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GC-MS Analysis of Essential Oil and Volatiles from Aerial Parts of *Peucedanum tauricum* M.B. during the Phenological Period

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Abstract: Widespread worldwide *Peucedanum* plants (Apiaceae) have been used for centuries as plant medicines. The polymorphism of this genus is consistent with chemotaxonomically and therapeutically significant differences in the composition of secondary metabolites. GC-MS of *Peucedanum tauricum* M.B. volatiles from the headspace (HS) and hydrodistilled essential oil (HD), both obtained simultaneously from flowers (FL), immature and ripe fruits (IF, MF) and leaves (L1-L3) collected at the time of harvesting of generative organs, show differences in the chemical profiles of HS and HD from the same parts of the plant, and between organs (FL, IF, MF vs. L1-L3). The presented studies on the variability of biometabolites in the phenological period indicated the optimal harvesting time, focused on two molecular chemotaxonomic markers of PT; guaia-1(10),11-diene and guaia-9,11-diene (in generative organs iHD at 25.5–32.1% and 26.8–33.6%; and in their HS at 29.4–41.3% and 25.0–29.4%, respectively). This is the first report on the analysis of fresh aerial parts of *Peucedanum* sp. in which GC-MS of HS and HD was performed simultaneously during the vegetation period. The importance, with possible limitations, of GC-MS analysis of HS and HD as an evaluation tool useful in the chemotaxonomy of *Peucedanum* plants was also discussed.

Keywords: biomolecules; *Peucedanum* sp.; headspace volatiles; HS-SPME; hydrodistillation; essential oil; GC-MS; chemotaxonomy; guaia-1(10),11-diene; guaia-9,11-diene

Citation: Bartnik, M. GC-MS Analysis of Essential Oil and Volatiles from Aerial Parts of *Peucedanum tauricum* M.B. during the Phenological Period. *Separations* **2023**, *10*, 484. https://doi.org/10.3390/separations10090484

Academic Editor: Paraskevas D. Tzanavaras

Received: 11 August 2023
Revised: 31 August 2023
Accepted: 2 September 2023
Published: 4 September 2023

1. Introduction

The genus *Peucedanum* (family Apiaceae) is a large group comprising more than 120 species (29 lists Flora Europaea), distributed widely in Europe, Asia and Africa [1,2]. The anatomical and morphological polymorphism of this genus is in accordance with differences in the composition of its secondary metabolites. The composition of the essential oils of many *Peucedanum* sp. have been studied previously (which will be discussed later in this paper) and the most common extraction technique used was hydrodistillation, the classic method recommended for many Pharmacopoeias. This time-consuming procedure is performed in the presence of boiling water, and the applied conditions (temperature, time and the presence of water) can cause degradation or chemical rearrangement of less stable compounds. Solid phase microextraction (SPME) has been introduced as a modern alternative to traditional sample preparation technology [3]. This technique eliminates the use of organic solvents and shortens the time of sample preparation before analysis. In one approach, a partitioning equilibrium between the sample matrix and the extraction phase is reached [4–6]. One of the modifications of SPME is a headspace technique (HS-SPME), which enables analysis of target compounds (volatiles) found in the space over the sample [7]. HS-SPME was successfully applied to many types of liquid and gaseous samples, including samples from food or from medicinal plants [8–10]. When applied to a living organism (e.g., a plant), the removal of only small amounts of the emitted analyte should not result in a significant disturbance of the homeostasis.

The presented work was focused on *Peucedanum tauricum* M.B. (=*Peucedanum tauricum* M.Bieb.) which is a rare perennial plant, growing in nature on dry hillsides and in

pinewoods in the Crimea, the Caucasus, and Romania [1,11,12]. Coumarins [13–19], phenolic acids [20], flavonoids [21] and essential oils (obtained by hydrodistillation of dried ripe fruit) [22] have been previously studied in this species, resulting in the isolation of two new compounds from the essential oil of its fruits [16,17]. Interesting changes of coumarin content during development of the plant were previously detected in aerial parts of *P. tauricum* [18]. The presented study is part of investigations into this genus with regards to the accordance of variability of secondary metabolites during plant development, which is interesting from phytochemical and chemotaxonomic points of view. This study indicated optimal harvesting time for the isolation of secondary metabolites, especially new compounds guaia-1(10),11-diene and guaia-9,11-diene, which can be used for further biological activity studies and could be considered as chemotaxonomic markers of this species. This is the first report on the analysis of fresh aerial parts of *Peucedanum* plants, in which GC-MS of HS and HD was performed simultaneously during the vegetation period. The importance, with possible limitations, of the GC-MS analysis of HS and HD as an evaluation tool useful in the chemotaxonomy of *Peucedanum* plants was also discussed.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *Peucedanum tauricum* M.B. (Apiaceae) were collected in the Botanical Garden of the Maria Curie-Skłodowska University (51°16′ N, 2°30′ E; 200 m AMSL, Lublin, Poland) every three weeks during the growing period (from 11 July to 3 September). The plant material was identified by taxonomist Krystyna Dąbrowska MSc. Representative samples of the leaves and the reproductive organs; leaves at the time of flowering (L1), flowers (FL), leaves when the fruits were immature (L2), immature but formed fruits (IF), leaves when the fruits were matured (L3), and mature fruits (MF) were collected and sampled in accordance with Polish Pharmacopoeia 6th ed. rules [23], and when fresh, were hydrodistilled (HD) or extracted by HS-SPME (HS).

