Chemical characterization and antioxidant activity of *Eryngium campestre* L., Apiaceae from Kosovo

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**Abstract**

This study is outlined to define the chemical composition and *in vitro* antioxidant activity of the extracts of aerial part and root of *Eryngium campestre* L. (Apiaceae) from Kosovo. Analysis of the chemical composition include determination of total ash, ash insoluble in hydrochloric acid, loss on drying and the content of water extract, as well as determination of flavonoids in aerial part and hemolytic activity of the root. The mineral composition (Zn, Fe, Cu, Mn, Ni, K, Co, Ph, Cd and Cr) in aerial parts and root has been studied using atomic absorption spectroscopy (AAS and ETAAS). Different part of *E. campestre* accumulate different amounts of investigated minerals. Antioxidant activity was determined by four various testing systems: DPPH assay, inhibition of production of hydroxyl radical, β-carotene-bleaching assay, and inhibition of lipid peroxidation (TBA test). In DPPH system, ethanol extract of root of *E. campestre* exhibited high radical-scavenging activity (IC₅₀ = 0.72 mg ml⁻¹) compared to the extract of the aerial part (IC₅₀ = 1.14 mg ml⁻¹). On the other hand, aerial part ethanol extract has exhibited stronger inhibition capacity on the production of hydroxyl radical in deoxyribose system than the root extract (50% and 45%, respectively). However, both ethanol extracts of *E. campestre* exhibited low antioxidant activity in β-carotene-bleaching assay as well as, low capacity for inhibition of spontaneous lipid peroxidation in rat liver homogenate.

**Key words:** *Eryngium campestre*, flavonoids, mineral content, DPPH assay, antioxidant activity, β-carotene-bleaching test, TBA test.

**Introduction**

*Eryngium campestre* L. (Apiaceae) (field eryngo) is perennial plant, spread in Spain, France, Germany, Balkan Peninsula and other scattered localities in Europe, and in Africa and Asia as well (Ingram, 2006). It has been used in folk medicine as an infusion to treat cough, whooping cough, urinary infections, disturbed functions of kidney, increased urine secretion, eliminating out stones and sand from kidney and bladder, against water retaining and other conditions on urinary tract, for regulation of the function of prostate.

The root of eryngo is known as antispasmodic, aromatic, diaphoretic, diuretic, expectorant, galactofuge and stimulant (Petkov, 1982). It promotes free expectoration and is very useful in the treatment of debility attendant on coughs of chronic standing in the advanced stages of pulmonary consumption. There are none known hazards of *Eryngium campestre*, even more young shoots and roots are edible parts of the plant which can be cooked and used as an asparagus substitute and as an easily digested vegetable (www.gardenzone.info).

