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Evaluation of the Chemical Composition and Antioxidant Activity of the Essential Oils of *Peucedanum longifolium* (Waldst. & Kit.) and *P. palimbioides* (Boiss.)

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Abstract: This study was designed to evaluate the chemical composition and antioxidant activity of the essential oils of *Peucedanum longifolium* and *P. palimbioides*. According to the GC-MS analysis, 35 compounds were accounted for 96.98% of the total oil of *P. longifolia*. Major components of the oil were 8-cedren-13-ol (33.74%), myristicin (8.03%), germacrene-D (7.73%) and Δ-3-carene (6.38%). In the case of *P. palimbioides*, 26 compounds were identified comprising the 98.96% of the total oil. The major compounds were described as α-pinene (35.45%), (E)-9-octadecenoic acid (23.57%) and β-pinene (20.19%). In general, *P. palimbioides* oil exhibited stronger than that of *P. longifolia* in all test systems. Especially in β-carotene/linoleic acid system, *P. palimbioides* showed strong activity against linoleic acid oxidation at 2.0 mg/mL concentration (90.58% ± 1.56). This value is too close to the synthetic antioxidants BHT and BHA at the same concentration (95.86% ± 0.23 and 93.05% ± 1.04, respectively). This plant also exhibited excellent chelating effect (90.39% ± 0.28) which is obviously greater than that of *P. longifolium* at 2.0 mg/mL concentration (24.12% ± 2.50). Chelating effect of the control agent EDTA was measured as 98.78% ± 0.78 (2.0 mg/mL).

Keywords: *Peucedanum longifolium; Peucedanum palimbioides;* antioxidant activity; essential oil; chemical composition.

1. Introduction

Reactive oxygen species (ROS) including singlet oxygen ($_1O^2$), superoxide ion (O_2^-), hydroxyl ion (OH·) and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules generated in cells

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under normal metabolic activities. However, in response to a variety of factors including tobacco smoke, pollutants, ionizing radiations, alcohol, synthetic pesticides, and solvent, their production increases [1]. ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA and they have also been linked to pathogenesis of oxidative stress [2]. Living cells possess an excellent scavenging mechanism to avoid excess ROS-induced cellular injury; however, with aging, and under influence of external stresses these mechanisms become inefficient and dietary supplementation of synthetic antioxidants is required. In recent years, due to toxicological concerns associated with the use of synthetic substances in food and increasing awareness about natural foods, there has been an increasing interest in the use of natural substances as food preservatives and antioxidants [3]. In this context, aromatic plants, particularly their essential oils, are being evaluated for antioxidant activity [4-6]. It is thus pertinent to evaluate the natural antioxidant activity of essential oils since they extensive use in the food and beverage industry.

The genus *Peucedanum* is a large group comprising more than 120 species, widely distributed in Europe, Asia and Africa. *Peucedanum longifolium* and *P. palimbioides* are species occurring naturally in Turkey but also in central and Eastern Europe. As with all plants belonging to the Apiaceae family, they are rich in coumarins and essential oils, but also some phenolic acids have been investigated in genus *Peucedanum*. However the chemical composition of *P. palimbioides* is not known.

In the present paper, we analyzed the antioxidant activity of the essential oils of two unknown member of the genus Apiaceae, *P. longifolia and P. palimbioides*, with their chemical compositions. By this way, we hope to bring the new natural sources to the literature as antioxidant agents.

2. Materials and Methods

2.1 Chemicals

Potassium ferricyanide, ferrous chloride, ferric chloride, methanol, and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt. Germany). 1.1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and α-tocopherol (TOC) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH. Sternheim. Germany). All other chemicals and solvents are of analytical grade.

2.2. Plant Material

P. longifolia was collected from Keltepe district, Karabuk-TURKEY (910 m) in 09 August 2006 (GPS coordinates: N: 41° 05 ' 18.5 " EO: 32 ° 30 ' 09.9 "). Locality for *P. palimbioides* was Kalecik district, Ankara-TURKEY (1149 m, GPS coordinates: N: 40° 08 ' 51.4 " EO: 33 ° 20 ' 45.4 "). This plant was collcted in 07 August 2006. The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: AA 4211 and AA 4208, respectively).