2.2. Reagents and Chemicals

Dichloromethane (>99.5%; GC) was purchased from J.T. Baker (Deventer, Netherlands). The mixture of *n*-alkanes (C8–C24) used for the retention index (RI) analyses were purchased from Sigma-Aldrich (Steinheim, Germany). An anhydrous sodium sulphate was of analytical grade (Merck, Germany). An ultrapure water (18.2 MΩ), as obtained from a Simplicity (Millipore, Molsheim, France) purification system was used.

2.3. Hydrodistillation of the Essential Oil

Hydrodistillation was performed using Deryng apparatus (3 h)—the procedure was carried out in accordance to method described in Polish Pharmacopoeia 6th ed. [23]. Accurately weighed fresh plant samples (leaves L1–L3; 30 g of each), flowers (FL; 15 g of each) and, independently, immature and mature fruit (IF and MF; 10 g of each, respectively) were hydrodistilled. After each hydrodistillation, the essential oil was kept in an amber vial over anhydrous sodium sulphate (and stored at temp. 4 °C until analysis, for no longer than 48 h). For each type of sample over mentioned procedure was repeated in the same conditions (n = 2). Before analysis the essential oil from each sample was diluted in dichloromethane (1:20, *v*/*v*). Quantitative estimation of the essential oil content (calculated on dry mass of the plant samples ± SD) was also performed.

2.4. HS-SPME Analysis of Plant Samples

A manual SPME apparatus Supelco (Bellefonte, PA, USA) was used. It consisted of a nonpolar polydimethylsiloxane (PDMS) 30 µm fiber (Supelco, USA) that was retracted inside a needle. It was exposed to the headspace above the sample matrix. A 0.1 g (±0.001) amount of *P. tauricum* solid samples (fresh plant material) was in each case hermetically sealed in a screw top amber vial (5 mL) with a polypropylene hole cap and PTFE septa and it was equilibrated during the equilibrium time at the desired temperature. Next, the SPME

device was inserted manually into the sealed vial by penetrating the septum and the fiber was exposed to the volatiles emitted to the headspace above the plant material during the extraction time. After sampling (maintaining the equilibrium between headspace and the polydimethylsiloxane coating of the SPME fiber) [24,25], the SPME needle was retracted from the vial and immediately manually inserted into the GC injector. The analytes were thermally desorbed from the PDMS-SPME fiber. Two replicates of samples were processed in the same way. The optimal temperature of SPME sampling (25 °C) and time of absorption (20 min.) were established during experiments (times 20 and 30 min, and temperatures 20, 25 and 30 °C were tested). Desorption in the GC port was set at 250 °C for 2 min [23,26,27]. Before each sampling, the fiber was reconditioned for 20 min in the GC injector port at 250 °C.

In the GC-MS analysis a response was based on the sum of the peak areas of the compounds. The fresh samples were chosen for experiments.

2.5. Gas Chromatography-Mass Spectrometry

GC-MS analyses were performed using gas chromatograph Thermo Trace GCQ with a mass spectrometer ITS-40 (Thermo Finnigan, San Jose, CA, USA). The DB-5 fused silica capillary column (30 m length, 0.25 mm I.D., 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used. A sample with a volume of 1 µL was injected in split mode 1:20. The flow rate of the carrier gas (He) was 1 mL/min. For the analysis of the HD and HS a different temperature program was set, taking into consideration different samples analyzed in HD and HS (HD in a liquid form, and HS as a gaseous phase evaporated from the SPME fiber) [16,26]. The temperature program for GC-MS analysis of the HD was isothermal for 2 min. at 35 °C, which was then raised to 300 °C at the rate of 4 °C/min. At 300 °C the temperature was kept constant for 15 min. The temperature program for GC-MS analysis of the HS started at 35 °C for 2.5 min. and then increased at the rate 8 °C/min to 250 °C, which was kept constant for 1 min. MS spectra were acquired using electron impact ionization (EI, 70 eV) over a range of 35–400 m/z in full-scan acquisition mode. The inlet temperature was 250 °C, ion source temperature was 220 °C and transfer line temperature 280 °C. The identification of the compounds was based on the comparison of the MS fragmentation pattern with those from the laboratory-built MS spectral database, and using the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) MS spectral database (62,000 spectra), the LIBR (TR) terpene library supplied by Finnigan MAT, and literature data [28,29]. The identification of the compounds was based on comparison of their retention indices (RI), obtained using various *n*-alkanes (C8–C24), which enables independent verification of the data, and cross-referencing between applications [30]. The relative amounts (Ras) of individual components were expressed as a percentage of peak areas relative to total peak area detected on the chromatogram.

