

## ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF FOUR *PEUCEDANUM* L. SPECIES

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The present study describes antioxidant activity of methanol, ethyl acetate, acetone and water extracts of the dried aerial parts of four *Peucedanum* species (*P. officinale*, *P. longifolium*, *P. aegopodioides* and *P. alsaticum*). The highest yields of extracts were obtained using polar solvents. The total phenolic content of the tested extracts varied from 52.18 to 118.32 mg of gallic acid equivalent (GA)/g of extract and the total flavonoid content from 4.43 to 234.67 mg of quercetin equivalent (Qu)/g of extract. Antioxidant activity was analyzed *in vitro* using DPPH reagent, activity expressed as EC<sub>50</sub> and obtained results ranged from 0.30 to 14.80 mg/mL and ABTS reagent, whose amounts ranged between 1.28 to 3.73 mg VitC/g. Synthetic antioxidant butylated hydroxyanisole (BHA) and Vitamin C were used as control. Antibacterial activity of all extracts tested showed minimal inhibitory concentration (MIC) which ranged between 5.0-160.0 mg/ml, while minimal bactericidal concentration (MBC) was 10.0-160.0 mg/ml. The results of antifungal activity of analyzed *Peucedanum* species showed antifungal activities in the MIC range of 10-160 mg/ml and MFC range of 20-160 mg/ml. This finding suggests that these species may be considered as a natural source of antioxidants and antimicrobial agents.

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### 1. Introduction

Members of the genus *Peucedanum* are characterised by flattened fruits with more or less developed lateral wings. The phylogenetic position of *Peucedanum sensu lato* and allied genera is placement in tribe Selineae (Apiaceae, subfamily Apioideae) [1].

*P. officinale* L. is glabrous perennial plant, growing to a height of 60-200cm with abundant fibres [2]. This species is distributed in Europe and western Asia [3]. The juice of this plant sometimes used in medicine against hypochondria, epilepsy, paralysis, anti-catarrh of the lungs, cramps in the uterus [4,5]. *P. officinale* has proved a significant antiinflammatory activity, therefore, it might be useful for patients unsuitable for classical antiinflammatory therapy [6].

*P. longifolium* Waldst. & Kit. has basal leaves like the former taxon, but leaf-lobes are rarely more than 1mm wide, keeled. Ripe fruit is about as long as pedicels [2]. This species is distributed in the northern part of the Balkan Peninsula and Romania [3].

*P. aegopodioides* (Boiss.) Vandas is glabrous perennial herb up to 100cm [2]. This species is distributed in the eastern part of Balkan Peninsula [3].

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*P. alsaticum* L. (Syn. *Johrenia pichleri* Boiss.; incl. *P. lubimenkoanum* Kotov) is glabrous, purplish perennial 30-180cm herb [2]. This species is distributed in Europe, Central and Southern Russia, Caucasus, Siberia (Altai area) [3].

Phenolic compounds, with the potential ability of losing a hydrogen atom and/or a single electron (due to the stability of resulting free radical) and with metal chelating properties (due to the presence of hydroxyl and carbonyl functional groups in their structures), exhibit considerable antioxidant activities [7,8]. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals [9,8]. Apart from that, there is a growing interest to use natural antibacterial compounds. In this context, the extracts of plants have gained considerable attention as antimicrobial and antioxidant compounds and have formed the basis of many applications including in raw and processed food preservation [10]. Today we know that some plant extracts have a broad spectrum activity against Gram-positive and Gram-negative bacteria of food-borne pathogenesis and they also perform antifungal activity [11,12].

