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VOLATILE GLUCOSINOLATE BREAKDOWN PRODUCTS AND THE ESSENTIAL OIL OF *DESCURAINIA SOPHIA* (L.) WEBB EX PRANTL (BRASSICACEAE)[†]

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Abstract. *Volatile constituents obtained by autolysis of aerial and underground parts of* D. sophia *and the essential oil obtained by hydrodistillation of whole plant samples were analyzed in detail by GC and GC-MS. In total, 71 constituents were identified, accounting for more than 90% of the total peak areas in the chromatograms. Both aerial and underground autolysates contained considerable amounts of lignan arctigenin and cuticular wax compounds. The essential oil was dominated by glucosinolate breakdown product 4-pentenenitrile. Glucosinolate degradation products identified in the essential oil and autolysates, 3-butenyl isothiocyanate, 4-pentenenitrile and allyl isothiocyanate, suggested the presence of gluconapin and sinigrin in this species as the most likely "mustard oil" precursors.*

Key words: Descurainia sophia, *essential oil, glucosinolates, isothiocyanates, arctigenin*

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1. INTRODUCTION

The genus *Descurainia* Webb et Berth. is represented in the Serbian flora by only one species – *Descurainia sophia* (L.) Webb ex Prantl 1891 (syn. *Sisymbrium sophia* L. 1753; *Discurea sophia* (L.) Schur 1866), commonly called flixweed or *strižica* in Serbian (Diklić, 1972). It is an annual weed plant commonly found in whole Europe, in Asia to India and the Himalayas, in Siberia, China, Japan, and North Africa, in North and South America and New Zealand. Flixweed is widespread in Serbia, where it usually occupies roadsides, walls and embankments, fields and gardens, dumps, rural roads and yards.

Previous phytochemical investigations of *D. sophia* led to the isolation and/or identification of flavonoids, coumarins, some cardiac glycosides, a norlignan and some other phenolic compounds (Chen et al., 1981; Mohamed and Mahrous, 2009; Sun and Li, 2003; Sun et al., 2003, 2004, 2005, 2006; Wang et al., 2004). The literature review also revealed that this species contains some lipids, fatty acids, alkanes and sterols (Afsharypuor and Lockwood, 1985; Bekker et al., 2005), while some previous investigations focused on flixseed essential oil (Li et al., 2010; Tavakoli et al., 2012), glucosinolates and their degradation products (Afsharypuor and Lockwood, 1985; Baslas, 1959; Chen and Guan, 2006; Cole, 1976; Daxenbichler et al., 1991; Fahey et al., 2001; Kjaer et al., 1953; Lockwood and Afsharypuor, 1986).

Biological activity assays showed that the metabolites of this plant species exhibit antioxidative, analgesic, antipyretic and anti-inflammatory effect (Mohamed and Mahrous, 2009; Shi, 2011). *Descurainia sophia* extract exhibited antibacterial activity against Gram-positive *Streptococcus pyogenes* (Aghaabbasi et al., 2014). Recently, Li *et al.* (2011) investigated allelopathic influence of *D. sophia* (one of the most troublesome weeds in wheat fields in China) volatile oils on wheat cultivars.

Descurainia sophia has been widely used in folk medicine. It is a plant with diverse ethno-medicinal uses – in Middle Asia, this species was used for the treatment of throat diseases and as an antipyretic for measles and smallpox, in Tibetan medicine for St. Anthony's fire and anthrax (Bekker et al., 2005), in Iranian traditional medicine mainly as a febrifuge (Afsharypuor and Lockwood, 1985), while Chinese traditional medicine used the seed of this plant as a remedy for treating cough, to prevent asthma, reduce edema, as a diuretic and, in mixtures with other Chinese herbs, for the prevention of lung cancer (Mohamed and Mahrous, 2009; Sun et al., 2004).

Bearing in mind the scarce literature data on the volatile chemistry of this plant species, this study was set to investigate, in detail, the volatile oil of this taxon originating from Serbia. Furthermore, as this species belongs to the Cruciferae family characterized by the presence of glucosinolates, another goal of this study was set to detect and identify volatile autolysis products of these metabolites by endogenous myrosinases.