3. Results

The amount of distilled essential oil from independent experiments for each part of the plant was calculated on the dry mass in mL/kg ± SD and was in leaves; L1: 0.21 ± 0.05; L2: 0.18 ± 0.02; L3: 0.27 ± 0.03; in flowers (FL) 11.30 ± 1.15; in immature fruits (IF) 17.20 ± 1.13; and in mature fruits (MF) 20.93 ± 1.87.

As the result of GC-MS analyses, 45 phytochemicals have been identified in the essential oil and the headspace above the plant matrix. The results are presented in Table 1. The compounds detected were predominantly sesquiterpenes (70.5%) and ca. 30% monoterpenes, mainly as alcohols and esters. The essential oil from leaves contained: trans-β-ocimene (3.9–22.7%), β-elemene (11.5–31.1%), germacrene D (13.3–31.4%), €-β-caryophyllene (2.7–9.2%), τ-cadinole (2.4–3.3%), α-selinene (2.1–6.9%) as the main compounds. The flower essential oil was characterized by myrcene (4–6.4%), guaia-1(10),11-diene (25.5–28.9%) and guaia-9,11-diene (24.2–26.8%). In the fruit essential oil, guaia-1(10),11-diene (26.1–32.1%), guaia-9,11-diene (28.6–33.6%), guaia-6,9-diene (2–2.4%) were detected.

Table 1. Compounds detected in GC-MS analysis of HS volatiles and HD essential oil from *P. tauricum* M.B. aerial parts.

Nº	Compound Name	RI	L1		FL		L2		IF		L3		MF	
			HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD
1	***cis*-3-hexen-1-ol**	846	**9.4**	-	-	-	-	-	-	-	-	-	-	-
2	1-hexanol	860	2.3	-	-	-	-	-	-	-	-	-	-	-
3	α-pinene	925	-	0.6	tr	0.2	-	0.2	tr	0.6	-	-	tr	0.7
4	camphene	942	-	0.8	-	0.2	-	-	-	-	-	-	-	-
5	β-pinene	978	0.3	-	0,1	-	-	-	-	-	-	-	-	-
6	**myrcene**	988	1.1	1.2	**5.0**	**6.4**	1.4	**6.0**	0.2	tr	0.9	0.2	0.2	0.5
7	***cis*-hexenyl 3-acetate**	995	**12.9**	-	-	-	-	-	-	-	-	-	-	-
8	Δ^3-carene	1004	-	0.8	-	0,3	-	-	-	-	-	-	-	-
9	hexenyl acetate	1007	2.3	-	-	-	-	-	-	-	-	-	-	-
10	p-cymene	1022	-	-	-	-	-	0.2	tr	-	-	-	-	-
11/12	limonene + β-felandrene	1026	1.8	0.4	1.3	1.4	0.4	2.0	0.1	0,1	0.3	0.4	0.1	0.2
13	*cis*-β-ocimene	1035	0.5	0.3	0.1	0.3	0.3	2.0	tr	-	0.3	-	tr	-
14	***trans*-β-ocimene**	1046	**6.3**	**3.9**	0.9	**3.6**	1.2	**22.7**	0.1	-	1.7	-	0.1	-
15	γ-terpinene	1057	0.3	-	0.1	-	-	-	-	-	-	-	-	-
16	terpinolene	1083	-	-	0.3	0.2	-	-	-	-	-	-	-	-
17	**camphor**	1140	**2.1**	-	**0.8**	-	-	-	-	-	-	-	-	-
18	bornyl acetate	1288	0.6	4.4	0.2	1.3	-	-	-	-	-	-	-	-
19	α-copaene	1380	0.7	0.4	0.4	0.1	0.6	0.5	0.1	tr	1.4	0.5	0.2	tr
20	NN	1385	0.4	-	0.1	-	0.5	1.0	0.2	-	0.9	2.1	0.2	0.3
21	β-bourbonene	1386	0.7	2.0	0.3	0.5	1.6	0.7	0.1	0.2	0.8	1.0	0.4	0.3
22	**β-elemene**	1392	**6.6**	**19.5**	**2.2**	**5.3**	**9.6**	**11.5**	**3.4**	**5.6**	**17.3**	**31.1**	**3.1**	**3.7**
23	**(E)-β-caryophyllene**	1423	2.9	**6.6**	2.7	1.6	**4.9**	2.7	1.3	1.7	**8.2**	**9.2**	1.7	1.1
24	**α-guaiene**	1439	0.2	-	0.6	1.8	-	-	0.5	0.2	-	-	0.5	0.2
25	**guaia-6,9-diene**	1445	1.9	-	4.0	-	-	-	3.5	2.4	-	-	3.8	2.0
26	NN	1454	8.6	-	8.1	3.6	-	-	6.8	4.4	-	-	6.9	3.5
27	**(Z)-β-farnesene** + α-humulene	1460	-	**7.8**	-	2.5	**56.6**	2.6	-	-	**7.3**	0.8	-	-
28	α-humulene	1460	0.8	-	0.6	-	0.5	-	1.8	2.7	0.8	-	2.1	2.1
29	NN	1478	1.8	-	9.7	5.0	0.4	0.1	8.7	6.3	0.7	0.3	10.2	6.0
30	NN	1482	0.6	-	2.8	1.8	-	-	2.6	2.2	-	-	2,4	2.0
31	**germacrene D**	1486	**18.2**	**31.4**	**2.7**	**1.8**	**17.8**	**13.3**	**0.7**	**1.1**	**52.2**	**25.4**	**0.5**	**0.3**
32	β-selinene	1493	0.3	0.6	1.3	0.9	-	0.2	1.3	1.2	-	1.0	1.4	1.3
33	NN	1495	-	-	-	-	-	-	-	0.4	-	-	-	1.1
34	α-selinene	1501	1.1	2.1	0.4	0.2	-	1.0	0.2	0.3	1.7	2.1	0.2	0.6
35	α-muurolene	1503	-	-	-	-	-	0.1	-	-	-	0.3	-	-
36	α-bulnesene	1506	-	-	0.6	0.5	0.3	-	0.8	0.7	-	-	0.7	0.7
37	germacrene A	1513	0.5	2.1	0.2	0.4	-	1.6	-	0.5	-	2.4	-	0.4
38	**guaia-1(10),11-diene**	1526	**3.7**	**-**	**29.4**	**25.5**	**0.9**	**-**	**41.3**	**32.1**	**1.3**	**-**	**34.7**	**26.1**
39	**guaia-9,11-diene**	1530	**2.8**	**-**	**25.0**	**26.8**	**0.7**	**0.4**	**25.0**	**33.6**	**0.8**	**-**	**29.4**	**28.6**
40	NN	1535	-	-	-	0.7	-	-	-	tr	-	-	-	1.3
41	4-β-hydroxygermacra-1(10),5-diene	1585	-	-	-	0.6	-	-	-	-		-	-	-
42	caryolan-1-ol	1583	-	-	-	-	-	-	-	-	-	-	-	tr
43	spathulenol	1584	-	-	-	-	-	1.6	-	-	-	0.8	-	-
44	caryophylene oxide	1589	-	0.4	-	-	-	3.8	-	-	-	2.5	-	0.6
45	NN	1601	-	-	-	-	-	-	-	0.1	-	-	-	1.4