The presence of bioactive secondary metabolites including saponins, phenolic acids, flavonoids, coumarins, essential oil, is considered to determine pharmacological...
activities of the plants of genus *Eryngium*, as well. Considering phenolics, flavonoids are the most investigated compounds. The literature data show that *Eryngium campestre* contain glycosides of kaempferol, isorhamnetin, luteolin and quercetin (Karting and Wolf, 1993; Nebija et al. 2006) and flavanolacyl glycosides (Hohman et al. 1997) while the other representatives of the genus such as *Eryngium planum* kaempferol and its glycosides (Stecka-Paszkiwczew, 1983, Zarnack et al. 1977), *E. maritimum* contains glycosides of kaempferol, isoquercetin and astragalin (Hiller et al. 1981) and *E. creticum* glycosides of quercetin (Al-Khail, 1994). *E. campestre* contains coumarins (Erdelmeier and Sticher, 1985), D-mannitol (Asenov and Gevrenova, 1991), cyclohexanone and cyclohexadienone glycosides (Erdelmeier and Sticher, 1986). Similar components were identified in other species of *Eryngium*, for example acetilenes in *E. creticum* (El-Gamal et al. 1978) and coumarins in *E. illicifolium* (Pinar and Galan, 1985). However, the most important class of secondary metabolites investigated in root of *Eryngium campestre* and other species of *Eryngium* were saponins. Thus, Kartal et al. were the first that had reported presence of two baringenol saponins in roots of *E. campestre*. These structures were elucidated by 2D NMR and mass spectrometry (Kartal et al. 2005). Five other saponins also glycosides of baringenol were reported one year latter (Kartal et al. 2006). Hiller at al. reported presence of eringyin saponins A, A1, A2 and B in root of *E. planum* (Hiller et al. 1972), then saponins of betulinic and oleanolic acids in *E. bromelifolium* (Hiller et al. 1974; 1976; 1978) and similar saponins in *E. maritimum* (Hiller et al. 1976). According to this, saponins were considered as the class of components responsible for anti-inflammatory effects of the root extract from *E. campestre*, but also for root and herb extracts of *E. maritimum*, *E. kotschyi*, *E. creticum* (Kupeli et al. 2006; Lisciani et al. 1984). Extracts of *E. creticum* could act beneficially against the hemolytic activities of snake and scorpion venoms (Alkohafi et al. 1997), while *E. foetidum* leaf extract acts as anticonvulsant, anti-inflammatory and analgesic agent (Simon and Singh, 1986; Saenz et al. 1998). Representatives of *Eryngium* species contain significant amounts of volatile oils and their oil composition and possible biological activity were investigated as well (Capetanos et al. 2007; Lerclercq et al. 1992; Pala-Paul et al. 2005; Pino et al. 1997a, 1997b; Wong et al. 1994). Finally, lypo-phylic extracts of species of genus *Eryngium* contain different phytosterols which are considered as important constituents for topical anti-inflammatory activity on acute and chronic inflammation models (Garcia et al. 1999).

*Eryngium campestre* is widely spread throughout the territory of Kosovo. Until now there is no data of chemical composition and biological activity of *Eryngii herba* or *Eryngii radix* originated from Kosovo. Having in mind all previously mentioned, the aim of the present research is determination of the chemical composition and possible antioxidant activity of different parts of *Eryngium campestre* from Kosovo.

## Experimental

### Plant material

The samples of the aerial parts in full blossom (*Eryngii herba*) were collected during summer in 2002 and 2003 on three different locations in Kosovo: 1) Prishtina, 2) Poduevo and 3) Lipjan. The roots (*Eryngii radix*) were collected at the same locations in autumn 2002 and 2003, as well. The samples were labeled with following marks for samples collected from 1) Prishtina, 2) Poduevo and 3) Lipjan, respectively:

- EH1/02, EH2/02 and EH3/02 for aerial plant material collected in 2002,
- EH1/03, EH2/03 and EH3/03 for aerial plant material collected in 2003,
- ER1/02, ER2/02 and ER3/02 for the roots collected in 2002, and
- ER1/03, ER2/03 and ER3/03 for the roots collected in 2003.

Herbarium voucher specimens with the same marks were deposited at the Institute of Botany, Section Pharmacy at the Faculty of Medicine in Prishtina, Kosovo. All samples were left to air dry and then put in a paper bag and stored at cool, dry and dark place, until analysis.

### Determination of basic chemical parameters

The contents of total ash and ash insoluble in hydrochloric acid as well as the loss on drying were determined using methods described in European Pharmacopoeia (Ph. Eur. 4). The content of total water extractive was determined by method in Ph. Jug. IV.

### Determination of flavonoids

The content of flavonoids was determined in the samples of *Eryngii herba* by UV/Vis spectrophotometry, using AlCl₃ as complexation reagent and method for determination of flavonoids described in Ph. Eur. 4. The spectrophotometer analysis was carried out in an Ultraviolet visible equipment (Perkin Elmer Lambda 16) at 425 nm. The results were expressed in percentage of flavonoids, calculated as quercetin, from the average of six determinations, using the calibration curve of quercetin (concentration range 0-100 μg.ml⁻¹; *y* = 0.0103*x* + 0.0066, *R²* = 0.9993).

