Chemical composition of volatile extract from *Inula aschersoniana* Janka var. *aschersoniana* growing in Bulgaria

MILKA TODOROVA[1], ANTOANETA TRENDAFILOVA[1,*], AND DIMITAR DIMITROV[2]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria*
[2] *National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd., 1000 Sofia, Bulgaria*
[*] *Corresponding author: trendaf@orgchm.bas.bg*

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The chemical composition of the volatile oil of *Inula aschersoniana* was studied by GC and GC-MS. Forty-five constituents representing 92.9% of the total oil were detected. The oil contained fatty acids (55.2%) and alkanes (14.1%), followed by oxygenated monoterpenes (9.6%), sesquiterpenoids (7.1%) and aromatic compounds (4.5%). It is characterized by relatively low content of terpenoids in total 16.7% only of which linalool (2.1%) and τ-cadinol (2.2%) were the dominant components in this class of compounds. Cluster analysis (CA) was used for determination of the relationship between the species in *Inula verbascifolia* aggregate.

Key words: *aschersoniana; volatile components; GC; GC-MS; CA*

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1. INTRODUCTION

The genus *Inula* (Asteraceae) consists of 100 species, some of which are known as plants used in traditional medicine. A literature survey revealed that *Inula* plants are rich source of biologically active compounds, mainly sesquiterpene lactones, flavonoids, and triterpenoids (Seca et al., 2014; 2015). Surprisingly, the information on essential oil composition of *Inula* species is scarce and only few biological activities have been reported – antibacterial (Deriu et al., 2008; Priydarshi et al., 2016), antifungal (Chauhan and Saxena, 1985; Haoui et al., 2016), and influence of *I. helenium* essential oil on electroencephalographic activity of the human brain (Sowndhararajan et al., 2016). *Inula aschersoniana* Janka is a species with areas of distribution in Bulgaria, Greece, Macedonia, and Turkey (*The Global Species*, 2009). Three varieties of *I. aschersoniana* – var. *madarense*, var. *macedonica* and var. *aschersoniana* are identified in Bulgaria (Delipavlov et al., 2003; Stojanov et al., 1967). Our previous phytochemical investigation of *I. aschersoniana* Janka var. *aschersoniana* afforded parthenolide, diepoxycostunolide, inusoniolide, chrysosplenol C and four pseudoguaiane derivatives (Trendafilova et al., 2014). The essential oil composition of *I. aschersoniana* has not been studied yet. In continuation of our phytochemical work on *Inula* essential oils, herein we report the chemical composition of the volatile fraction of *I. aschersoniana* var. *aschersoniana* growing in Bulgaria.

2. MATERIALS AND METHODS

1. Plant material

Flowers of *I. aschersoniana* were collected in July 2015 near Marziganiza hut (41°53′32.8″ N 24°52′47.8″ E) in Rhodope Mts, Bulgaria. A voucher specimen (SOM169980) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

2. Preparation of the essential oil

Air-dried flowers were subjected to a micro hydrodistillation-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 0.6 mg/g.

3. Gas chromatography (GC) and Gas chromatography - mass spectrometry (GC-MS)

The GC analysis was performed with a Shimadzu 17A gas chromatograph equipped with FID and an HP5-MS fused-silica capillary column ((5%-phenyl)-methylpolysiloxane, 30 m x 25 mm i.d., film thickness 0.25 µm). The column temperature was programmed from 50°C (4 min isotherm), to 240°C at a rate of 4°C/min, to 300°C at a rate of 10°C/min, and held at this temperature for 10 min, the injector and detector temperatures were 260°C, carrier gas - N_2 (linear velocity, 33 cm/s), split ratio, 1:100. Relative percentage amounts were calculated from peak area without the use of correction factors.