Table 1. *Cont.*

Nº	Compound Name	RI	L1		FL		L2		IF		L3		MF	
			HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD	HS SPME	HD
46	humulene epoxide	1617	-	-	-	-	-	0.2	-	tr	-	-	-	0.6
47	NN	1620	-	-	-	-	-	-	-	0.2	-	-	-	3.1
48	germacrene D-4-ol	1630	-	-	-	-	-	-	-	0.2	-	0.5	-	0.5
49	NN	1630	-	-	-	-	-	-	-	-	-	0.3	-	-
50	NN	1634	-	-	-	-	-	-	-	tr	-	-	-	2.7
51	cubenol	1635	-	-	-	-	-	tr	-	-	-	tr	-	-
52	NN	1637	-	-	-	-	-	-	-	0.8	-	-	-	1.5
53	eudesmol	1640	-	-	-	2.3	-	-	-	-	-	-	-	-
54	τ-cadinol	1653	-	-	-	-	-	3.3	-	0.3	-	2.4	-	0.4
55	eudesm-3-en-7-ol	1655	-	0.6	-	0.6	-	-	-	-	-	-	-	-
66	NN	1656	-	-	-	-	-	-	-	0.1	-	-	-	1.9
57	α-cadinol	1665	-	0.3	-	-	-	1.5	-	0.1	-	1.7	-	tr
58	NN	1673	-	0.4	-	-	-	-	-	0.9	-	-	-	1.3
59	NN	1679	-	-	-	-	-	3.0	-	-	-	1.4	-	-
60	NN	1719	-	-	-	-	-	2.6	-	-	-	0.8	-	-
61	NN	1737	-	-	-	-	-	2.7	-	-	-	1.3	-	-

NN—not identified, tr—traces (less than 0.01%), HS-SPME—headspace volatiles, HD—hydrodistilled essential oil, L1—leaves in time of flowering, FL—flowers, L2—leaves in time of immature fruit, IF—immature fruit, L3—leaves in time of mature fruit, MF—mature fruit, (n = 2).

Representative TIC chromatograms obtained for samples L2 (HS) and IF (HS) are presented in Figure 1a,b, respectively.

