Analysis of volatile compounds from Angelica seeds obtained by headspace method

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The study was performed in order to determine the profile of volatile components of the garden angelica (*Angelica archangelica L.*) and wild angelica (*A. sylvestris L.*) seeds. The volatile ingredients were isolated by headspace techniques and analyzed by means of gas chromatography–flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS). It was found that *A. archangelica* seed consists of 29 components among which the dominant is *β*–phellandrene (84.7%), followed by *α*–phellandrene (3.4%), α–pinene (2.5%), myrcene (2.1%) and *α*–copaene (1.3%). However, the *A. sylvestris* seed consists of 22 volatile compounds, and the dominant are limonene (62.7%) and *α*–pinene (28.0%), followed by camphene (2.6%), *α*–phellandrene (2.0%) and *β*–pinene (1.0%).

Keywords: *Angelica archangelica*, *Angelica sylvestris*, headspace, seeds, volatile compounds

1. Introduction:

Genus Angelica has more than 40 species, but only *Angelica archangelica* L., also called European or garden angelica, is officially used in medicine (Eur.Ph 2004). This plant possesses anxiolytic (Kumar and Bhat 2011), anticancer (Sigurdsson et al. 2004; Sigurdsson et al. 2005a), hepatoprotective (Yeh et al. 2003) and imunostimulant (Modaresi 2013) activities. Apart from this, it acts as antibacterial (Rather et al. 2013), antifungal and antiaflatoxigenic (Prakash et al. 2015) as well as antioxidative (Wei and Shibamoto 2007) and insecticidal agent (Pavela and Vrchotova 2013). This indicated that *A. archangelica*, apart from pharmacological importance, has a high potential for being applied as a natural preservative as well as natural biocontrol agent in agro-food industry.

A. archangelica is indigenous to Northern Europe, but it is also widespread in Temperate Asia at high altitudes (1000–4000 m). The wild grown type is rarely present in Serbian flora (Josifovic 1970 – 86),

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it was recorded in Mt. Radan (Ranđelović et al. 2005), and there are attempts for its cultivation in the mountainous regions of Central Serbia (Vukomanovic and Stepanovic 1996). However, it is commercially grown in Belgium, Hungary and Germany (Peter 2004).

A. sylvestris L., wild or woodland angelica, has relatively similar morphological traits to *A. archangelica* (Yankova and Cherneva 2007; Dihoru et al. 2011). This species usually grows in moist rough grassland, in shaded areas, on the banks of rivers and streams at altitudes up to 850 m (Grime et al. 1988). *A. sylvestris* is not an official drug, but it has been used in traditional medicine in European countries (Sarker et al. 2003). The new investigation with this plant showed that its root and seed possess good antimicrobial activity (Canli et al. 2016; Aćimović et al. 2016). However, both species are very similar, often mistaken for one another. The morphological differences are mostly quantitative: stem size, number of rays in the composite umbel, size of leaf sheaths (Yankova and Cherneva 2007).

According to Dihoru et al. (2011) these two species can be properly distinguished only by mature fruit material. *A. archangelica* L. has ellipsoid to semiglobose flattish fruit. From the dorsal side there are three intermedial low crowded ridges, two lateral ridges, while the ventral side is wing-shaped without visible vittae. Fruits are 7.0–8.0 mm in length and 4.0–5.0 mm in width. The surface of the whole fruit is light brown (Bojnansky and Fargasova 2007). On the other side, *A. sylvestris* L. fruit is ovoid to ellipsoid and flattish. From the dorsal side there are prominent crowded ridges, low and slightly winged, while lateral ridges are very broadly winged. On the ventral side there are two vittae. Fruits are 4.5–5.5 mm in length and 3.7–4.3 mm in width. The surface of fruit is dull, glabrous, valleculae dark brown, while the ridges are pale brown. Nevertheless, the position of the seed in the plant affected their size and proportion (Pop et al. 2011).

However, there are papers about differences between these two species according to the morphological traits (Yankova and Cherneva 2007; Bojnansky and Fargasova 2007; Dihoru et al. 2011), but there is no data about the comparative differences between their essential oil compositions. Techniques based on Headspace are important for studying the composition of the volatile fraction, in addition to or as an alternative to other sampling techniques; monitoring the biological phenomena involved with the volatile fraction of a plant; discriminating between species, subspecies, varieties, cultivars, or chemotypes; and quality control of plant samples (Belliardo et al. 2006). Apart from this, Headspace sampling for gas chromatographic analysis has many advantages, the most important being the elimination of many interferences arising from the sample matrix. In addition, the standard methods for headspace sampling have been accepted because they are simple and inexpensive (Fakhari et al. 2005).

In the study conducted in China, headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) was used for the analysis of the volatile constituents present in *A. pubescens* and *A. sinensis* roots. HS-SPME is a powerful tool for determining the volatile constituents present in the medicinal plants (Song et al. 2004). Because of this, the aim of our investigation was to determine the composition of volatile compounds of *A. archangelica* and *A. sylvestris* seed by using headspace extraction.