The more interesting pharmacological properties of the species belonging to *Peucedanum* genus are due, in particular, to the presence, among several metabolites, of furocoumarins and pyranocoumarins. Some of them have antimicrobial and cytotoxic activities [13]. Some of compounds (e.g. peucedanin and 8-methoxypeucedanin, which are both linear furanocoumarins of a rare structural type with restricted occurrence in *P. officinale* and the closely related species *P. coriaceum* and *P. longifolium*) could be important from chemotaxonomic aspect. Peucedanin is effective at induction of apoptosis and inhibition of heat-shock protein expression in HeLa cells [14]. Acetone extract of *P. nebrodense* has been shown antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. tropicalis* and *Streptococcus agalactia* [13]. *P. ostruthin* contained ostruthin (6-geranyl-7-hydroxy coumarin), imperatorin and umbelliferon, of which ostruthin showed strong activity against some species of mycobacteria (MICs 3.4 -107.4 $\mu$ M) and umbelliferon displayed only weak activity (MIC=0.79mM) [15]. Seed methanol extract of *P. graveolens* had moderate activity on *Salmonella thyphi* with zone inhibition diameter  $\leq$ 5-9mm [16].

In this work the antioxidant and antimicrobial activity of *Peucedanum officinale*, *P. longifolium*, *P. aegopodioides* and *P. alsaticum*) is reported. As far as we know this is the first report of the antioxidant activity of these species.

## 2. Materials and Methods

### 2.1 Plant material

Aerial plant parts *P. officinale* were collected in July 2003 from Kosovska Mitrovica, *P. longifolium* were collected in June 2003 from Orijen mountain, *P. aegopodioides* were collected in July 2003 from Kopaonik mountain and *P. alsaticum* were collected in July 2003 from Fruška Gora mountain. A voucher specimens for *P. officinale* (PO 60322), *P. longifolium* (PL 60319), *P. aegopodioides* (PA 60320), *P. alsaticum* (PA 60318) have been deposited at the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU).

### 2.2 Chemical reagents

Organic solvents were purchased from „Zorka pharma“ Sabac, Serbia. Gallic acid, 3-tert-butyl-4-hydroxyanisole (BHA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent was purchased from Merck, Darmstadt, Germany. Sodium carbonate anhydrous (Na<sub>2</sub>CO<sub>3</sub>), potassium acetate (C<sub>2</sub>H<sub>3</sub>KO<sub>2</sub>), potassium peroxodisulphate (K<sub>2</sub>O<sub>8</sub>S<sub>2</sub>) and L(+)- Ascorbic acid (Vitamin C) were purchased from AnalaR Normapur, VWR, Geldenaaksebaan, Leuven Belgium. Aluminium nitrate nonahydrate (Al(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O) was purchased from Fluka Chemie AG, Buchs, Switzerland. ABTS and quercetin hydrate were obtained from TCI Europe NV, Boerenveldsweg, Belgium. All other solvents and chemicals were of analytical grade.

## 2.3 Sample preparation

Plant material was air dried in the dark and ground to a powder. The aerial plants parts (10g) were extracted with 100ml methanol, ethyl acetate, acetone and water. The mixture was exposed to ultrasound for 30min and after 24h standing in the dark was filtered. The methanol, ethyl acetate and acetone solvents were removed by evaporation under the reduced pressure, at maximum temperature of 40°C. Water extract was frozen and later dried by freeze-drying. After evaporation of the solvent the crude extract was subjected to subsequent analysis.

## 2.4 Phytochemical screening

### 2.4.1 Determination of Total Phenolic Content

The total phenolic content of extracts was determined spectrophotometrically by Folin-Ciocalteu method according to the procedure reported by Singleton, Orthofer, & Lamuela-Raventos (1999) with some modifications [17]. Briefly, 300µl of different extracts solution (methanol, ethyl acetate, acetone and water) and 1500µl of 1:10 Folin-Ciocalteu reagent were mixed and after 6 minutes in the dark were added 1200µl of sodium carbonate (7.5%). After 2h in the dark of incubation at room temperature, the absorbance at 740nm was measured (Shimadzu, UV-Visible PC 1650 spectrophotometer). The total phenolic concentration was calculated from gallic acid (GA) calibration curve (10-100mg/l). Data were expressed as gallic acid equivalents (GA)/g of extract averaged from 3 measurements.