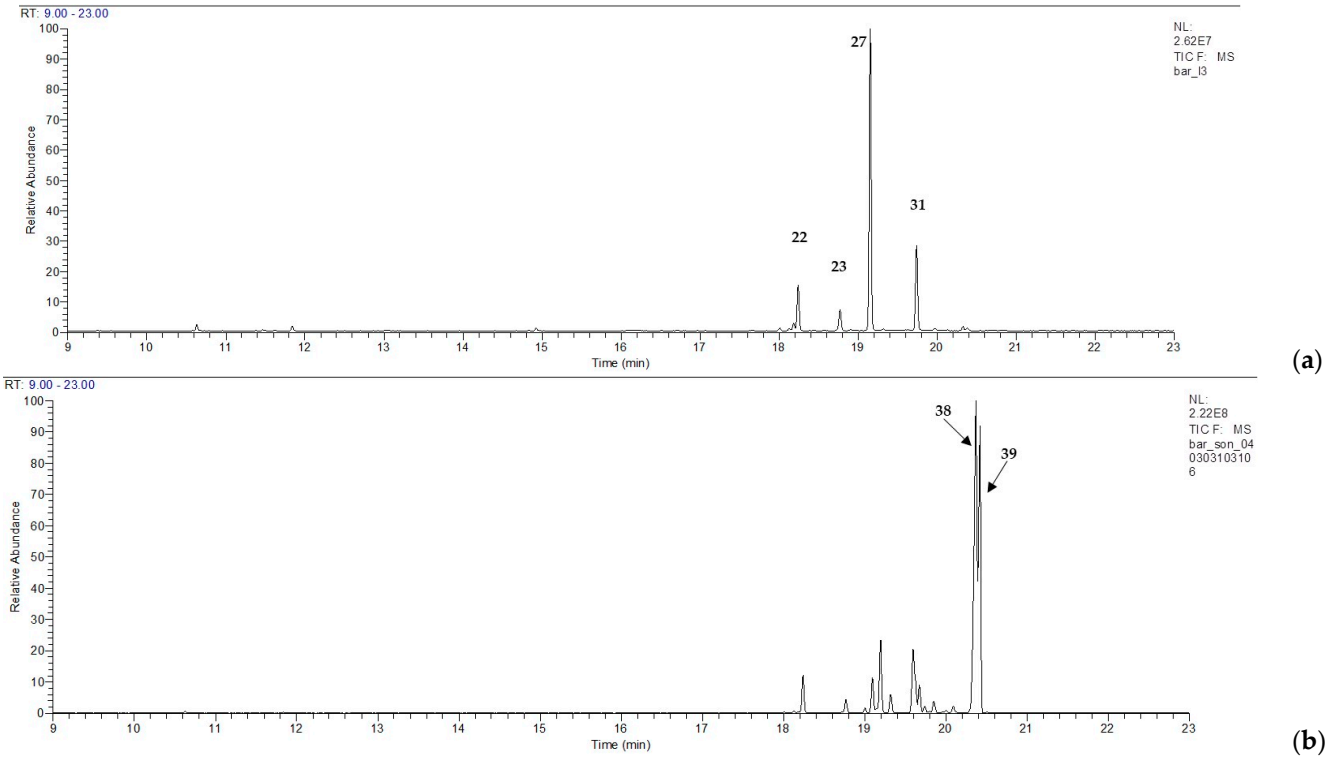

Figure 1. Representative TIC chromatograms obtained in GC-MS analysis of the headspace of the samples L2 (**a**), IF (**b**) of *P. tauricum* M.B. The compounds were numbered as in the Table 1.

In the analyzed samples we could observe only in HS of the leaves in time of flowering the presence of *cis*-3-hexen-1-ol (9.4%) and *cis*-hexenyl 3-acetate (12.9%).

α-Pinene was detected in traces in HS of generative organs (FL, IF, MF) and in the corresponding HD only in a small amount (less than 0.7%), and *ß*-pinene was detected in HS only in leaves in time of flowering (L1 and FL samples).

Myrcene was found mainly in flowers HS and HD (5.0 and 6.4%, respectively) and also in L2 HD in time of fruit maturation (6.0%). The amount of myrcene in IF and MF was not higher than 0.2% in HS. Only traces of this compound were found in the HD of IF and 0.5% was found in the HD of MF. *Trans-ß*-ocimene was present in a high amount (22.7%) in leaves (L2) during the time of immature fruit. Camphor was found to be emitted only into the HS and constituted 2.1% of L1, and 0.8% of FL from all of the detected HS volatiles. *ß*-Elemene was detected in leaves in bigger amount than in generative organs (in L1: 6.6% in HS and 19.5% in HD; in L2: 9.6% in HS and 11.5% in HD; in L3: 17.3% in HS and 31.1% in HD). It was also observed, that amount of this compound increases during vegetation period of the plant. In generative organs *ß*-elemene constituted in FL; 2.2% in HS (and 5.3% in HD); in IF 3.4% in HS (and 5.6% in HD); and in MF 3.1% in HS (and 3.7% in HD).