2. Materials and methods:

2.1. Plant Material and Extraction

The seed of *A. archangelica* L. (Budapesti Kertimag ZRT, Hungary) was bought at local a market, while the seeds of *A. sylvestris* were collected from wild grown plants at locality Borkovac, Serbia (45°02' N, 19°49' E, 169 m asl) (Picture 1).

The dried seed of both species were ground in a mill just prior to the headspace sampling. Each sample (1.0 g) was filled into a 10 mL vial respectively, and then the vials were hermetically sealed with a PTFE coated rubber septum and an aluminum cap (Agilent Technologies GC sampler 80 with headspace).

2.2. GC and GC/MS analysis

Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, the USA), in positive ion electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 μm film thickness. The GC oven temperature was programed from 60°C to 285°C at a rate of 3°C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 ml/min at 210°C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: MS source temperature, 230°C; MS Quad temperature, 150°C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

3. Results and discussion:

3.1.Results

It is found that *A. archangelica* seed consists of 29 components among which the dominant is *β*–phellandrene (84.7%), followed by *α*–phellandrene (3.4%), α–pinene (2.5%), myrcene (2.1%) and *α*–copaene (1.3%). All other compound was presented less than 1% (Table 1). A typical chromatogram of the *A. archangelica* seed volatile compounds is shown in Picture 2.

The *A. sylvestris* seed consists of 22 volatile compounds and the dominant are limonene (62.7%) and *α*–pinene (28.0%), followed by camphene (2.6%), *α*–phellandrene (2.0%) and *β*–pinene (1.0%). All other compound was presented less than 1% (Table 1). A typical chromatogram of the *A. sylvestris* seed volatile compounds is shown in Picture 3.

3.2. Discussion

According to a study dealing with *A. archangelica* grown wild in Lithuania, the seed essential oil obtained by hydrodistillation contains 67 compounds, in which *β*–phellandrene was the dominant constituent (33.6–63.4%), followed by *α*–pinene (4.2–12.8%), *α*–phellandrene (2.6–7.4%), sabinene (2.5–4.6%) and *d*–germacrene (0.4–3.0%) (Nivinskienë et al. 2005). The seed of *Angelica archangelica* from Siberian Region contains 21 compounds in the essential oil, with *β*–phellandrene (49.3%), *α*–pinene (15.1%), *β*–pinene (7.79%) and Δ^3–carene (7.5%) as the main compounds (Shchipitsyna and Efremov 2011). The essential oil of Angelica seed from Iceland contains *β*–phellandrene (up to 55.2%) and *α*–pinene (14.4–41.4%) in different relative amounts depending on chemotype (Sigurdsson et al. 2005b).

However, in Turkey it was established that the composition of the fruit essential oil of *A. sylvestris* L. var. *sylvestris* depends on the isolation method (Özek et al. 2008). According to this study, *α*–pinene (9.2–36.2%), bornyl acetate 4.3–7.3%, *α*–champigrene (3.4–9.1%), *β*–phellandrene (3.2–9.9%), *β*–sesquiphellandrene (2.5–8.7%), limonene (2.1–5.6%), myrcene 1.3–4.4% and camphene (1.2–4.7%) were found as the main constituents in the oil.

Previous study with other aromatic plants established that many factors influence the essential oil composition, among which are the growing location, i.e. bioclimatic area, as well as extraction techniques (Jemia et al. 2014, Aliboudhar and Tigrine-Kordjani 2014). According to this, the differences which occurred between our results and the results of other researchers can be the consequence of this.

4. Conclusions:

There are 29 volatile extracted by headspace technique in the seed of *A. archangelica*, among which the dominant is *β*–phellandrene (84.7%), followed by *α*–phellandrene (3.4%), α–pinene (2.5%), myrcene (2.1%) and *α*–copaene (1.3%). The seed of *A. sylvestris* consists of 22 volatile compounds, and the dominant are limonene (62.7%) and *α*–pinene (28.0%), followed by camphene (2.6%) *α*–phellandrene (2.0%) and *β*–pinene (1.0%).