### 2.4.2 Determination of Total Flavonoid Content

The total flavonoid content was evaluated using aluminium nitrate nonahydrate according to the procedure reported by Woisky & Salatino (1998) with some modifications [18]. The sample for determination was prepared by mixing a 600µl of different extracts solution (methanol, ethyl acetate, acetone and water) and 2580µl of mixture (80% C<sub>2</sub>H<sub>5</sub>OH, 10% Al(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O and 1MCH<sub>3</sub>CO<sub>2</sub>K). After 40min of incubation at room temperature, the absorbance at 415nm was measured (Shimadzu, UV-Visible PC 1650 spectrophotometer). The total flavonoid concentration in different extracts was calculated from quercetin hydrate (Qu) calibration curve (10-100mg/l) and expressed as quercetin equivalents (Qu)/g of dry extract. Measurements were done in triplicates.

## 2.5 Antioxidant testing

### 2.5.1 DPPH radicals-scavenging assay

The antioxidant activity of extract was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. This spectrophotometer assay uses stable radical DPPH as reagent [19]. Methanolic, ethyl acetate, water and acetone solution of investigated extracts (300µl) (the extracts concentrations between 31 and 9500µg/ml) was added to 2700µl methanolic solution of DPPH radical (concentration of 0,04mg/ml) and after shaking reaction mixture was left to react in the dark for 30 minutes at room temperature.

Absorbance of remaining DPPH radical was measured on 517nm after that time (A1) on Shimadzu, UV-Visible PC 1650 spectrophotometer. Every concentration was done in triplicate and the same was done with Vitamine C and BHA, known antioxidants. Blank probes were done in the same way using methanol instead of investigated solution (A0). The decrease of absorption of DPPH solution is calculated by equation:

$$\text{Percentage of absorption decrease (on 517 nm)} = (A0 - A1) \times 100 / A0$$

Concentrations which decrease absorption of DPPH solution for 50% (EC<sub>50</sub>) were obtained from the curve dependence of absorption of DPPH solution on 517nm from concentration for each compound and standard antioxidant. For calculation of these values, Origin 7.0 soft ware was used. Tests were cared out in triplicate.

### 2.5.2 ABTS radical-scavenging assay

For ABTS radical-scavenging activity, the procedure followed the method of Miller & Rice-Evans (1997) with some modifications [20]. The ABTS<sup>+</sup> solution was prepared by mixing 19.2mg of ABTS with 5ml of potassium persulfate (2.46mM). The solution was held at room temperature in the dark for 12-16h before use. The ABTS<sup>+</sup> solution (1ml) was diluted with 100-110ml water, in order to obtain an absorbance  $0.7 \pm 0.02$  at 734nm. Fresh ABTS<sup>+</sup> solution was prepared for each analysis. Antioxidant or standard solutions, 75 $\mu$ l, were mixed with 3ml of diluted ABTS<sup>+</sup> solution and incubated at 30°C for 30'. The absorbance at 734nm was measured (Shimadzu, UV-Visible PC 1650 spectrophotometer). Water was used as a blank. ABTS radical scavenging activity in different extracts was calculated from Vitamin C (VitC) calibration curve (0-2mg/l) and expressed as Vitamin C (VitC)/g of dry extract. Measurements were done in triplicates.

### 2.6 Antimicrobial assays

For the bioassays we used eight bacteria species including four Gram-negative: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (human isolate) and Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (human isolate), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538).

Eight fungal species were used: *Aspergillus ochraceus* (ATCC12066), *A. niger* (ATCC 6275), *A. fumigatus* (ATCC 9142), *A. versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *P. ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and *Candida albicans* (ATCC 10231). All of the organisms tested were from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research «Siniša Stanković», Belgrade, Serbia. The micromycetes were maintained on malt agar (MA), bacteria on Mueller-Hinton agar (MH) and cultures were stored at +4°C and subcultured once a month [21].

