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Research Article

Chemical composition, antiradical and phytotoxic activity of the essential oil from *Peucedanum ostruthium* W.D.J.Koch leaves

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Abstract

Peucedanum ostruthium W.D.J.Koch, commonly known as masterwort, is a flowering perennial species in the Apiaceae family with known medicinal and aromatic properties. This study was designed to chemically characterize the essential oil (EO) obtained from the leaves and investigate some aspects of its bioactivity. Thirty-two compounds were detected by gas chromatography-mass spectrometry analysis and sesquiterpenoids identified as the dominating group of compounds. The major ones were caryophyllene oxide (20.7%) and spathulenol (17.2%), followed by cubenol (8.7%), δ-cadinene (6.1%) and humulene epoxide II (5.6%). EO was evaluated *in vitro* by ABTS·⁺ (2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) and DPPH· (2,2-diphenyl-picryl hydrazyl) assays, showing a marked scavenging ability, in particular towards the ABTS·⁺ radical cation (2.02 ± 0.00 μM Trolox eq/mL). EO was also screened for phytotoxic activity against mono- and dicotyledonous weeds. It exhibited significant effects by reducing the growth of *Lolium multiflorum* Lam. and *Sinapis alba* L. seedlings up to 90.7% and 76.6%, respectively.

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1. Introduction

Peucedanum ostruthium W.D.J.Koch (syn. *Imperatoria ostruthium* L.), commonly referred to as masterwort, is a perennial herbaceous species belonging to the Apiaceae family. Its native range is the mountains of central and southern Europe. In Italy, it grows spontaneously in the central and northern regions between 1000 and 2000 m. a.s.l. *P. ostruthium* is characterized by white flowers collected in umbrellas, an erect stem up to 40 cm high and tripartite leaves with a deeply serrated edge [1, 2]. Flowers, leaves and rhizomes are used in folk medicine for remedies administered both internally and externally to treat cardiovascular, digestive, integumentary, musculo-skeletal, respiratory and urogenital disorders. The rhizome is also appreciated for its flavoring properties exploited in the preparation of some drinks such as grappas and liqueurs [3-5].

Peucedanum species are known to possess a broad spectrum of pharmacological activities and many of them are due to the presence of coumarins, flavonoids, phenolics and essential oils [6]. The latter were investigated for their antimicrobial, antioxidant and cytotoxic [7-10] as well as pesticidal [11] effects obtaining promising results. The bioactivity of *P. ostruthium* essential oil (EO) has never been studied. Therefore, this work was designed to examine its antiradical and phytotoxic activity, after the characterization of the chemical composition.

2. Materials and methods

2.1 Plant material collection

The leaves of *P. ostruthium* were collected in August 2018 in the Valle di Champorcher (Valle d'Aosta, Italy) at 1955 m a.s.l and air dried, then stored in paper bags until pulverization with a laboratory blender. The species was identified according to Flora d'Italia [2] and the herbarium sample (No. PO-VC-VDA-18) was deposited at the Department of Agricultural and Environmental Sciences of the Milan State University (Italy).

2.2 Seeds

The seeds of *S. alba* were purchased from the company "Padana Sementi" situated in Tombolo (Padova, Italy) while those of *L. multiflorum* were supplied by the organic rice farm "Terre di Lomellina", located in Candia Lomellina (Pavia, Italy). After an appropriate selection, they were surface sterilized with a 1% bleach solution for 10 minutes, then rinsed repeatedly with distilled water until the disinfectant was completely removed.

2.3 Essential oil distillation

EO of the dried leaves from *P. ostruthium* was obtained by 3 h hydrodistillation in a Clevenger-type circulatory apparatus with 25 g of material and a yield of 0.68% w/w. Then, it was collected and combined with anhydrous sodium sulfate to remove water. EO was stored at 4 °C in a sealed vial until use.

2.4 Chemical analysis

To performe the analyses, a Clarus 500 model Perkin Elmer (Waltham, MA, USA) gas chromatograph coupled with a mass spectrometer and equipped with an FID (flame detector ionization) was used. Chromatographic separation was performed with a Varian Factor Four VF-1 capillary column flushed with helium at a flow rate of 1 mL/min. The applied operative conditions followed those reported in some previous works [12,13]. The mass spectra were obtained in the electron impact mode (EI), at 70 eV in scan mode in the range 35-400 m/z. The identification of volatile compounds was performed by matching their mass spectra with those stored in the Wiley 2.2 and Nist 02 mass spectra libraries database and by comparison of their linear retention indices (LRIs), relative to C8-C25 *n*-alkanes, with those available in the literature. To express the quantity of the components, we used the percentage values calculated in relation to the total area of the chromatogram by normalizing the peak area without the use of an internal standard and any factor correction. The analysis was carried out in triplicate.