GC-MS analysis was performed on a HP 6890 Plus instrument with MS detector 5973 of the same company. Mass detector was operated in electron ionization mode (70 eV) at a mass range of 30–450 Da. All chromatographic conditions and the column were as described above (for GC Analysis) except for the carrier gas, which was He. 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 (van Den Dool and Dec. Kratz, 1963). The individual components were identified by their RI, referring to known compounds from the literature (Adams, 2009; Tkachev, 2008) and by comparison of their mass spectra with those of NIST 98, WILEY and homemade MS databases.

4. Statistical analysis

The single linkage clustering method based on linkage distances was used to determine similarities between the examined samples (Statistica 10.0 package).

3. RESULTS AND DISCUSSION

Hydrodistillation of *I. aschersoniana* flowers gave pale yellow oil, which was further analyzed by GC and GC–MS. Forty-five volatile constituents in concentration more than 0.1%, representing 92.9% of the total oil were listed in Table 1. Forty-three compounds were identified, and the chemical type of two components was determined. The oil was primarily composed of fatty acids (55.2%) and alkanes (14.1%), smaller amounts of oxygenated monoterpenes (9.6%), sesquiterpenoids (7.1%) and aromatic compounds (4.5%). It should be noted the abundance of fatty acids, of which octanoic, dodecanoic, and decanoic acids were principal constituents of the oil (16.8, 13.7 and 9.3%, respectively). The aliphatic hydrocarbons pentacosane (5.4%), heptacosane (3.5%) and nonacosane (2.5%) also presented in the oil in significant concentrations.

The oil is characterized by relatively low content of terpenoids in total 16.7% only. Linalool (2.1%) and τ-cadinol (2.2%) were the dominant components in this class of compounds. Megastigmatrienone-2 and megastigmatrienone-4 were detected now for the first time in *Inula* species. Megastigmatrienones are dinor-isoprenoids derived from carotenoids and have been found mainly in members of the Solanaceae (D'Abrosca et al., 2004; Osorio, 2003) but also have been identified in Lamiaceae (*Stachys palustris*) (Senatore et al., 2007) and Asteraceae (*Bidens pilosa*) (Deba et al., 2008) essential oils.

The literature review showed that the rare essential oil component β-damascenone accounted for 0.2% in the studied oil is not unusual for the genus Inula. It has been detected in volatile extracts of *I. verbascifolia* ssp. *parnassica*(Tzakou et al., 2001) , *I. cappa* (Priydarshi et al., 2016), *I. crithmoides* (Tsoukatou and Roussis, 1999), and *I. viscosa* (Madani et al., 2014). Methyl jasmonate, detected in remarkable concentration of 1.0% in the studied oil is a compound isolated from Jasmine flowers (Adams, 2009). It has been previously found also in oils from species of Asteraceae family, like *Artemisia absinthium* (Ueda and Kato, 1980), *A. kermanensis* Ganjali and Pourramezani Harati (2012), *A. aucheri* (Badrabadi et al., 2015), *Rhaponticum acaule* (Boussaada et al., 2008), etc., but it was detected now for the first time in *Inula* species. *I. aschersoniana* Janka (syn. *I. verbascifolia* subsp. *aschersoniana* Janka) belongs to *I. verbascifolia* aggregate that includes also *I. verbascifolia* (Willd.) Hausskn., *I. fragilis* Boiss. & Hausskn., *I. heterolepis* Boiss. (syn. *I. verbascifolia* subsp. *heterolepis* (Boiss.) Tutin), *I.* methanaea Hausskn. (syn. *I. verbascifolia* subsp. *methanaea* (Hausskn.) Tutin) and *I. parnasica* Boiss. & Heldr. (*I. verbascifolia* subsp. parnassica (Boiss. & Heldr.) Tutin) (*Euro+Med*, 2006). The literature survey revealed that only the volatile fractions from *I. verbascifolia* (**Iv**) of Croatian origin (Fontana

