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RESEARCH ARTICLE

Volatile compounds of *Peucedanum chryseum* (Boiss. et Heldr.) Chamberlain Fruits

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Abstract

In this study, we report the isolation of volatile constituents of *Peucedanum chryseum* (Boiss. et Heldr.) Chamberlain fruits collected from Honaz, Denizli in Turkey. The volatile constituents obtained from whole fruits by micro-hydrodistillation were analyzed by GC and GC/MS, simultaneously. Thirty nine components of the whole fruits were characterized representing 99.4% of the sample. The major components of the whole fruits were α-pinene (72.8%) and β-pinene (20.4%). Monoterpene hydrocarbons were observed as the main constituents in the sample.

Keywords: *Peucedanum chryseum*; Gas Chromatography; Gas Chromatography/Mass Spectroscopy; pinene; monoterpene

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Introduction

Peucedanum L. belongs to the family Apiaceae and comprises about 200 species, which occur largely in Europe, Asia and Mediterranean region (Zargari, 1993). Davis, (1972) reported that the genus *Peucedanum* was represented in Turkey by 16 taxa of which 5 were endemic. But recent studies have shown that *Peucedanum* species in the Turkish flora are represented by 23 taxa of which 9 are endemic (Davis, 1972; Duran, 2014). The genus *Peucedanum* is rich in aromatic plants, but taxonomically many of its species are still unresolved. Because characterization of each *Peucedanum* species by anatomical and morphological features is difficult, chemical analysis of essential oils has played an important role in identifying chemotaxonomic markers (Skalicka-Wozniak, Los, Glowniak, & Malm, 2008; Sarkhail, 2014).

Fruits of several members of the family Apiaceae are used as carminatives as they are rich in essential oil and have an antispasmodic effect. Many of these species are also important as spice or condiment (Heinrich, Barnes, Gibbons, & Williamson, 2004; Tanker, Koyuncu, & Coşkun, 2007). Some *Peucedanum* species have been used in local medicine to treat various conditions, including sore throat, coughs, colds, headaches, asthma, angina, cramps, epilepsy, gastrointestinal disorders, rheumatism, gout, and cardiovascular problems, and as a chemopreventive agent and antifebrile (Sarkhail, 2014). Because of extensive uses in folk medicine, the phytochemical studies are still promising for these species.

Coumarins (Chen et al., 1996; Urbain, Marston, & Hostettman, 2005; Skalicka-Wozniak, Mroczek, Garrard, & Glowniak, 2009; Yu et al., 2012) and essential oils (Fraternale, Giamperi, Ricci, & Manunta, 2000; Rustaiyan, Komeilizadeh, Mojab, & Khazaie, 2001; Cisowski, Sawicka, Mardarowicz, Asztemborska, & Luczkieewicz, 2001; Masoudi, Akhgar, & Rustaiyan, 2004; Skalicka-Wozniak, Los, Glowniak, & Malm, 2009) are considered to be the main constituents in nearly all *Peucedanum* plants and can be responsible for many of their biological and pharmacological activities. According to the published data, *P. chryseum* was studied for its fatty acid composition by Akpınar et al. (2012). But the volatile composition of *P. chryseum* has never been studied before. Our results can be useful for the chemotaxonomic point of view of *Peucedanum* species (Akpinar et al. 2012; Sarkhail, 2014).

Materials and Methods

Plant Material

The fruits of *P. chryseum* (2 g) were collected from Honaz, Denizli, Menteş Village in Turkey on 17th July 2008 (GPS data: 37 44 147 N, 29 20 026 E). The plant was identified by one of us (AD). Voucher specimens are kept at the Herbarium of Selçuk University, Konya, Turkey (AD 8068).

Isolation of the volatile compounds

The whole fruits were subjected to micro-hydrodistillation. Micro-hydrodistillation method which is applicable to very small amounts of plant material (<1 g of material), is reliable and faster than other classical techniques. It is also used for the isolation of volatiles for subsequent analysis of aromatic materials in minute amounts (Bicchi, D'amato, Nano, & Frattini, 1983; Baser, Demirci, Demirci, Kirimer, & Hedge, 2001). The microscale hydrodistillation of the sample is performed using a 20 mL crimp-cap glass vial with a Teflon®-lined rubber septum containing 10 mL water and <1 g of material to be investigated. This vial, which is placed in a heating block, is connected with a cooled receiver vial by a 0.32 mm I.D. fused silica capillary. By temperature-programmed heating of the sample vial, the water and the volatile constituents are vaporized and passed through the capillary into the cooled receiver vial. There, the volatiles as well as water are condensed and the essential oil collected in *n*-hexane for further analysis. The received analytical results have been compared to results from identical samples obtained by conventional hydrodistillation showing a good correlation of the qualitative and quantitative composition (Kubeczka, 2010).