(E)-*β*-caryophyllene is the compound found in all of tested samples, however the detected amount in leaves was higher than in generative organs. In L1: 6.6% in HD and 2.9% in HS; in L2: 2.7% in HD and 4.9% in HS; in L3: 9.2% in HD and 8.2% in HS.

α-Guaiene was present in the HS and HD of FL, IF and MF, and in a low amount in the HS of L1 samples (0.2%). Similarly, guaia-6,9-diene was detected in both, HS and HD, mainly in IF and MF samples, in FL only in HS (4.0%), and in small amount (1.9%) in volatile fraction of the HS of leaves in the time of flowering.

(Z)-*ß*-farnesene was found in the HD of L1 (7.8%), L2 (2.6%) and in L3 (0.8%). It should be noticed, that the amount of this compound constituted 56.6% of the volatile fraction (HS) in L2 (when fruits are immature), and much lower (7.3% in HS) in L3, when fruits are already matured.

Germacrene D was detected mainly in leaves; in HS (L1; 18.2%; L2 17.8%; L3 52.2%), and in HD (L1 31.4%; L2 13.3%; L3 25.4%). In generative organs only FL contained germacrene D in HS (2.7%) and in HD (1.8%). IF and MF, in both HS and HD contained less than 1.1% of this compound.

The presence of guaia-1(10),11-diene (G1/10/11) and guaia-9,11-diene (G9/11) was detected in the HS (FL 29.4–25.0%; IF 41.3–25.0% and in MF 34.7–29.4%), and in the HD essential oil (FL 25.0–26.8%; IF 32.1–33.6% and in MF 26.1– 28.6%) in the generative organs, where they were predominant compounds. In leaves these sesquiterpenoids were found only in HS in amount not higher than 3.7% (G1/10/11) and 2.8% (G9/11).

As we could observe the generative plant organs (FL, IF, MF) show different profile, of the essential oil components and headspace volatiles, as compared to leaves collected in the respective time of vegetation period (in the time of flowering; L1, in the time of immature fruits; IF, and in the time of mature fruits; MF).

4. Discussion

As was reported, *Peucedanum* plants' essential oil could be a source of active ingredients such as e.g., in the case of *P. dhana* A. Ham, where essential oil is active against *E. coli*, *P. aeruginosa* and *E. aerogenes*, and also possesses significant cytotoxic activity against human colon adenocarcinoma SW480 [31], or in the case of *P. oeroselinum* (L.) Moench essential oil, which was found to be a source of P-glycoprotein inhibitors in in vitro tests [32]. Therefore, the isolation and analysis of essential oil composition is of great interest not only from a chemotaxonomic but also from a biomedical point of view.

There are differences in the mono- and sesquiterpene compositions in *Peucedanum* plants. In some species e.g., *P. dhana* A. Ham HD essential oil from fruits contains mainly monoterpenes (98.25% of the total essential oil composition) [31]. The other *Peucedanum* species e.g., *P. cervaria* or *P. alsaticum*, are constituted mainly from sesquiterpenoids or in equal amounts of mono- and sesquiterpenoid compounds. The composition of the

headspace and the essential oil of the *P. tauricum* plant parts is constituted mainly of sesquiterpenoids, however in leaves, especially in time of flowering we observe almost equal amount of both groups.

In the time of fruit maturation composition of the HS is different when compared with time of flowering or with time when fruits are matured and vegetative period ends. As we know plants could release some substances (e.g., coumarins) on his surface as the part of plant defense system [33]. Some components presented in the headspace of *P. tauricum* leaves could play a similar preventive role in the time of fruit maturation, as in the case of the high presence (56.6%) of Z-(ß)-farnesene in the L2 HS (leaves in period of immature fruits). The presence of this compound decreases to 7.3% in L3 HS (leaves in time where fruits are finally mature). It should be noticed, that this compound is believed to involve in a plant chemical defence, possess DPPH free radical scavenging, antibacterial and antifungal activity, and demonstrates, dose-related neuroprotective effects on cultured rat primary cortical neurons in in vitro study [34]. *Cis*-3-hexen-1-ol, 1-hexanol, *cis*-3-hexenyl acetate, hexyl acetate, β-pinene, γ-terpinene and camphor were detected only in headspace of *P. tauricum*, and pinenes (α- and β-pinene) were found only in small amounts in HS and HD essential oil in contrast to *P. palimbioides* where in aerial parts α-pinene constituted 35.45% and β-pinene 20.19% of the total hyrodistilled essential oil [35]. Similarly in *P. scoparium* where in the aerial parts amounts of pinenes was 39.6% (α-pinene) and 23.9% (β-pinene) [36]. Additionally, in *P. officinale* from Serbia, pinenes were predominant compounds in all of the investigated organs (leaves, flowers, stems and rhizome) being; 24.8, 7.2, 5.2, 28.1% (α-pinene) and 17.6, 9.9, 8.7, 2.7% (ß-pinene), respectively [37]. In *P. cervaria* from Vienna (Austria) predominant monoterpenes in the essential oil were pinenes (7–58% β-pinene and 7–22% α-pinene). Similarly in *P. alsaticum* oils were made up mainly by α-pinene (11–40%), sabinene (16–34%) and β-phellandrene (12–31%) [38].