Table 1. Percentage composition of *Inula ashersoniana* essential oil

RI[a]	Compounds	%
965	hexanoic acid	6.0
1069	*cis*-linalool oxide	0.7
1085	*trans*-linalool oxide	0.7
1093	linalool	2.1
1108	phenylethyl alcohol	0.2
1135	$C_{10}H_{18}O$ [b]	1.1
1168	octanoic acid	16.8
1189	α-terpineol	1.4
1226	nerol	1.8
1251	geraniol	1.8
1268	nonanoic acid	0.5
1365	decanoic acid	9.3
1384	β-damascenone	0.2
1411	methyl eugenol	0.2
1455	*allo*-aromadendrene	0.8
1511	tridecanal	0.5
1515	γ-cadinene	0.3
1564	dodecanoic acid	13.7
1567	*cis*-3-hexenyl benzoate	0.7
1580	megastigmatrienone-2	0.5
1585	caryophyllene oxide	0.8
1626	megastigmatrienone-4	0.3
1644	τ-cadinol	2.2
1650	methyl jasmonate	1.0
1660	α-cadinol	1.2
1700	*(Z,Z)*-farnesol	0.3
1750	$C_{15}H_{24}O$ [c]	0.3
1757	benzyl benzoate	1.7
1762	tetradecanoic acid	2.7
1845	6,10,14-trimethyl-2-pentadecanone	0.9
1858	2-phenylethyl benzoate	0.2
1863	pentadecanoic acid	0.8
1865	benzyl salicylate	1.5
1916	*(E,E)*-farnesyl acetone	0.2
1960	hexadecanoic acid	4.5
2140	*(Z,Z)*-9,12-octadecadienoic acid	0.8
2146	*cis*-9-octadecenoic acid	0.1
2300	tricosane	1.0
2400	tetracosane	0.4
2500	pentacosane	5.4
2600	hexacosane	0.5
2700	heptacosane	3.5
2800	octacosane	0.5
2900	nonacosane	2.5
3100	hentriacontane	0.3
	Total	92.9

[a]Retention indices to C_8–C_{30} n-alkanes on HP-5MS column.
[b]*m/z* (rel. int.): 154 (4, $[M]^+$), 139 (4), 136 (1), 126 (42), 111 (30), 95(8), 81 (20), 67 (100), 39 (32)
[c]*m/z* (rel. int.): 220 (36, $[M]^+$), 205 (38), 202 (16). 187 (20), 177 (17), 159 (42), 145 (38), 133 (40), 121 (43), 105 (62), 93 (84), 79 (68), 67 (69), 55 (76), 41 (100).

et al., 2014), *I. verbascifolia* subsp. *methanaea* (**Ivm**) and *I. verbascifolia* subsp. *parnassica* (**Ivp**) both from Greece (Tzakou et al., 2001) have been studied so far. Comparison of the results for Bulgarian *I. ashersoniana* (**Ia**) essential oil with those published for **Iv** (Fontana et al., 2014), **Ivp** and **Ivm** (Tzakou et al., 2001) showed a big variety in their chemical composition (Fig. 1). Thus, high concentrations of alkanes (18.8% and 14.1%) and

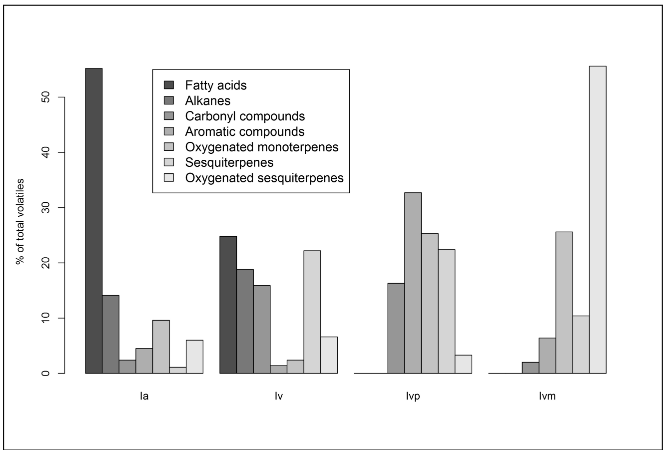

Fig. 1. Comparison of the main classes of compounds found in *I. aschersoniana* (**Ia**) and other related species *I. verbascifolia* (**Iv**), *I. verbascifolia* subsp. *methanaea* (**Ivm**) and *I. verbascifolia* subsp. *parnassica* (**Ivp**) published in the literature (Tzakou et al., 2001; Fontana et al., 2014)