The distillation procedure is described at below:

The volatiles were obtained by microdistillation of dry whole fruits (500 mg) using an Eppendorf MicroDistiller® with 10 mL distilled water per sample vial (See in Figure 1). The sample vial was heated to 108°C at a rate of 20°C/min and kept at this temperature for 90 min, then heated to 112°C at a rate of 20°C/min and kept at this temperature for 30 min. The sample was subjected to a final post-run for 2 min under the same conditions. The collecting vial, containing a solution of sodium chloride (2.5 g) and water (500 µL) plus *n*-hexane (350 µL) to trap volatile components, was cooled to –5°C during distillation. After the distillation was completed, the organic layer in the collection vial was separated and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) systems, simultaneously. The distillation procedure was duplicate.

GC and GC/MS analyses

The GC analysis was carried out using an Agilent 6890N GC system. Flame ionization detector (FID) temperature was 300°C. To obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m*/*z* 35 to 450.

Identification of the volatile compounds

The components of the sample were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty & Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Alkanes (C8-C21) were used as reference points in the calculation of relative retention indices (RRI) (Curvers, Rijks, Cramers, Knauss, & Larson, 1985). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Results and Discussion

Thirty eight components of the whole fruits were characterized representing 99.4% of the sample. The major components were α-pinene (72.8%) and β-pinene (20.4%). The detailed information is given in Table 1. Table 1 summarizes the identified compounds with their retention indices and relative percentages of the total volatile compounds. Monoterpene hydrocarbons were observed as the main constituents in the sample.

Literature research indicated that the essential oil composition of the genus *Peucedanum* is important to reveal chemotaxonomic variations. In *P. scoparium* oil collected from Sanandaj to Divandareh, province of Kordestan, 33 components, which represented about 99.3% of the total composition of the oil, were identified. The oil of *P. scoparium* consisted mainly of 12 monoterpene hydrocarbons (86.8%), 11 oxygenated monoterpenes (8.3%) and 10 sesquiterpenes (4.2%). α-Pinene (39.6%), β-pinene (23.9%) and β-phellandrene (9.5%) were the major components (Masoudi, Akhgar, & Rustaiyan, 2004). An analysis of leaf and fruit essential oils of *P. austriacum* from Serbia by GC and GC/MS resulted in identification of 141 different components, representing 93.6% and 96.1% of the total oils. The most abundant class of compounds in fruit essential oil was monoterpenoids (64.0%) and in the leaf essential oil sesquiterpenoids (82.0%). The major contributors of fruit essential oil were β-phellandrene (45.2%) and α-pinene (10.1%). The major constituents of leaf essential oil were caryophyllene oxide (23.1%), germacrene D (12.2%) and (*E*)-caryophyllene (10.2%) (Jovanovic et al. 2013). The oils constituents from rhizomes, leaves and fruits of *P. petiolare* (DC.) Boiss. collected from Tehran province have been studied, the major constituents of rhizome oil were found to be β-bisabolene (31.3%), (*E*)-sesquilavandulol (20.5%), geranyl acetate (5.7%), citronellyl acetate (5.2%) and sabinene (5.2%); the main components of leaf oil were sabinene (42.3%), α-pinene (42.6%) and limonene (2.6%) and in the fruits, α-pinene (47.3%) and sabinene (45.9%) were the major volatile compounds (Mirza, Navaei, & Dini, 2005). Rustaiyan et al. (2001) reported essential oil of the aerial parts of *P. petiolare*. Among the ten components (97.6%) which he identified in this oil, sabinene (57.8%) and δ-3-carene (36.2%) were found to be the major constituents (Rustaiyan et al. 2001). A very recent study reported the chemical composition of *P. longifolium* Waldst. & Kit. essential oil and headspace (HS) volatiles obtained from the fresh aerial parts collected from Serbia. The major contributors of the essential oil were found as β-elemene (24.7%), (*E*)-β-ocimene (11.7%), *cis*-linalool oxide (furanoid) (5.7%), (*Z*)-β-ocimene (5.6%) and α-humulene (5.2%). MS analysis gave the identification of thirty-one components, representing 98.7% of the total volatiles. The main constituents were: (*E*)-β-ocimene (28.5%), (*Z*)-β-ocimene (11.9%), α-pinene (8.9%), myrcene (8.5%), *p*-cymene (6.4%), limonene (6.3%), sabinene (6.0%) and α-phellandrene (5.6%) (Jovanovic, Zlatkovic, Jovanovic, Petrovic & Stojanovic, 2015). Bartnik et al. (2015) recently isolated two compounds, guaia-1(10),11-diene and guaia-9,11-diene, from the fruits of *P. tauricum* M. B. (Bartnik & Komsta, 2015). The herbal parts of *Peucedanum luxurians* which is endemic to Armenia was found to be rich in *trans*-α-farnesene, germacrene D, α-zingiberene and β-caryophyllene while the fruits were rich in α-phellandrene, γ-elemene, β-curcumene (Widelski, Grzegorczyk, Malm, Chinou, & Glowniak, 2015).