2.5 Antiradical assays

DPPH assay

The radical-scavenging capacity of EO against DPPH· was assessed following Iriti et al. [14], with some modifications. Briefly, the DPPH· stock solution (0.35 g/L) was diluted with methanol to an absorbance of 1.00 ± 0.03 at 517 nm. Then, 50 µL of EO was added to 2.45 mL of this solution. After a reaction time of 30 min in the dark and at room temperature, the absorbance changes were monitored using a Jenway 6310 spectrophotometer (Keison, Chelmsford, Essex, UK). The obtained results are expressed as µM eq Trolox mL^{-1} EO. A DPPH· solution without EO was used as a control. The assay was performed in triplicate.

ABTS assay

The test was carried out following Iriti et al. [14]. The $ABTS^{+}$ radical cation was produced by reacting a solution of ABTS 7 mM with potassium persulfate 2.45 mM and keeping the mixture in the dark at room temperature for at least 6 h before use. The $ABTS^{+}$ solution was diluted with ethanol to an absorbance of 0.7 (±0.02) at 734 nm. Then, 1 mL of the working solution was mixed with 10 µL of EO for 30 s and the absorbance was read at 734 nm after another 20 s using a Jenway 6310 spectrophotometer (Keison, Chelmsford, Essex, UK). An $ABTS^{+}$ solution without EO was used as a control. The results were expressed as µM eq Trolox mL^{-1} EO. The assay was performed in triplicate.

2.6 Phytotoxicity Test

A dose-response assay was carried out following Vitalini et al. [15], with some modifications. 2 or 20 µL of EO was placed in an aluminum container inside 9 cm diameter Petri dishes to avoid direct contact with the seeds distributed on a layer of filter paper wetted with 4 ml of deionized water. The Petri dishes were

prepared in a vertical laminar flow hood and sealed with a double layer of parafilm to prevent the volatile compound escape. Then, they were transferred in a growth chamber at 16/8 h of light/dark photoperiod with a temperature of 25/18 °C for 7 days. The experimental design was as follows: 15 seeds of *L. multiflorum* or *S. alba* × 2 doses of EO or distilled water as a control × 3 replicates × 2 runs.

The seed germination (radicle ≥ 1 mm) was recorded daily. Root and shoot lengths of *L. multiflorum* and *S. alba* seedlings were measured at the end of the tests, after a week. The collected data were used to calculate four indices.

(i) Germination percentage (G) = Germinated seed number)/(Seed total number) × 100

(ii) Coefficient of Velocity of Germination (CVG) = N1 + N2 + . . . + Ni/100 × N1T1 +. . . + NiTi, where N is the number of seeds germinated every day; T is the number of days from seeding corresponding to N [16]

(iii) Mean Germination Time (MGT) = (∑D × Germinated seed number)/(∑Germinated seed number), where D is the number of days from the beginning of germination, plus the number of seeds germinated on day D [17]

(iv) Seedling Vigor Index (SVI) = (Mean Root length + Mean Shoot length) × Germination % [18].

3. Results and Discussion

3.1 Chemical analysis

The GC-MS technique was used to describe the chemical profile of *P. ostruthium* leaf EO. In total, 32 compounds, accounting for 99.6% of the total composition, were identified, and their average percentage values are shown in Table 1. EO was characterized by a sesquiterpene content exceeding the monoterpenic one and the main two components were caryophyllene oxide (20.7%) and spathulenol (17.2%), followed by cubenol (8.7%), δ-cadinene (6.1%), humulene epoxide II (5.6%) and *cis*-*p*-mentha-2,8-dien-1-ol (5.4%). All detected monoterpenes ranged from 0.1 to 0.9%. Two phthalates, namely diisobutyl phthalate (4.6%) and diethyl phthalate (1.3%), two fatty acids such as decanoic acid (0.3%) and palmitic acid (0.7%), and a diterpenoid, 13-epimanool (3.8%), were also found. The GC-FID chromatogram was reported in Fig. 1.

Table 1. Chemical composition (percentage values ± standard deviation) of *P. ostruthium* leaf essential oil.