2. MATERIAL AND METHODS

2.1. Plant material

Plant samples (aerial and underground parts) of *D. sophia* utilized in this research were collected from its natural surroundings during the flowering stage in the vicinity of Niš, Serbia, in June 2010. Voucher specimens were deposited in the Herbarium collection of the Faculty of Sciences and Mathematics, University of Niš.

2.2. Essential oil isolation

Fresh plant material (three batches of about 250 g of entire plants) was subjected to hydrodistillation with ca. 2 L of distilled water for 2.5 h using the original Clevenger-type apparatus (Clevenger, 1928). The obtained oils were separated by extraction with freshly distilled diethyl ether (Merck, Germany), dried over anhydrous magnesium sulfate (Aldrich, USA) and immediately analyzed. The yield was 0.022% (w/w, fresh weight basis).

2.3. Isolation of the autolysis volatiles

Hydrolysis of glucosinolates was catalyzed by endogenous myrosinases according to the following procedure (three repetitions for each sample): homogenized fresh plant samples (100 g of aerial and underground parts, respectively) were mixed with a sufficient quantity of distilled water to make a paste (250 mL). According to a modified procedure reported by Vaughn and Berhow (2005), diethyl ether (100 mL) was added to each sample, the all-glass vessels were sealed, and the flasks were placed in an incubator shaker set at 25 °C and 200 rpm for 8 h. Following hydrolysis, sodium chloride (60 g) was added and mixed thoroughly. The diethyl ether layer was then separated from the brine solution and filtered through a Whatman No. 1 filter paper and the residual plant mass was extracted additional three times with excess diethyl ether. The combined crude ether extracts were dried over anhydrous magnesium sulfate, evaporated to dryness on a rotary evaporator at 25 °C, redissolved in diethyl ether (10 mL) and immediately analyzed.

2.4. GC-FID and GC-MS Analyses

Analyses of the essential oil and autolysates were carried out by GC and GC-MS. The GC-MS analyses (three repetitions) were performed on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975C mass selective detector from the same company. The injector and interface were operated at 250 and 320 °C, respectively. Oven temperature was raised from 70 to 315 °C at a heating rate of 5 °C/min and then isothermally held for 10 min. As a carrier gas, helium at 1.0 mL/min was used. The samples, 1 μL of the sample solution in diethyl ether (1:100), was injected in a pulsed split mode (split ratio 40:1). Mass selective detector electron impact was operated at the ionization energy of 70 eV, in the 35–650 amu range and scanning speed of 0.32 s. GC-FID analysis was carried out under the same experimental conditions using the same column as described for the GC-MS.

Qualitative analysis of the autolysates and essential oil were based on the comparison of the essential-oil constituent linear retention indices (determined relative to the retention times of C_7–C_{33} *n*-alkanes on the DB-5MS column (Van den Dool and Kratz, 1963)), to those reported in the literature (Adams, 2007) and by comparison of their mass spectra to those of authentic standards, as well as those from Wiley 6, NIST05 and MassFinder 2.3 libraries. In addition, a homemade MS library with the spectra corresponding to pure substances and components of known essential oils was used, and finally, wherever possible, the identification was achieved by co-injection with an authentic sample. The percentage composition was computed from the GC-FID peak areas without the use of correction factors.

2.5. Silylation of the autolysate

Chlorotrimethylsilane (trimethylsilyl chloride, 0.1 mmol) was added with stirring to a mixture of the autolysate (*ca.* 100 mg), triethylamine (0.15 mmol) and dimethyl sulfoxide (0.01 mmol) in diethyl ether (10 mL). The temperature of the mixture was kept at 10 °C by occasional cooling. After one hour, the reaction mixture was poured into ice-water (10 mL). After washing the ethereal solution with water, the extract was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The obtained residue was completely dissolved in dry diethyl ether and subjected to the GC–MS analysis as previously described.

