, analogously to their lactone profiles. Thus, gaillardin, the basic lactone and pulchellin C are common compounds in both samples. However, ergolide, a component of **Ir** sample [17] was not found in **Bg** [1]. Instead, four new lactones from plant growing in Bulgaria were isolated [1]. The discussed differences in the chemical composition of the lactone and volatile profiles of these two samples revealed intraspecific variation, which is probably due to specific ecological conditions in the two countries and / or to genetic factors. Moreover, quite a lot unusual for the genus *Inula* components were detected in this sample.

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