Microdilution method. In order to investigate the antimicrobial activity of the extracts tested, the modified microdilution technique was used [22,23]. Bacterial species were cultured overnight at 37°C in Tryptic Soy Broth (TSB) medium. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The fungal and bacterial cell suspension was adjusted with sterile saline to a concentration of approximately  $1.0 \times 10^5$  in a final volume of 100 $\mu$ l per well. The inocula were stored at +4°C for further use. Dilutions of the inocula were cultured on solid MH for bacteria and solid MA for fungi to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The extracts were dissolved in 30% methanol, 30% ethanol and 5% DMSO (20mg/ml) and added in broth medium with inoculum. The microplates were incubated for 48h at 37°C for bacteria and or 72h at 28°C, for fungi. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The minimum bactericidal concentrations (MBCs) and fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2ml into microtitre plates containing 100mL of broth per well and further incubation for 48h at 37°C or 72h at 28°C, respectively. The lowest concentration with no visible growth was defined as MBC/MFC respectively, indicating=99.5% killing of the original inoculum. Ampicillin and commercial fungicide, ketokonazole, were used as positive controls (0.1-5mg/ml).

## 3. Results and discussion

### 3.1 Total phenolic and total flavonoid contents

The antioxidant potential of different extracts of four *Peucedanum species* was estimated by determining their total phenolic and flavonoid contents and their ability for free radical scavenging. The results are shown in Table 1.

Table 1. The comparison of the total phenolic content and the total flavonoid, antioxidant capacities by ABTS assays content of the different *Peucedanum* extracts.

		Extracts concentration (mg/ml)	Total phenolic content	Total flavonoid content	ABTS assay
<b>Methanol extracts</b>	<i>P. officinale</i>	1	100.61±0.001	40.75±0.003	3.35±0.012
	<i>P. longifolium</i>	2	60.36±0.002	12.55±0.002	1.90±0.004
	<i>P. aegopodioides</i>	1	52.18±0.007	21.52±0.002	1.87±0.016
	<i>P. alsaticum</i>	1	62.55±0.018	20.48±0.001	1.91±0.003
<b>Ethyl acetate extracts</b>	<i>P. officinale</i>	5	79.35±0.002	215.11±0.000	2.39±0.003
	<i>P. longifolium</i>	5	67.85±0.004	74.74±0.003	2.00±0.009
	<i>P. aegopodioides</i>	–	–	–	–
	<i>P. alsaticum</i>	5	99.78±0.001	234.67±0.000	2.92±0.016
<b>Water extracts</b>	<i>P. officinale</i>	1	91.30±0.003	20.87±0.002	3.16±0.000
	<i>P. longifolium</i>	2	52.49±0.004	4.43±0.001	2.15±0.001
	<i>P. aegopodioides</i>	1	54.08±0.002	15.21±0.005	2.27±0.013
	<i>P. alsaticum</i>	1	67.77±0.004	15.08±0.003	2.54±0.003
<b>Acetone extracts</b>	<i>P. officinale</i>	1	118.32±0.003	119.32±0.003	3.73±0.005
	<i>P. longifolium</i>	2	56.72±0.003	23.86±0.008	1.28±0.011
	<i>P. aegopodioides</i>	1	78.89±0.004	–	–
	<i>P. alsaticum</i>	1	67.32±0.004	54.60±0.008	1.70±0.024
<b>BHA</b>		0.1	63.31±0.001	–	2.66±0.005
<b>Vitamin C</b>		0.1	40.91±0.002	–	–

Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

The total phenolic content of studied different *Peucedanum* extracts was determined by Folin-Ciocalteu method. The amount of phenolic compounds varied from 52.18 to 118.32mg GA/g of extract. The highest phenolic content was found in *P. officinale* and the lowest in *P. longifolium* (except methanol extract of *P. aegopodioides* which had the value 52.18mg GA/g of extract). The amount of phenolic compounds in the same species but in the different extracts was approximately the same. For ethyl acetate extracts it was not possible to accurately measure the amount of phenolic compound because they could not be mixt with water.

The total flavonoid content was evaluated using aluminium nitrate nonahydrate. The amount of flavonoid compounds ranged from 4.43 to 234.67mg Qu/g of *Peucedanum* extract. The highest flavonoid content was identified in *P. officinale* and the lowest in *P. longifolium* except in ethyl acetate extract where *P. alsaticum* had bigger amount of flavonoid compounds than *P. officinale*. All extracts of *P. alsaticum* and *P. aegopodioides* had approximately the same values of the total flavonoid content.