3. RESULTS AND DISCUSSION

Table 1 presents the results of detailed GC and GC-MS analyses of the volatile constituents obtained by autolysis of aerial (stems, flowers and leaves) and underground parts (roots) of *D. sophia* and the essential oil obtained by hydrodistillation of whole plant samples. The analyses allowed the identification of 71 compounds in total, accounting for more than 90% of the total peak areas in the chromatograms. Both aerial and underground autolysates contained a lignin, arctigenin, and considerable amounts of cuticular wax compounds – the most abundant compounds in the autolysate isolated from the aerial part of the plant were the lignan arctigenin (39.0%) and cuticular wax alkanes hentriacontane (29.9%) and nonacosane (9.1%), while a glucosinolate breakdown product, 3-butenyl isothiocyanate (27.3%), as well as arctigenin (14.5%) and palmitic acid (13.3%) were the main constituents of flixweed root autolysate. Glucosinolate breakdown products localization in roots was not surprising since it is well known that glucosinolate-containing plants accumulate these compounds in their reproductive organs and plant organs which are most exposed to herbivores and pathogens (Redovnikovic et al., 2008).

The essential oil was dominated by the glucosinolate breakdown product 4-pentenenitrile (68%), together with considerable amounts of terpenoids such as *cis*-chrysantenyl acetate (4.7%), hexahydrofarnesyl acetone (4.5%) and (*E*)-phytol (4.4%), and cuticular wax compounds (nonacosane (3.0%), hexadecanoic acid (2.4%) etc.). Noticeable differences between the essential oil composition reported in the current study and those previously reported are observable. Li *et al.* (2010) investigated two samples collected from two different locations in China. Contrary to our results, both of these oils were almost completely consisted of mono- and sesquiterpenoids although they identified only *ca.* 2/3 of the total peak areas. Predominance of terpenoids was also noticeable in the composition of *D. sophia* essential oil from Iran, in the study conducted by Tavakoli *et al.* (2012). One of possible explanation for the absence of even traces of glucosinolate degradation products could be misidentification or, most likely, that these compounds remained unidentified in the mentioned cases.

Table 1 Percentage composition of *D. sophia* autolysates isolated from the aerial (A) and underground plant parts (B), as well as of the essential oil (C) of the same species

RI[a]	Compound	Percentage (%)[b]			Method of identification[c]
		A	B	C	
763	4-Pentenenitrile	-	-	68.0	MS
773	Toluene	-	t	-	RI, MS, Co-GC
810	Butyl acetate	-	5.9	-	RI, MS, Co-GC
884	Allyl isothiocyanate	-	0.3	-	RI, MS, Co-GC
903	Cyclohexanone	-	0.2	-	RI, MS, Co-GC
985	3-Butenyl isothiocyanate	-	27.3	-	RI, MS
1069	1-Octanol	-	t	-	RI, MS, Co-GC
1114	2,6-Dimethylcyclohexanol (isomer)	-	-	t	RI, MS
1151	Camphor	-	-	t	RI, MS, Co-GC
1163	*cis*-Chrysanthenol	-	-	0.5	RI,MS
1182	Terpinen-4-ol	-	-	t	RI, MS, Co-GC
1204	Safranal	-	-	t	RI, MS
1225	β-Cyclocitral	-	-	1.0	RI, MS
1262	*cis*-Chrysanthenyl acetate	-	-	4.7	RI, MS
1293	Dihydroedulan[d]	-	-	t	RI, MS
1303	Teaspirane[d]	-	-	t	RI, MS
1319	Teaspirane[d]	-	-	t	RI, MS
1400	Tetradecane	-	t	-	RI, MS, Co-GC
1460	2-Methyltetradecane	-	-	t	RI, MS
1487	Germacrene D	-	0.3	-	RI, MS
1490	(*E*)-β-Ionone	-	-	4.0	RI, MS, Co-GC
1491	(*E*)-β-Ionone-5,6-epoxide	-	-	0.5	RI, MS
1766	Myristic acid	-	1.1	-	RI, MS, Co-GC
1800	Octadecane	-	t	-	RI, MS, Co-GC
1841	Neophytadiene (isomer 1)	0.3	-	-	RI, MS
1846	Hexahydrofarnesyl acetone	-	-	4.5	RI, MS
1865	Pentadecanoic acid	-	t	-	RI, MS, Co-GC
1866	Neophytadiene (isomer 2)	0.1	-	-	RI, MS
1884	Neophytadiene (isomer 3)	0.1	-	-	RI, MS
1900	Nonadecane	-	t	-	RI, MS, Co-GC
1921	(*E*,*E*)-Farnesyl acetone	-	-	t	RI, MS
1942	Palmitoleic acid	-	1.3	-	RI, MS
1961	Hexadecanoic acid	0.6	13.3	2.4	RI, MS, Co-GC
2000	Eicosane	-	0.7	t	RI, MS, Co-GC
2086	1-Octadecanol	-	1.7	-	RI, MS, Co-GC
2100	Heneicosane		t	t	RI, MS, Co-GC
2116	(*E*)-Phytol	t	-	4.4	RI, MS, Co-GC
2135	Linoleic acid	0.1	3.1	-	RI, MS, Co-GC
2140	Oleic acid	0.4	5.2	-	RI, MS, Co-GC
2145	Linolenic acid	-	6.1	-	RI, MS, Co-GC
2164	Stearic acid	0.7	2.1	-	RI, MS, Co-GC
2200	Docosane	-	t	t	RI, MS, Co-GC
2287	1-Eicosanol	-	1.7	-	RI, MS
2300	Tricosane	0.1	0.3	t	RI, MS, Co-GC
2364	Arachidic acid	0.1	0.8	-	RI, MS, Co-GC