Aerial parts of the plant species were submitted for 3 hours to water-distillation using a Clevenger-type apparatus (yields 0.75%, and 0.53% v/w, respectively). The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4 °C until tested and analysed.

2.3. Gas Chromatography-Mass Spectrometry (GC/MS)

GC–MS analyses were performed by using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m · 0.32 mm i.d., film thickness 0.25 lm). The carrier gas used was helium, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 ^{o}C. One microliter essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 ^{o}C for 2 min and then increased to 260 ^{o}C with a 5 ^{o}C/min heating ramp and subsequently kept at 260 ^{o}C for 13 min. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator.

2.4. Total antioxidant activity by β-Carotene–linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [7]. A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade). 25 µL linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added with vigorous shaking; 2.5 mL of this reaction mixture was dispersed to test tubes and 0.5 ml of essential oils (2.0 mg/mL) in methanol were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of *β*-carotene disappeared. The bleaching rate (R) of *β*-carotene was calculated according to Eq. (1).

$$R = \ln (a/b)/t \qquad (1)$$

Where, ln=natural log, *a*=absorbance at time 0, *b*=absorbance at time *t* (120 min) [8]. The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using Eq. (2).

$$AA = [(R_{Control} - R_{Sample}) / R_{Control}] \times 100 \qquad (2)$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 2.0 mg/mL and blank consisting of only 0.5 mL methanol.

2.5. Scavenging effect on DPPH

The hydrogen atoms or electrons donation ability of the corresponding samples were measured from the bleaching of purple colored methanol solution of DPPH. The effect of essential oils on DPPH radical was estimated according to Hatano et al. [9]. 4 mL of various concentrations (0.25-1.50 mg/mL) of the essential oils in methanol was added to a 1 mL of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$$I\% = 100 \times (A_{Control} - A_{Sample}) / A_{Control}$$

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. BHT and BHA were used as a control.

2.6. Reducing power

The reducing power was determined according to the method of Oyaizu [10]. Each sample (1-6 mg/mL) in methanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6)

and 2.5 mL of 1% potassium ferricynide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of 10% TCA were added, and the mixture was centrifuged at 200g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. BHT and BHA were used as a control.

2.7. Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. [11]. Briefly, 2 mL of essential oils (2.0 mg/mL) in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Total volume was adjusted to 5 mL with methanol and then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometricaly at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\textit{Metal chelating effect (\%)} = [(A_{Control}/A_{Sample})/A_{Control}] \times 100$$

Where $A_{Control}$ is the absorbance of control (The control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{Sample} is the absorbance of the test compound. Ethylenediaminetetraacetic acid (EDTA) was used as a control.

3. Results and Discussion

3.1. Chemical composition of the essential oil

GC-MS analysis of the essential oils of *P. longifolium and P. palimbioides* are confirmed by FID and the results are presented in Table 1.

In the first case, 35 compounds were accounted for 96.98% of the total oil (Table 1). As can be seen from these results, major components of the oil were 8-cedren-13-ol (33.74%), myristicin (8.03%), germacrene-D (7.73%) and Δ-3-carene (6.38%). Total percentages of monoterpenes, monoterpenoids, sesquiterpenes and sesquiterpenoids were calculated as 9.83, 1.58, 18.61, and 49.57%, respectively.

In the case of *P. palimbioides*, 26 compounds were identified including two unknown compounds comprising the 98.96% of the total oil (Table 1). As can be seen from the table, the major compounds were described as α-pinene (35.45%), (E)-9-octadecenoic acid (23.57%) and β-pinene (20.19%). According to the results presented in the table, monoterpenes were represented in the oil in highest percentage (65.27%). Sesquiterpenes were represented in a small quantity in the oil (0.07%).

To the best of our knowledge, essential oil composition of the some members of the genus *Peucedanum* (*P. cervaria, P. officinale, P. alsaticum, P. oreoselinum, P. austriacum, P. cervariifolium, P. petiolare, P. scoparium, P. ostruthium, P. verticillare, P. palustre*) have previously been reported [12-25].