### 3.2 Antioxidant capacity by DPPH and ABTS assays

Free radical scavenging capacities of the tested extracts was measured by DPPH assay. A radical scavenging method has been chosen in this study because radical scavenging is the main mechanism by which antioxidants act in foods. According to the results obtained, methanol extracts was found active with EC<sub>50</sub> value for *P. officinale* 0.470mg/ml of solution, for *P. longifolium* 4.062mg/ml of solution, for *P. aegopodioides* 1.174mg/ml of solution and EC<sub>50</sub> value for *P. alsaticum* 0.946mg/ml. Ethyl acetate extracts were found active with EC<sub>50</sub> value for *P.*

*longifolium* 14.804mg/ml of solution, for *P. aegopodioides* 4.942mg/ml of solution and EC<sub>50</sub> value for *P. alsaticum* 2.550mg/ml. Water extracts were found active with EC<sub>50</sub> value for *P. officinale* 0.308mg/ml of solution, for *P. longifolium* 4.912mg/ml of solution, for *P. aegopodioides* 1.360mg/ml of solution and EC<sub>50</sub> value for *P. alsaticum* 0.612mg/ml. Acetone extracts was found active with EC<sub>50</sub> value for *P. officinale* 0.542mg/ml of solution, for *P. longifolium* 11.057mg/ml of solution, for *P. aegopodioides* 1.136mg/ml of solution and EC<sub>50</sub> value for *P. alsaticum* 1.197mg/ml. EC<sub>50</sub> values of the synthetic antioxidants BHA was 0.093mg/ml and Vitamin C 0.054mg/ml were determined in parallel experiments. Extracts of *P. officinale* possessed the strongest antioxidant activities compared to other species. Therefore, it was deduced that the activity was due to polyphenolic compounds. This species is used in traditional medicine against hypochondria, epilepsy, paralysis, anti-catarrh of the lungs, cramps in the uterus [4,5].

The results from the ABTS assay are shown in **Table 1**. The amount ranged from 1.28 to 3.73mg VitC/g of *Peucedanum* extract. The highest content of activity was identified in *P. officinale* and the lowest in *P. longifolium*.

### 3.3 Correlations

The total phenolic content correlated well with the DPPH values for different extracts (methanolic extracts had reasonably well correlation  $R^2 = 0.7629$  (**Fig. 1**), water and acetone extracts had highly correlation  $R^2 = 0.9826$  and  $R^2 = 0.9273$ ). The total flavonoid content also correlated well with the DPPH values for different extracts (water extracts had reasonably well correlation  $R^2 = 0.7392$ , methanolic (**Fig. 2**) and acetone extracts had highly correlation  $R^2 = 0.9691$  and  $R^2 = 0.9871$ ). However, the correlation between ABTS values and the total phenolic contents were very high (for methanolic extracts were  $R^2 = 0.9662$  (**Fig. 3**), for ethyl acetate extracts were  $R^2 = 0.9948$ , for water extracts were  $R^2 = 0.9935$  and for acetone extracts were  $R^2 = 1.00$ ). The correlation between ABTS values and the total flavonoid contents were not so high (for methanolic extracts were  $R^2 = 0.882$  (**Fig. 4**), for ethyl acetate extracts were  $R^2 = 0.7711$ , for water extracts were  $R^2 = 0.6817$  and for acetone extracts were  $R^2 = 0.9746$ ). The results indicated that significant correlation coefficient between DPPH / ABTS activities and the total phenolic content/total flavonoids content of different extract four *Peucedanum* species. Therefore, total phenolic components can be used as an indicator in assessing the antioxidant activity of fruits and vegetables, including *Peucedanum* species.

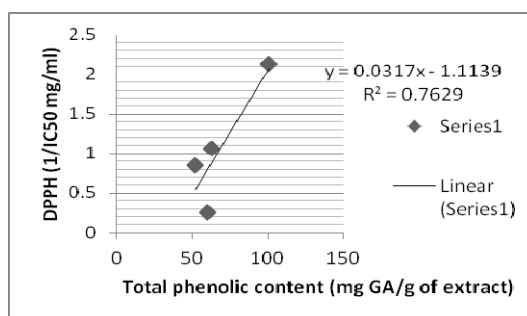


Fig. 1. Correlation between total phenolic and DPPH activities (methanolic extracts).