As the result of the present study it was found, that no sabinene was found neither in *P. tauricum* HD essential oil nor in the analysed HS. As was detected in previous studies, sabinene is present in essential oils from some *Peucedanum* species e.g., *P. alsaticum, P. oreoselinum, P. cervaria, P. grande, P. verticillare* and *P. petiolare* [39–45].

Only small amount of limonene and β-phellandrene were observed in *P. tauricum* HD essential oil. The presence of limonene characterize some essential oils from plants of *Peucedanum* genus; e.g., *P. grande*—10.14% [43], *P. zenkeri*—23.2% [46] or *P. oreoselinum*—44.1–82.4% [40], *P. palustre*—87.53% [47], and *P. officinale* from Serbia (11.3–28.2%) [37]. In contrast in *P. tauricum* less than 1.8% of limonene was detected (in fruits even less than 0.2%).

The changes in the composition of the HD essential oil and the HS during development of the *P. tauricum* plant were observed; e.g., in leaves, germacrene D content increases in HS during the time of mature fruits to 52.2% from ca. 18% in leaves collected during the earliest phases of plant vegetation. In contrast, the relatively low content of this compound in flowers (2.7%) decreases in immature fruits (IF) to 0.7% and in mature fruits (MF) to 0.5%. If compared to, e.g., *P. alsaticum* fruits with a high germacrene D content in their HS (6.9–8.7%) and HD essential oil (7.9%) [39], in *P. tauricum* fruit HS and HD essential oil, a relatively low content of this compound could be significant. In is worth to underline, that also in *P. ruthenicum* M.B. which is chemotaxonomically close to *P. tauricum*, germacrene D was detected only in the essential oil from flowers (1%) [48].

The presence of β-elemene in leaves of *P. tauricum* increases in HS from 6.6% in the time of flowering, 9.6% in time of immature fruits to 17.3% in leaves harvested when fruits were completely matured (RAs in HD essential oil from leaves were; 19.5%, 11.5% to 31.1%, respectively). In reproductive organs content of β-elemene was about 3% in the HS, and in FL, IF and in MF RAs in HD essential oil were; 5.3%, 5.6% and 3.7%, respectively. As we observed in each plant part the amount of the β-elemene detected in HD is much higher than in respective HS. It should be noted, that β-elemene could be formed by rearrangement from germacrene A during distillation (as shown in composition of the HD essential oil

from leaves), and probably also during GC analysis, due to the high temperature of the injector [49–52].

HS-SPME technique of sampling, when compared with HD, could show differences which could be considered as artifacts formed in process of extraction of the essential oil by hydrodistillation in the water environment and in the heat conditions, and also during analysis.

However, data interpretation and comparison in case of the essential oil and HS studies has several limitations, which should be always taken into consideration. The evaluation of the composition of essential oil from different species from the same family depends on many factors (especially as we consider quantitative composition of the investigated essential oil) such as genotype of the plant (chemotypes), environmental conditions (stress factors—biological, mechanical and chemical, soil composition, climate and pollution), seasonal variations and finally plant organ used for evaluation [53].

Extraction method has also influence on the essential oil composition—especially hydrodistillation, which is an aggressive and time consuming method, resulting in decomposition of some unstable compounds and its chemical rearrangement [54,55]. Additionally, using either fresh or dried samples of the plant for analysis results in different observations. Some differences between fresh plant samples and plant samples after drying were observed e.g., in case of *P. verticillare* where fresh fruit essential oil contained mainly sabinene (63%), whereas in the essential oil of dry fruit β-caryophyllene (24%) and α-phellandrene (21%) were predominant compounds [44].

Endemic species are characterized by different profile of secondary metabolites, e.g., endemic species from Corsica; *P. paniculatum* Loisel. essential oil is characterized by lavandulyl- and cyclolavandulyl esters [56]. In *P. cervariifolium* from Iran, a high amount of essential oil α-guaiene was observed in the aerial parts (11.9%), but it was not indicated in which aerial part of the plant [57].

P. tauricum, as also an endemic plant, growing in wild in Romania and Caucasus, is characterized by an unique profile of the essential oil and headspace secondary metabolites. The presence of sesquiterpenes as; α-guaiene, guaia-6,9-diene and two major compounds: guaia-1(10),11-diene and guaia-9,11-diene, previously detected [16,21], isolated and identified as new compounds found only in the essential oil from *P. tauricum* [16,17] chemotaxonomically characterize investigated essential oil (and the HS) if compared to those extracted from other plants from *Peucedanum* L. genus; e.g., closely related *P. ruthenicum* M.B., without guaienes and with major compounds such as; thymol (18.29% in leaves), myrcene (10.68%) and germacrene B (10.06%) in flowers, caryophyllene oxide (13.65%), 1–8 cineole (11.15%) and 8,9-dehydroisolongifolene (11.33%) found in fruits [48]. All of these compounds are absent in *P. tauricum* essential oil from respective parts of the plant, and also in the respective headspace. On the other hand, the β-selinene absent in *P. ruthenicum* fruits is present at a volume of ca. 1.3% in all of the reproductive organs of *P. tauricum*.