RI[a]	Compound	Percentage (%)[b]			Method of identification[c]
		A	B	C	
2400	Tetracosane	t	0.1	-	RI, MS, Co-GC
2500	Pentacosane	0.3	0.6	0.7	RI, MS, Co-GC
2568	Behenic acid	t	0.8	-	RI, MS, Co-GC
2600	Hexacosane	t	t	-	RI, MS, Co-GC
2634	Tetracosanal	t	-	-	RI, MS, Co-GC
2662	2-Methylhexacosane	0.1	t	-	RI, MS
2700	Heptacosane	1.3	0.9	0.8	RI, MS, Co-GC
2764	2-Methylheptacosane	t	-	-	RI, MS
2773	3-Methylheptacosane	0.1	-	-	RI, MS
2800	Octacosane	0.1	0.3	-	RI, MS, Co-GC
2834	Squalene (all *E*)	0.1	0.2	-	RI, MS
2838	Hexacosanal	0.1	-	-	RI, MS
2862	2-Methyloctacosane	1.3	t	t	RI, MS
2900	Nonacosane	9.1	4.4	3.0	RI, MS, Co-GC
2937	Heptacosanal	0.1	-	-	RI, MS
2961	2-Methylnonacosane	0.1	-	-	RI, MS
2972	3-Methylnonacosane	0.7	-	-	RI, MS
3000	Triacontane	1.1	-	t	RI, MS, Co-GC
3042	Octacosanal	0.2	-	-	RI, MS
3066	2-Methyltriacontane	2.6	-	-	RI, MS
3100	Hentriacontane	29.9	-	1.1	RI, MS, Co-GC
3115	Arctigenin (4-[(3,4-dimethoxyphenyl)methyl]-3- [(4-hydroxy-3-methoxyphenyl)methyl]-2-tetrahydrofuranone)[d]	39.0	14.5	-	MS, TMS
3160	2-Methylhentriacontane	0.5	-	-	RI, MS
3176	3-Methylhentriacontane	1.0	-	-	RI, MS
3200	Dotriacontane	1.4	0.9	-	RI, MS, Co-GC
3300	Tritriacontane	1.7	-	-	RI, MS, Co-GC
	Total	93.3	94.1	95.6	

[a] Linear retention indices experimentally determined on the DB-5MS column. [b] Values are means of three individual analyses. [c] RI – Retention indices matching with literature data; MS – mass spectra matching; Co-GC – co-injection with pure reference compound; TMS – chemical transformation to trimethylsilyl ether derivative. [d] Correct isomer not determined. t – Trace amounts (<0.05%).

Together with the above-mentioned glucosinolate breakdown products, 3-butenyl isothiocyanate in the root autolisate and 4-pentenenitrile in the essential oil, another glucosinolate degradation product, allyl isothiocyanate (0.3% in the root autolysate), was also identified. The occurrence of these compounds suggests the presence of possible glucosinolate precursors – 3-butenyl glycosinolate (gluconapin) and allyl glucosinolate (sinigrin) (Fig. 1). A literature survey concerning the previously isolated/identified glucosinolates in this species showed that this taxon also produces the aliphatic, branched-chain glucosinolate glucocochlearin, aromatic glucosinolates glucotropaeolin, glucosinalbin and gluconasturtiin, side-chain-sulphur-containing glucosinolates 3-methylthiopropyl, 4-methylthiobutyl, 3-hydroxy-5-(methylsulphonyl)pentyl and 3-hydroxy-5-(methylsulphinyl)pentyl glucosinolate, and oxo-aliphatic glucosinolate glucocappasalin.