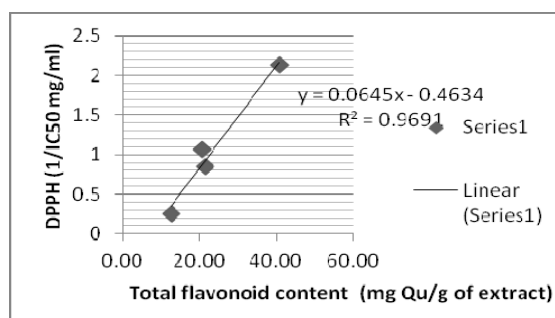


Fig. 2. Correlation between total flavonoid and DPPH activities (methanolic extracts).

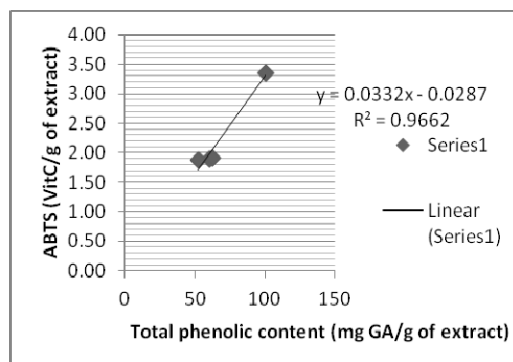


Fig. 3. Correlation between total phenolic and ABTS activities (methanolic extracts).

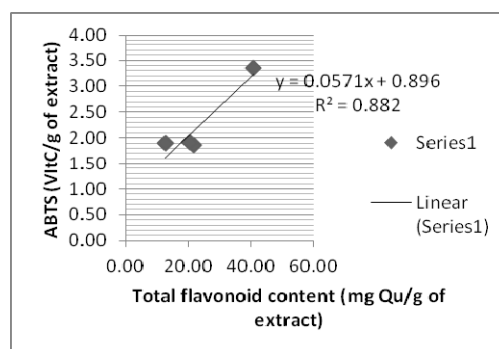


Fig. 4. Correlation between total flavonoid and ABTS activities (methanolic extracts).

Different solvents, such as water, methanol, acetone, ethyl acetate, (ranged from higher polarity to lower polarity) extracts were used for the study of antioxidant activity. The solvents were used to achieve extraction of active substances with diversity in their polarity. Polarity is an important consideration here. Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethylether, or ethyl acetate, while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol-water mixtures. Glycosides have increased water solubility and aqueous alcoholic solutions are suitable [24]. According to our results, water, methanol and acetone extracts showed similar antioxidant activity while ethyl acetate extract possessed the lowest activities.

### 3.4 Antimicrobial activity

Antibacterial activity of all extracts tested of different species of genus *Peucedanum* are presented in **Table 2**. The tested *Peucedanum* extracts showed antibacterial activity against all bacteria strains, but on different level. Minimal inhibitory concentration (MIC) was ranged between 5.0-160.0mg/ml, while minimal bactericidal concentration (MBC) was of 10.0-160.0mg/ml. Their antibacterial potential of the extracts can be presented as follows: acetone > ethyl acetate > methanol > water. The best antibacterial activity was obtained for acetone extract of *P. longifolium*, MIC ranged 5.0-30.0mg/ml and MBC from 10.0-40.0mg/ml. The lowest antibacterial activity among all the extracts tested in here was obtained for water extracts of all *Peucedanum* species. The most sensitive bacterial species was *S. typhimurium* with MIC at 15.0-120mg/ml and MBC at 20.0-160.0mg/ml. *L. monocytogenes* was the most resistant species with inhibitory activity between 20.0-160.0mg/ml, and starting bactericidal with 40mg/ml to more than >160mg/ml for water extracts. Ampicillin, standard drug used as a positive control, was also active against all the bacteria. Range of MIC for ampicillin was 0.1-0.3mg/ml and MBC 0.15-0.5mg/ml. Comparing biological activity of extracts with commercial antibiotics, it can be seen that tested samples showed lower antibacterial potential (**Table 2**).