Table 1. *A. archangelica* and *A. sylvestris* seed essential oil composition

Compound	R.t.	RI	*A. archangelica*	*A. sylvestris*
α–pinene	5.789	932	2.5	28.0
camphene	6.189	941	0.1	2.6
sabinene	6.861	966	0.4	0.4
β–pinene	6.970	974	0.4	1.0
myrcene	7.344	988	2.1	0.9
α–phellandrene	7.800	1003	3.4	2.0
Δ^3–carene	7.999	1008	0.1	nd
p–cymene	8.482	1022	0.8	0.3
limonene	8.744	1025	nd	62.7
β–phellandrene	8.745	1025	84.7	nd
cis–β–ocymene	8.917	1030	0.2	nd
trans–β–ocymene	9.294	1040	0.2	nd
terpinolene	10.833	1084	0.1	0.1
NI	14.039	1166	nd	0.1
NI	14.416	1177	0.1	0.2
cryptone	14.896	1182	0.1	nd
carvone	17.275	1240	nd	0.2
o–actenil–*p*–cresole	18.788	1271	nd	0.1
bornyl acetate	19.139	1283	nd	0.4
NI	20.440	1313	0.1	tr
NI	21.378	1321	0.1	nd
NI	22.893	1366	0.1	nd
α–copaene	23.277	1371	1.3	nd
daucene	23.311	1377	nd	0.1
β–bourbonene	23.672	1383	0.1	nd
trans–caryophyllene	25.160	1419	0.1	0.3
β–copaene	25.583	1427	0.2	nd
α–humulene	26.624	1452	0.6	0.1
trans–β–farnesane	26.777	1456	nd	0.4
γ–muurolene	27.806	1480	0.4	tr
NI	27.860	1481	0.1	nd
α–zingiberene	28.421	1495	0.4	nd
α–muurolene	28.622	1500	0.1	nd
β–bisabolene	28.977	1508	0.2	nd
NI	29.603	1523	0.1	tr
γ–bisabolene	30.257	1542	nd	0.1
β–germacrene	30.939	1555	0.3	nd
NI			0.5	0.3
Monoterpene hydrocarbons			**0.6**	**0.3**
Oxygenated monoterpenes			**95.0**	**98.0**
Sesquiterpene hydrocarbons			**0.1**	**0.7**

Oxygenated sesquterpenes	**3.7**	**1.0**
Total	**99.4**	**100**

R.t. (Retention time) is in correlation with RI (Kovats Retention Index) for HP-5MS capillary column; percentages of single components was computed from the GC peak areas; tr-compound present less than 0.1% (in traces), nd-compound not detected.

Figure 1. Seeds of *A. archangelica* (left) and *A. sylvestris* (right).

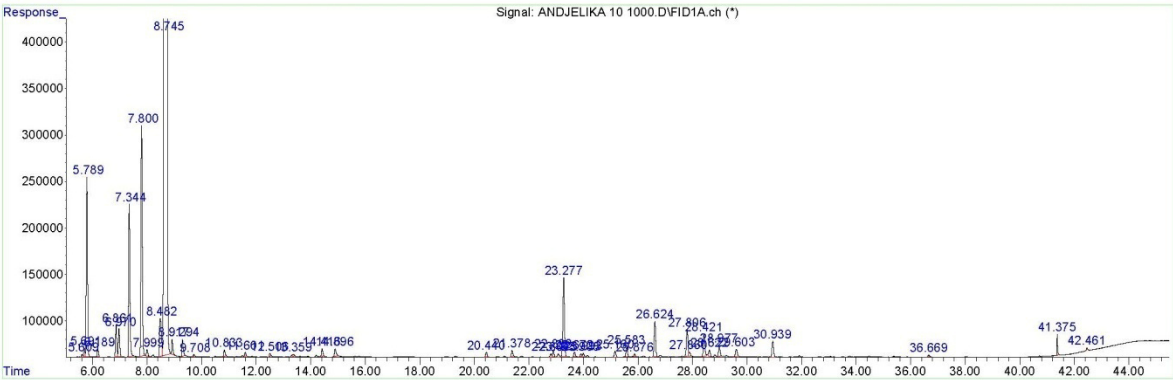

Figure 2. A typical chromatogram of the *A. archangelica* seed volatile compounds.

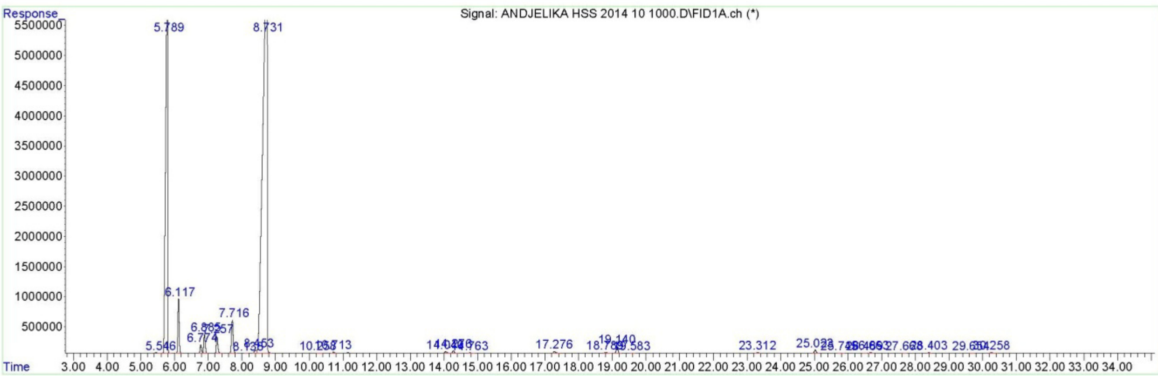

Figure 3. A typical chromatogram of the *A. sylvestris* seed volatile compounds.

Competing interests

The authors declare that they have no competing interests.

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