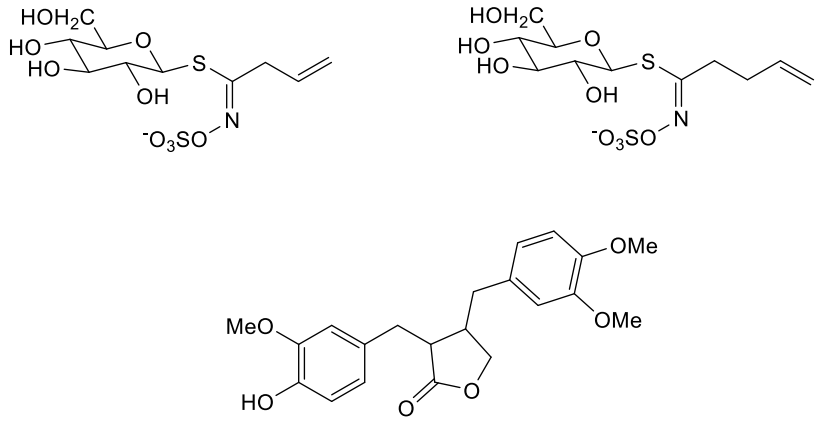

Fig. 1 Chemical structures of *D. sophia* metabolites: glucosinolates sinigrin and gluconapin (upper part), "mustard oil" precursors of the identified glucosinolate breakdown products and the lignan arctigenin (lower part)

Arctigenin was detected in both *D. sophia* autolysates (Fig. 1). The tentative identification of this metabolite was further confirmed by a GC-MS analysis after derivatization with chlorotrimethylsilane to the corresponding TMS ether. The obtained results additionally confirmed the structure of arctigenin through the shift in their RI values and mass fragmentation data from the obtained mass spectrum. Unfortunately, due to the lack of literature information about the GC retention index data and an unsuccessful isolation of the compound in question, the relative stereochemistry remained undetermined. The structure of this compound and its glucoside arctiin were known since 1930's; for the first time, arctiin, *i.e.* arctigenin were isolated from *Arctium lappa*, Compositae, and afterwards from many other taxa (Omaki, 1935 and ref. therein). According to literature data, this lignan displays important biological activities, such as antioxidative, anti-HIV-1 and anti-inflammatory effects (Eich et al., 1996; Predes et al, 2011; Zhao et al., 2009). A significant level of selective cytotoxicity of arctigenin was proved in the case of lung, stomach and liver cancers but it did not exert such an effect towards several normal cell lines (Susanti et al., 2012). Its derivatives possess anticancer activity as well (Chen et al., 2012). The induction of apoptosis appears to be the key mechanism for anticancer effect of arctigenin (Susanti et al., 2013). The relatively high abundance of arctigenin in the *D. sophia* aerial parts autolysate suggests that this species could serve as a good source of this compound.

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ISPARLJIVI PROIZVODI RAZLAGANJA GLUKOZINOLATA I ETARSKO ULJE VRSTE *DESCURAINIA SOPHIA* WEB EX PRANTL (BRASSICACEAE)

Detaljnim GC i GC-MS analizama etarskog ulja i isparljivih sastojaka dobijenih autolizom nadzemnog dela i korena vrste Descurainia sophia *identifikovano je ukupno 71 jedinjenje, odnosno više od 90% ukupno detektovanih pikova hromatograma. Oba autolizata su sadržala znatne količine sastojaka kutikularnog voska i lignana arktigenina. Najzastupljeniji sastojak etarskog ulja je bio proizvod razlaganja glukozinolata 4-pentenonitril. Degradacioni proizvodi glukozinolata identifikovani u autolizatima i etarskom ulju, 3-butenil-izotiocijanat, 4-pentenonitril i alil-izotiocijanat, ukazuju na prisustvo glukonapina i sinigrina u tkivima ove biljne vrste.*

Ključne reči: Descurainia sophia, *etarsko ulje, glukozinolati, izotiocijanati, arktigenin*