In recently analyzed hydrodistilled essential oil from aerial parts (herb) of *P. luxurians* Tamamsch., the main compounds were; *trans*-ß-farnesene (16.35%), germacrene D (13.76%), α-zingiberene (10.58%), ß-sesquiphellandrene (3.72%), ß-caryophyllene (8.84%), δ-elemene (5.10%) and myrcene (1.33%) [58].

The HS-SMPE method is non-aggressive, and it is possible to establish comparable conditions for each evaluated sample, and also observe in situ plant expression of volatiles because the SPME device is suitable to bring it on the place of plant growth. As reported previously *P. cervaria* Lap. (L.) [42] and *P. alsaticum* L. [39], and essential oil and headspace of dry fruits from cultivated plants and plants from the natural site were analyzed. In case of *P. cervaria* HD and HS the main components were α-pinene (31.3% and 32.7%), ß-pinene (21.7% and 19.6%) and sabinene (31.0% and 38.6%), with little presence of limonene (3.6% and 3.3%), and with presence of germacrene D and ß-caryophyllene in very little amount (0.1–0.3%). In case of *P. alsaticum* similar composition was observed with predominant amount of sabinene (22.0% and 27.6%), limonene and ß-phellandrene (18.7% and 13.9%), with presence of germacrene D and ß-caryophyllene in less than 9%. These results indicate

close chemotaxonomic relation between these two species, and also with other selinene and pinene containing plants from *Peucedanum* genus such as *P. oreoselinum*, *P. petiolare*, *P. scoparium* and *P. schottti* [42].

Additionally, in *P. longifolium* (Waldst. & Kit.) an analysis of HD and volatiles from HS was performed [59]. Similar components in HS and HD were detected, however they were found in a bigger amount in the HS as compared to HD essential oil (HS vs. HD, in %); limonene (6.3 vs. 3.4), α-pinene (8.9 vs. 2.2), p-cymene (6.4 vs. 0.4), myrcene (8.5 vs. 3.1), (E)-β-ocimene (28.5 vs. 11.7), (Z)-ß-ocimene (11.9 vs. 5.6), sabinene (6.0 vs. 3.7) and α-phellandrene (5.6 vs. 0.5). In the HD the predominant compounds were; β-elemene (24.7%), cis-linalool oxide (furanoid, 5.7%), and α-humulene (5.2%).

The plant material consisted of fresh leaves and young stems. As the result it was found, that monoterpenes constituted 87.1% of the HS and only 46.0% of the HD.

In this work, for the first time, the analysis of volatiles from the plant matrix (HS) and hydrodistilled essential oil (HD) was carried out, comparing material from fresh parts of *P. tauricum*, which was collected during the vegetation period, and the subject of the study were leaves, flowers and fruits (immature and mature). This work brings new data to the chemical characterization of *Peucedanum* plants, which is still important from the aspect of chemotaxonomy of this botanical genus [2,60,61].

5. Conclusions

The HS-SPME technique combined with GC-MS allowed the observation of changes in the composition of the analyzed headspace during the vegetation period of the plant and, compared to hydrodistillation, to inform about the decomposition of the essential oil during the classic distillation procedure. In this context, it can be considered as a useful tool for chemotaxonomic studies of *Peucedanum* species. HD and HS-SPME can be used as complementary extraction techniques to obtain broad characterization of plant volatiles in both hydrodistilled oil and emitted to the space above plant matrix.

As a time and solvent saving extraction technique, HS-SPME when used in chemotaxonomic studies can be used to obtain a quick fingerprint of the plant's headspace and can be helpful in protecting the biodiversity of the plant kingdom when rare or endangered plants are the subject of research.

The presence of the main guaienes (guaia-1(10),11-diene and guaia-9,11-diene) in the analyzed *P. tauricum* samples, both in the HS and HD essential oils, confirms that they are not artifacts and can be isolated from hydrodistilled essential oils without their decomposition under the applied conditions. It should be noted that immature but fully formed fruits can be considered the best plant material for the isolation of essential oil rich in these main components for further analysis of their possible biological activity.

Funding: GC-MS experiments and data acquisition was financed by Medical University statutory funds DS 26.

Data Availability Statement: Not applicable.

Acknowledgments: The author sincerely thanks the Botanical Garden UMCS for enabling collection of the plant material for experiments. The author, with the consent of the recipient, sincerely thanks Marek Mardarowicz for information on GC-MS analyses.

Conflicts of Interest: The author declares no conflict of interest.

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