Compounds[1]	LRI[2]	LRI [3]	Area (%)
α-Pinene	938	945	0.4±0.03
cis-Linalool oxide	1052	*	0.1±0.02
Linalool	1092	1088	0.4±0.02
Chrysanthenone	1106	1103	1.0±0.03
Cis-*p*-mentha-2,8-dien-1-ol	1120	1116	5.4±0.05
Dehydrolinalool	1121	1116	0.5±0.02
Trans-*p*-mentha-1(7),8-dien-2-ol	1171	1165	0.9±0.02
α-Terpineol	1183	1182	0.7±0.04
p-Cymen-8-ol	1186	1185	0.7±0.03
Trans-sabinene hydrate	1190	*	1.0±0.05
2,5-Bornanedione	1268	1264	0.5±0.03
Decanoic acid	1355	1353	0.3±0.02
α-Copaene	1378	1388	0.5±0.03
(-)-*β*-Bourbonene	1392	1390	0.5±0.02
Trans-geranyl acetone	1430	1432	0.2±0.02
β-Caryophyllene	1431	1434	3.5±0.02
Cis-muurola-3,5-diene	1451	1447	0.8±0.02
Humulene	1475	1473	3.0±0.03
Germacrene D	1493	1489	0.6±0.03
Valencene	1517	1515	2.7±0.02
δ-Cadinene	1521	*	6.1±0.02
epi-Cubebol	1530	*	3.4±0.03
Diethyl phthalate	1555	1551	1.3±0.02
Spathulenol	1578	1571	17.2±0.05
Caryophyllene oxide	1583	1580	20.7±0.04
Humulene epoxide II	1615	*	5.6±0.02
Cubenol	1635	1631	8.7±0.02
Ledene oxide II	1678	*	3.1±0.04
Hexaydrofarnesyl acetone	1855	1846	0.7±0.06
Diisobutyl phthalate	1868	1871	4.6±0.03
Palmitic acid	1978	1973	0.7±0.03
13-Epimanool	2060	2056	3.8±0.03
Total			**99.6**
Monoterpenoids			6.4
Sesquiterpenoids			77.1
Diterpenoids			3.8
Others			12.3

[1]The components are reported according to their elution order on apolar column; [2]Linear Retention Indices measured on apolar column; [3]Linear Retention Indices from literature; * LRI not available; [4]Percentage mean values of *P. ostruthium* EO components.