Essential oil composition of *P. longifolium* has previously been reported by Kapetanos et al. [16]. According to this report, monoterpene hydrocarbones had identified as the main components. In contrast to this finding, we determined the sesquiterpenoids as the main components in *P. longifolium.* But in the case of *P. palimbioides*, monoterpenes were determined in a percentage of 65.27 in the oil. Several researchers concerned with chemical compositions of the essential oils of *Peucedanum* species have indicated the abundance of monoterpenes in the oils [15, 16, 23].

Table 1. Chemical composition of the essential oil of *P. longifolium* and *P. palimbioides.*

Compounds	**RT** [a]	**RI** [b]	***P. longifolium***	***P. palimbioides***
α-Thujene	4.93	930	-	1.94
α-Pinene	5.19	939	0.13	35.45
Camphene	5.59	956	0.07	3.11
Sabinene	6.31	974	0.11	-
β-Pinene	6.29	977	-	20.19
Myrcene	6.87	993	1.46	1.15
α-Phellandrene	7.31	1003	1.68	-
Limonene	7.95	1033	-	3.27
Δ-3-Carene	8.16	1035	6.38	-
E-β-Ocimene	8.67	1054	-	0.16
α-Pinene oxide	10.48	1099	-	0.23
α-Campholenal	11.67	1131	-	0.30
trans-Verbenol	12.41	1149	-	0.43
Unknown	12.69	1156	-	0.75
Pinocarvone	13.13	1166	-	0.86
Terpinen-4-ol	13.98	1182	-	0.30
Myrtenal	14.57	1198	-	0.51
Methyl chavicol	14.85	1201	-	3.27
Unknown	15.20	1213	0.60	-
Thymol, methyl ether	16.74	1231	-	0.14
Exo-Fenchyl Acetate	16.36	1232	0.12	-
Unknown	17.49	1268	0.31	-
Perilla aldehyde	18.40	1275	1.46	-
Bornyl acetate	18.33	1287	-	0.68
Trans-pinocarvylacetate	20.12	1302	-	0.37
Δ-Elemene	20.83	1337	1.24	-
α-Cubebene	22.09	1356	-	0.07
Unknown	21.41	1361	0.34	-
Unknown	22.80	1392	0.54	-
β-Elemene	23.21	1392	0.32	-
E-Caryophyllene	24.23	1428	0.62	-
β-Gurjunene	24.21	1438	0.39	-
δ-Elemene	24.91	1442	0.29	-
α-Humulene	25.70	1459	2.08	-
δ-Muurolene	26.45	1482	0.23	-
Germacrene D	26.87	1489	7.73	-
Bicyclogermacrene	27.40	1505	0.49	-
trans-β-Guainene	27.83	1507	1.77	-
Unknown	28.54	1512	0.56	-
Myristicin	29.01	1518	8.03	-
Myristicin	28.44	1523	-	0.27
Elemicin	29.78	1558	-	0.08
Germacrene B	29.83	1564	3.45	-
Unknown	30.26	1570	1.79	-
Spathulenol	31.07	1579	3.52	-

1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-,7-diene	31.36	1606	3.24	-
Isopathulenol	32.00	1622	3.93	-
8-Cedren-13-ol	33.35	1682	33.74	-
Unknown	34.62	1694	-	0.10
Unknown	34.64	1695	1.17	-
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	35.34	1715	2.64	-
7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	36.41	1746	2.50	-
Unknown	38.11	1793	3.27	-
Unknown	44.34	1981	0.78	-
n-Hexadecanoic acid	44.64	1990	-	1.16
(E)-9-Octadecenoic acid	50.17	2173	-	23.57
Unknown	57.37	2434	-	0.21
Unknown	58.56	2480	-	0.39
		Total Identification	**96.98**	**98.96**
		Compound Classes		
		Monoterpenes	9.83	65.27
		Monoterpenoids	1.58	7.09
		Sesquiterpenes	18.61	0.07
		Sesquiterpenoids	49.57	25.08

[a]RT, Experimental Retention Time (as minutes)
[b]RI, Experimental Retention Indices

3.2. Antioxidant activity

Antioxidant activities of the essential oils of *P. longifolium* and *P. palimbioides* were determined by four different test systems named as β-carotene/linoleic acid, DPPH free radical scavenging, reducing power and chelating effect. Results are presented in Table 2. To the best of our knowledge, no literature data is available on the antioxidant activities of the essential oils of plant species presented here. However, some other members of this genus had previously been reported with their antioxidant activities [26-29].