Kapetanos et al. (2008) reported that the essential oil compositions of six *Peucedanum* species from Central Balkans. In all *Peucedanum* species, the dominant component was (-)-α-pinene (4.0 – 38.7%). *P. alsaticum* L., *P. austriacum* (Jacq.) Koch, and *P. oreoselinum* (L.) Moench are characterized by the presence of (+)-α-pinene (15.0, 8.0, and 15.2%, resp.). Only *P. cervaria* (L.) Cuss stands out from the other species of the genus *Peucedanum,* with a domination of (+)-limonene (11.5%), *p*-cymene (7.7%), and α-phellandrene (3.5%). According to their results, monoterpene hydrocarbons were identified as the main components for these species (Kapetanosa, Kariotia, Bojovic, Marinc, Veljic, & Skaltsa, 2008).

Some *Peucedanum* species growing in Turkey were investigated for their essential oil compositions. Tepe et al., (2011) revealed the esssential oil compositions of aerial parts from *P. longifolium* (Waldst. & Kit.) and P. palimbioides (Boiss.) collected from Karabuk and Ankara, Turkey, respectively. The *P. longifolium* essential oil was found to be rich in sesquiterpenoids. The major volatiles were 8-cedren-13-ol (33.7%), myristicin (8.0%), germacrene-D (7.7%) and δ-3-carene (6.4%). The essential oil of *P. palimbioides* was found to be rich in monoterpenes with major compounds followed by α-pinene (35. 5%), (*E*)-9-octadecenoic acid (23.6%) and β-pinene (20.2%) (Tepe, Akpulat, & Sokmen, 2011).

Based on the literature search, monoterpene hydrocarbons are the main components of *Peucedanum* oils occurring in many *Peucedanum* species, including *P. officinale*, *P. schotti, P. scoparium, P. petiolare P. alsaticum*, *Peucedanum austriacum*, *Peucedanum oreoselinum*, *Peucedanum longifolium* and *Peucedanum cervaria*, α-pinene is the major component of essential oils. However, some species are dominated by other monoterpenes or sesquiterpenes (Skalicka-Wozniak, Los, Glowniak, & Malm, 2008; Tepe et al., 2011; Sarkhail, 2014). According to our results, the fruit oils of *P. chryseum* obtained by micro-hydrodistillation are rich in α-pinene and β-pinene.

Table 1. Composition of the volatile compounds of *P. chryseum* (Boiss. et Heldr.) Chamberlain

Retention time	**RRI**	**Volatile Compounds**	**A %**	**Method of identification**
9.0	1032	α-Pinene	72.8	t_R , MS
10.8	1076	Camphene	1.0	t_R , MS
12.7	1118	β-Pinene	20.4	t_R , MS
15.4	1174	Myrcene	0.6	t_R , MS
16.2	1188	α-Terpinene	0.2	t_R , MS
17.0	1203	Limonene	1.5	t_R , MS
17.6	1218	β-Phellandrene	0.6	t_R , MS
18.8	1246	(*Z*)-β-Ocimene	0.1	t_R , MS
19.2	1255	γ-Terpinene	0.4	t_R , MS
19.7	1266	(*E*)-β-Ocimene	< 0.1	MS,
20.4	1280	*p*-Cymene	0.1	t_R , MS
20.9	1290	Terpinolene	0.2	t_R , MS
23.2	1348	6-Methyl-5-hepten-2-one	< 0.1	MS,
24.6	1382	*cis*-Alloocimene	0.1	MS,
27.2	1452	*p*-Cymenene (dehydro-*p*-cymene)	< 0.1	MS,
27.7	1466	α-Cubebene	< 0.1	MS,

28.3	1482	(*Z*)-3-Hexenyl-2-methyl butyrate	< 0.1	MS,
28.9	1498	Campholenal	< 0.1	MS,
29.0	1499	α-Copaene	0.1	MS,
30.6	1550	β-Cubebene	< 0.1	MS,
30.7	1553	Linalool	< 0.1	t_R , MS
31.8	1586	Pinocarvone	< 0.1	MS,
31.9	1590	Fenchyl alcohol	< 0.1	MS,
32.0	1591	Bornyl acetate	0.1	MS,
32.3	1600	β-Elemene	< 0.1	MS,
32.6	1611	Terpinen-4-ol	0.3	t_R , MS
32.7	1612	β-Caryophyllene	0.2	t_R , MS
33.7	1648	Myrtenal	< 0.1	MS,
34.4	1670	*trans*-Pinocarveol	< 0.1	t_R , MS
35.5	1706	α-Terpineol	0.4	t_R , MS
36.0	1711	Germacrene D	0.1	t_R , MS
37.1	1763	Naphthalene	< 0.1	t_R , MS
37.3	1773	δ- Cadinene	0.1	t_R , MS
38.2	1804	Myrtenol	< 0.1	MS,
39.1	1838	(*E*)-β-Damascenone	< 0.1	MS,
39.3	1845	*trans*-Carveol	< 0.1	t_R , MS
39.9	1868	(*E*)-Geranyl acetone	< 0.1	MS
43.5	2008	Caryophyllene oxide	0.1	t_R, MS
TOTAL			**99.4**	

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