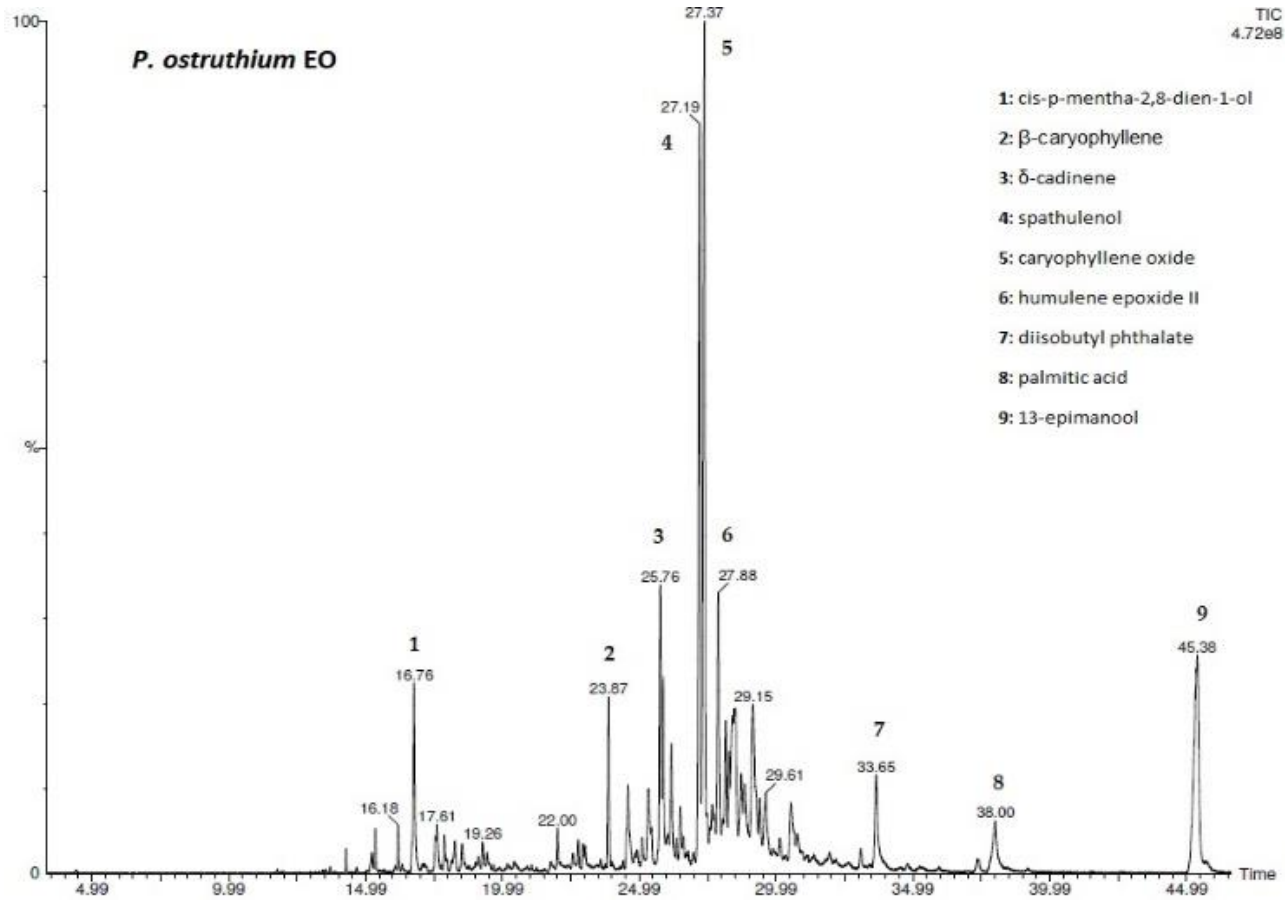

Figure 1. GC-FID Chromatogram of *P. ostruthium* leaf essential oil.

The prevalence of sesquiterpenoid compounds in *P. ostruthium* leaf EO composition confirmed the data previously published in the only work present in the literature where Cisowski and co-authors [19] analyzed, in addition to the rhizome EO, the EO obtained from the herb (it is not better specified, the whole aerial parts including the leaves are assumed) of *P. ostruthium* collected in the Sudety mountains (Karpacz area, Poland). In that case, the main components were *β*-caryophyllene (16.1%), *α*-humulene (15.8%) and germacrene D (9.6%), and the monoterpenoid fraction was more abundant than that of the *P. ostruthium* EO under investigation (25.9% vs 6.4%). Nevertheless, many compounds, both mono- and sesquiterpenoids, were found to be common, albeit, in some cases, in significantly different amounts. Differently, caryophyllene oxide was identified as the main compound (23.1%) in the EO of *P. austriacum* leaves, followed by germacrene D and (E)-caryophyllene [20]. Leaf EOs obtained from other *Peucedanum* species such as *P. zenkeri* Engl., *P. petiolare* Boiss., *P. officinale* L. and *P. cervaria* (L.) Lapeyr. were instead characterized by a higher content of monoterpenes, mainly sabinene, *α*- and *β*-pinene, limonene, myrcene and *β*-phellandrene [21-24].

3.2 P. ostruthium radical scavenging activity

The values reported in Table 2 showed a greater ability of the *P. ostruthium* leaf EO to scavenge the $ABTS^{\cdot+}$ radical cation rather than the DPPH· free radical. In terms of percentage inhibition, $ABTS^{\cdot+}$ was reduced by 2.2 times more than DPPH· (91.0% vs 41.2%). The difference could be attributed to the different solubility of EO in the test systems and can be considered as an indication of the relative reactivity of its active compounds as it occurs for resinous exudates or other extracts [25, 26].

Few previous reports have documented the antiradical and/or antioxidant activity of *Peucedanum*

species. Masuda et al. [27] reported a weak ability of the methanolic extract of *P. japonicum* leaves to inhibit DPPH. A few years later, Hisamoto and collaborators [28] obtained a very active butanol fraction from the leaves of the same species. Hence, some compounds including rutin and three isomers of caffeoylquinic acid were isolated and found to be the major antioxidant constituents. Only the antiradical activity of the *P. litorale* EO seems to have been studied [29]. The data shows that a comparison is rather difficult. The type of sample, the different origin, the method of extraction and analysis are just some factors that influence its bioactivity.

3.3 P. ostruthium phytototoxicity

Appreciable results were obtained from screening of the phytotoxic activity of *P. ostruthium* leaf EO against both target species at the two tested doses (Table 3). It was found to be less effective in influencing seed germination (reduced by 39.6% and 33.3%, respectively) than seedling growth. In this case, the 2 µL dose had greater effects towards *S. alba* (SVI, -36.8 % vs -10.9%; root length, -31.8% vs -8.3%; shoot length, -24.1% vs -7.7%) while the 20 µL dose showed a better effect against *L. multiflorum* (SVI, -90.7% vs -76.6%; root length, -90.4% vs -67.6%; shoot length, -76.4% vs -60.4%). Under the effect of 20 µL of EO, the CVG and MGT indices of *L. multiflorum* were also the most affected, decreasing by 64.2% and increasing by 16.7%, respectively.