Polyunsaturated fatty acids, such as linoleic acid, are easily oxidized by the oxygen in the air. This auto-oxidation leads to the occurrence of chain reactions with the formation of coupled double bonds, and at a later stage also to obtaining secondary products, such as aldehydes, ketones, and alcohols. Using the β-carotene/linoleic acid method, essential oils of *P. longifolium* showed excellent antioxidant activity profile at 2.0 mg/mL concentration (Table 2). In the case of *P. palimbioides* (Table 2), essential oil showed strong activity against linoleic acid oxidation (90.58% ± 1.56). This value is too close to the synthetic antioxidants BHT and BHA (95.86% ± 0.23 and 93.05% ± 1.04, respectively).

The radical scavenging of the essential oils were tested using a methanolic solution of the ‘‘stable’’ free radical, DPPH. Unlike laboratory-generated free radicals such as the hydroxyl radical

and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition [30]. A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale.

The effective concentrations of the essential oils and reference compounds required to scavenge DPPH radical, the scavenging values as percentage; are presented in Table 2. As can be seen from the table, activity was increased with the increasing concentration of the oil samples. DPPH free radical scavenging abilities of the oils are almost equal to each other and they showed moderate activity profile when compared with the synthetic antioxidants.

Table 2 is also show the reducing power of the essential oils of plant species as a function of their concentration. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form.

The reducing power of the oils increased with concentration. Reducing power of *P. palimbioides* oil was found superior to *P. longifolium*. On the other hand, none of them showed activity as strong as the synthetic antioxidants.

Ferrous ions could stimulate lipid peroxidation by Fenton reaction, and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [31]. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions [32]. Accordingly, it is suggested that the low-to-moderate ferrous ions chelating effects of these extracts would be somewhat beneficial to protect against oxidative damage.

Table 2. Antioxidant activity of the essential oils of *P. longifolium* and *P. palimbioides* [a]

Assay	Concentration (mg/mL)	*P. longifolium*	*P. palimbioides*	BHT	BHA
β-Carotene Linoleic Acid (%)	2.00	70.73 ± 1.31	90.58 ± 1.56	95.86 ± 0.23	93.05 ± 1.04
DPPH (%)	0.20	8.59 ± 0.36	10.67 ± 1.63	93.85 ± 0.67	94.98 ± 0.14
	0.40	18.12 ± 0.36	22.12 ± 1.60	-	-
	1.00	41.87 ± 1.85	47.26 ± 1.52	-	-
Reducing power (absorbance, nm)	0.20	0.044 ± 0.015	0.035 ± 0.006	1.313 ± 0.043	2.495 ± 0.044
	0.40	0.078 ± 0.007	0.104 ± 0.006	-	-
	1.00	0.104 ± 0.006	0.248 ± 0.003	-	-
Chelating effect (%)	2.00	24.12 ± 2.50	90.39 ± 0.28	**EDTA** 98.78 ± 0.78	

[a] Values expressed are means ± S.D. of three parallel measurements

As can be seen from the table, essential oil of *P. palimbioides* showed obviously greater chelating effect (90.39% ± 0.28) than that of *P. longifolium* (24.12% ± 2.50). Chelating effect of the control agent EDTA was measured as 98.78% ± 0.78.

4. Conclusion

Searching wild sources may bring new natural products into the health industries with safer and better antioxidants that provide good protection against the oxidative damage, which occurs both in the body and our daily foods. Therefore, new unexplored plant species, as natural sources, should be identified. As far as our literature survey could as certain, there is no information about the antioxidant activities of the essential oils of *Peucedanum* species presented here. From this point of view, this study could be assumed as the first report on these species.

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