fatty acids (22.8% and 55.2%) are characteristic features for **Iv** and **Ia**. These classes of constituents are not present in **Ivm** and **Ivp**. On the other hand, Croatian sample (**Iv**) was rich in sesquiterpene hydrocarbons (22.2%) and carbonyl compounds (15.9%) like **Ivp** (22.4% and 16.3%, respectively), while these types of compounds in **Ia** were 1.1% and 2.4%, respectively. In addition, **Ivp** contained relatively high amounts of aromatic compounds (32.7%) and oxygenated monoterpenes (25.3%). *cis*-Chrysanthenol (17.3%), caryophyllene (13.2%) and methyl salicylate (23.4%) were the most abundant constituents in **Ivp**. Further, **Ivm** mis distinguished from the others as its oil was mainly consisting of terpenoids (91.6%). Linalool (21.2%) was the principal component among oxygenated monoterpenoids (25.6%), while τ-cadinol (19.5%) and *(Z)*-nuciferol (16.6%) dominated in the sequiterpene group (55.6%). It should be noted, that the lack of monoterpene hydrocarbons was a common feature for these four species. The observed differentiation of the samples was further supported by cluster analysis (Fig. 2), which showed three distinct groups based on the Euclidian distance. The first group (**Iv** and **Ia**) was characterized by high content of alkanes and fatty acids. The second cluster (**Ivp**) contained almost equal amounts of oxygenated monoterpenes, sesquiterpene hydrocarbons and aromatic compounds. The third cluster (**Ivm**) contained primarily terpenoids (91.6%).

CONCLUSION

In conlucion, the volatile components discussed above showed significant variety in chemical composition of the studied up to now species from *I. verbascifolia* aggregate. Further investigation of oil composition of *I. fragilis* Boiss. & Hausskn., *I. heterolepis* Boiss. (syn. *I. verbascifolia* subsp. *heterolepis*) as well as *I. aschersoniana* var. *madarense* and *I. aschersoniana* var. *macedonica* will complete the information on the relationship between the species in this aggregate.

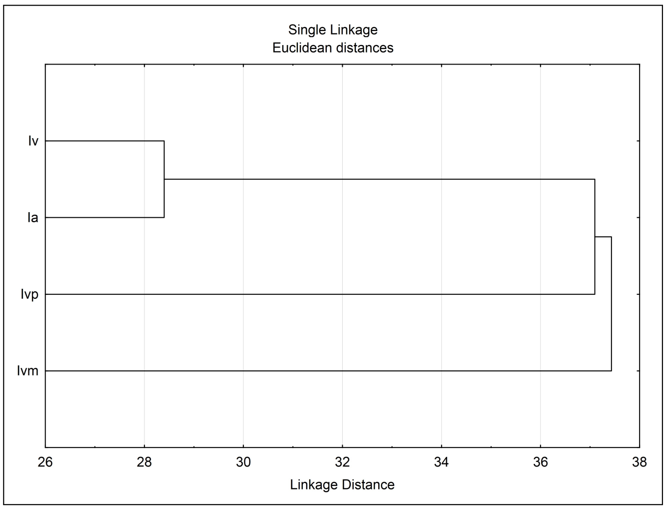

Fig. 2. Dendrogram performed from essential oils components of *I. aschersoniana* (**Ia**) and other related species *I. verbascifolia* (**Iv**), *I. verbascifolia* subsp. *methanaea* (**Ivm**) and *I. verbascifolia* subsp. *parnassica* (**Ivp**) published in the literature (Tzakou et al., 2001; Fontana et al., 2014)

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