Table 2. Antiradical activity of essential oil from *P. ostruthium*

	ABTS (µM Trolox eq/mL)	DPPH (µM Trolox eq/mL)
EO	2.02±0.00	0.77±0.01

Values are mean ± standard deviation.

species. In fact, the aqueous extracts and powders of inflorescences, leaves and rhizomes of *P. ostruthium* from the same geographical area were able to significantly reduce both the germination and the development of two important monocotyledonous weeds, namely *Echnochloa oryzoides* (Ard.) Fritsch and *L. multiflorum*. 5-Caffeoylquinic acid was the main phenolic component in all plant organs, but other chemical classes such as chlorogenic acids, flavonol glycosides, coumarins, and furanocoumarin glycosides were detected. Among the compounds identified in *P. ostruthium* leaf EO, some in particular might be responsible for the inhibitory activity. Caryophyllene oxide and spathulenol, the two most abundant, have been reported to possess a variety of biological properties [31,32], including allelopathic one [33-35]. Besides them, cubenol was responsible for the allelopathic effect of *Sinapis arvensis* var. *orientalis* EO [36], δ-cadinene was identified as one of the exudates released by the roots of *Chrysanthemoides monilifera* spp. *rotundata* (DC.) T. Norl and found in the extracts of its growth soil characterized by phytotoxic activity [37].

Table 3. Germination and growth parameters of two target species *L. multiflorum* and *S. alba* under the phytotoxic effects of *P. ostruthium* essential oil.

Target species	EO (µL)	G (%)	CVG	MGT	SVI	Root (mm)	Shoot (mm)
L. multiflorum	0	96.0±4.0	109±7.0	4.8±0.0	12538±1183	75.9±13.7	54.7±11.2
	2	93.0±0.0	93±5.5	5.0±0.0	11169±946	69.6±8.5	50.5±1.2
	20	58.0±14.0	39±3.0	5.6±0.1	1172±244	7.3±1.9	12.9±1.9
S. alba	0	90.0±5.0	122±6.2	4.1±0.1	4999±384	34.3±6.7	21.2±4.7
	2	80.0±5.0	112±10.0	4.2±0.1	3160±195	23.4±4.6	16.1±5.0
	20	60.0±0.0	68±3.00	4.4±0.1	1170±112	11.1±1.6	8.4±1.0

Values are mean ± standard deviation. G%, Germination percentage; CVG, Coefficient of Velocity of Germination; MGT, Mean Germination Time, SVI, Seedling Vigor Index

These results supported previously published data on the phytotoxic potential of *P. ostruthium* [30] and proved its herbicidal action against dicotyledonous

Other sesquiterpenoids of the EO were recognized as allelochemicals (e.g., *β*-caryophyllene) or detected as the major compounds of EOs with significant allelopathic behavior (e.g., humulene) [38,39].

Allelopathy of monoterpenes is also documented [40]. For example, α-pinene is a compound present in EOs with high activity [41]. This component has an important ecological role on the plant-plant allelopathic interference. Pinenes were found to reduce chlorophyll content in leaves, cell respiration, enzymatic activity of proteases, α- and β-amylases as well as root and coleoptile length [42]. Therefore, the phytoxicity of *P. ostruthium* leaf EO could be linked to the presence of both monoterpenes and sesquiterpene, whose effectiveness can in some cases be improved by a synergistic interaction [41].

4. Conclusions

To conclude, this is one of the very few works on *P. ostruthium* and the first on the antiradical and phytotoxic activity of its EO. The obtained results are promising and deserve to be corroborated with further tests. *P. ostruthium* is confirmed as a good source of compounds, which have proved to be useful both as antioxidants and as allelochemicals.

Author Contributions

Conceptualization, S.V. and M.I.; Methodology, S.V., S.G. and M.I.; Validation, S.V. and S.G.; Investigation, S.V. and S.G.; Resources, S.G. and M.I.; Data Curation, S.V. and S.G.; Writing – Original Draft Preparation, S.V. and S.G.; Writing – Review & Editing, S.V., S.G. and M.I.

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Conflicts of Interest

The authors declare no conflict of interest.

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