Table 2. Minimal inhibitory (MIC mg/ml) and bactericidal concentration (MBC mg/ml) of extracts tested.

		<i>B. c</i>	<i>M. f</i>	<i>S. a</i>	<i>L. m</i>	<i>E. c</i>	<i>E. cl</i>	<i>P. a</i>	<i>S. t</i>
		MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC
		MBC	MBC	MBC	MBC	MBC	MBC	MBC	MBC
Methanol extracts	<i>P. officinale</i>	20	40	20	40	30	20	20	20
		30	50	30	60	40	30	30	30
	<i>P. longifolium</i>	20	30	20	40	30	20	20	30
		30	40	30	60	40	30	30	40
	<i>P. aegopodioides</i>	20	40	20	40	30	20	20	20
30		50	30	60	40	30	30	30	
<i>P. alsaticum</i>	30	30	30	30	30	30	30	30	
	50	50	50	50	50	50	40	60	
Ethyl acetate extracts	<i>P. officinale</i>	20	30	20	40	20	15	20	15
		30	40	30	60	40	20	20	20
	<i>P. longifolium</i>	20	40	30	30	30	20	15	15
30		40	40	40	40	30	20	20	
<i>P. alsaticum</i>	20	20	15	20	20	15	15	15	
	30	40	20	40	40	20	20	20	
Water extracts	<i>P. officinale</i>	140	160	140	140	-	120	120	120
		160	-	-	-	-	-	-	160
	<i>P. longifolium</i>	120	160	120	140	160	120	160	120
		-	-	-	-	-	-	-	-
<i>P. aegopodioides</i>	120	160	140	140	140	120	120	120	
	160	-	-	-	-	-	140	160	
<i>P. alsaticum</i>	140	-	-	160	-	140	-	140	
	-	-	-	-	-	-	-	-	
Acetone extracts	<i>P. officinale</i>	20	30	20	30	15	15	10	15
		30	40	30	40	20	20	15	20
	<i>P. longifolium</i>	5	20	15	20	20	10	15	15
		10	30	20	40	30	15	20	20
	<i>P. alsaticum</i>	10	20	15	20	20	10	10	20
		15	30	20	40	40	15	15	30
Ampicillin	0.1	0.1	0.1	0.15	0.15	0.15	0.3	0.1	
	0.15	0.15	0.15	0.3	0.2	0.2	0.5	0.2	

- no activity

*Escherichia coli* (*E. c*), *Pseudomonas aeruginosa* (*P. a*), *Salmonella typhimurium* (*S. t*), *Enterobacter cloacae* (*E. c*), *Listeria monocytogenes* (*L. m*), *Bacillus cereus* (*B. c*), *Micrococcus flavus* (*M. f*) and *Staphylococcus aureus* (*S. a*).

In a recent study, it was shown that antimicrobial activity of hexane extracts from fruits of *P. alsaticum* and *P. cervaria* possess inhibitory effects against Gram-positive strains tested with different MIC values (0.25–2 mg/ml); however, extract from *P. alsaticum* possessed stronger antibacterial properties and a broader spectrum. The growth of Gram-negative bacteria and *Candida* spp. was not inhibited even at the highest extract concentration used (MIC > 4 mg/ml) [25].



The results of antifungal activity of investigated *Peucedanum* species are presented in **Table 3**. All tested extracts showed antifungal activities in the MIC range of 10-160mg/ml and MFC range of 20-160mg/ml. The antifungal potential ranged in following order: acetone > ethyl acetate > methanol > water extracts. The best antifungal activity was obtained for the acetone extract of *P. alsaticum* with inhibitory activity of 10-40mg/ml and fungicidal was 20-60mg/ml. The lowest antifungal activity can be seen for the water extract of all the extracts tested where MIC is starting 120mg/ml and MFC 160mg/ml. For many fungal species did not determinate inhibitory and fungicidal activity for water extracts. *A. ochraceus* was the most sensitive tested fungus with MIC 10-120mg/ml and MFC at 20-160mg/ml, while *C. albicans* was the most resistant species with MIC 40-160mg/ml and MFC at 60mg/ml to more then >160mg/ml. Ketoconazole possessed inhibitory activity of 0.15-2.5mg/ml and fungicidal of 0.5-3.0mg/ml. The synthetics fungicides exhibited better antifungal activity as extracts tested.

Table 3. Minimal inhibitory (MIC mg/ml) and fungicidal concentration (MFC mg/ml) of extracts tested.

		<i>P. f</i>	<i>P. o</i>	<i>T. v</i>	<i>A. f</i>	<i>A. n</i>	<i>A. v</i>	<i>A. o</i>	<i>C. a</i>
		MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC
Methanol extracts	<i>P. officinale</i>	20	20	20	20	10	20	20	50
		60	50	60	60	50	40	50	70
	<i>P. longifolium</i>	20	15	20	20	20	15	15	40
		60	40	50	50	50	40	40	80
	<i>P. aegopodioides</i>	20	20	20	20	20	20	20	40
60		60	50	50	50	40	50	60	
<i>P. alsaticum</i>	20	20	20	30	10	20	20	40	
	60	50	50	60	40	50	40	60	
Ethyl acetate extracts	<i>P. officinale</i>	40	20	10	20	40	10	15	40
		60	30	20	50	60	20	20	70
	<i>P. longifolium</i>	40	20	20	20	30	20	20	50
60		60	50	60	60	60	60	60	
<i>P. alsaticum</i>	30	20	20	20	20	10	10	50	
	60	30	30	40	30	30	20	60	
Water extracts	<i>P. officinale</i>	140	140	-	160	120	120	120	140
		-	-	-	-	-	160	160	-
	<i>P. longifolium</i>	160	160	-	160	160	120	120	160
		-	-	-	-	-	-	-	-
<i>P. aegopodioides</i>	140	140	140	140	120	140	120	140	
	-	-	160	-	-	160	140	-	
<i>P. alsaticum</i>	160	140	100	160	160	120	120	160	
	-	-	-	-	-	160	160	-	
Acetone extracts	<i>P. officinale</i>	40	20	20	30	30	20	10	50
		60	40	50	60	60	40	40	80
	<i>P. longifolium</i>	15	20	20	20	15	15	15	50
30		30	40	50	30	20	20	70	
<i>P. alsaticum</i>	15	15	15	20	15	15	10	40	
	40	30	40	40	40	20	20	60	
ketokonazole	0.2	0.5	2.5	0.2	2.5	0.2	1.5	0.2	
	0.5	1	3	0.5	2.5	0.5	2	0.5	

- no activity

*Aspergillus ochraceus* (*A. o*), *A. niger* (*A. n*), *A. fumigatus* (*A. f*), *A. versicolor* (*A. v*), *Penicillium funiculosum* (*P. f*), *P. ochrochloron* (*P. o*), *Trichoderma viride* (*T. v*) and *Candida albicans* (*C. a*).

It can be seen that the growth of tested bacteria and fungi responded differently to the compounds tested, which indicates that different components might have different modes of action or that the metabolism of some microorganisms was able to overcome the effect of the agents or adapt to it.

#### 4. Conclusion

All the extracts evaluated from different *Peucedanum* species could be used as protective reagents against oxidative stress provoked by DPPH and hydroxyl radicals. All of them except for ethyl acetate extracts have relatively good antioxidant activity in comparison with synthetic antioxidants BHA and Vitamine C. *P. officinale* showed the highest antioxidant activity in both methods. Therefore, they should be considered to be tested in the food systems under various processing and storage conditions. On the other hand, the best antibacterial activity was obtained for acetone extract of *P. longifolium*, while the best antifungal activity was obtained for the acetone extract of *P. alsaticum*. Generally, all tested species can be used in food as good antioxidants, as well as therapeutic purposes as a good antimicrobial agent.

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