### Determination of hemolytic activity

Haemolytic activity (expressed as HA) was evaluated for the root of *Eryngium campestre* by the method given in the Pharmacopoeia Jugoslavica (Ph. Jug. 4) using the Saponin-standard (HA = 30000) as the reference substance. The value of hemolytic activity (SU/g) was calculated using following equation:

\[
HA = a \cdot c / b \cdot d
\]

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where:  
\[ a = \text{minimum volume (ml) of saponin-standard solution that provoke total hemolysis} \]  
\[ b = \text{minimum volume (ml) of extract that provoke total hemolysis} \]  
\[ c = \text{mg of saponin-standard in 100 ml standard solution} \]  
\[ d = \text{g of plant material in 100 ml extract} \]  

**Determination of mineral content**

**Sample preparation.** Microwave-assisted digestion in a Milestone Touch Control microwave digestion system was used for mineralization purposes. To 0.5 g herbal material 2 ml of conc. HNO₃ and 1 ml of 30% H₂O₂ were added and the mixture was subjected to microwave digestion with following program:

| Step | Temperature/°C | Duration/min | Power/W |
|------|----------------|--------------|---------|
| 1    | 180            | 10           | 800     |
| 2    | 180            | 15           | 800     |

After cooling, the obtained solution was transferred into 50 ml volumetric flask and filed up with 4% HNO₃. With each set of digested samples, a blank sample was run through the digestion procedure.

**Instrumentation condition.** Macrolelements were determined by atomic absorption spectrometry (AAS). A Varian SpectrAA 640Z Zeeman electrothermal atomic absorption spectrophotometer with a Varian PSD-100 Autosampler and Varian SpectrAA 880 with deuterium correction (for flame determination) were used. Hollow cathode lamps were used as a source. Operating conditions for the determination of Pb, Co, Ni, Cr, Fe, Mn, Na, K, Cd and Zn are given in Tables 1 and 2.

**Determination of the radical scavenging and antioxidant activity**

**Sample preparation.** Dried powdered plant material (10 g) was extracted by continual mixing in 100 ml

| Table 1. Instrumental parameters for determination of Na, K, Ni, Fe, Mn, Cu and Zn by flame AAS |
| Element | Wavelength, nm | Slit, nm | Lamp current, mA |
|---------|----------------|----------|-----------------|
| Na      | 589.0          | 0.2      | 5               |
| K       | 766.5          | 1.0      | 5               |
| Ni      | 232.0          | 0.2      | 4               |
| Fe      | 248.3          | 0.2      | 5               |
| Mn      | 279.5          | 0.2      | 5               |
| Cu      | 324.8          | 0.2      | 5               |
| Zn      | 213.9          | 1.0      | 5               |

| Gas mixture | Acetylene/air |
|-------------|---------------|

| Table 2. Optimal parameters for Co, Ni, Pb, Cd and Cr determination by Zeeman ETAAS |
| Parameters | Co | Ni | Pb | Cd | Cr |
| Wavelength, nm | 242.5 | 232.0 | 283.3 | 228.8 | 357.9 |
| Slit, nm | 0.2 | 0.2 | 0.5 | 0.5 | 0.5 |
| Lamp current, mA | 7.0 | 4.0 | 5.0 | 4.0 | 7.0 |
| Calibration mode | GAS | GAS | GAS | GAS | GAS |
| GAS | Absorbance, peak hight | Argon |
| Temperature (°C) | 120 | 120 | 120 | 120 | 120 |
| Ramp time (s) | 45 | 45 | 45 | 45 | 45 |
| Hold time (s) | 10 | 10 | 10 | 10 | 10 |
| CHARING | | | | | |
| Temperature (°C) | 750 | 800 | 400 | 250 | 100 |
| Ramp time (s) | 5 | 6 | 5 | 5 | 5 |
| Hold time (s) | 32 | 2 | 32 | 2 | 3 |
| ATIMIZING | | | | | |
| Temperature (°C) | 2300 | 2400 | 2100 | 1800 | 2500 |
| Ramp time (s) | 1.1 | 1 | 1 | 0.7 | 1.2 |
| Hold time (s) | 2 | 2 | 2 | 0 | 2 |
| CLEANING | | | | | |
| Temperature (°C) | 2300 | 2400 | 2100 | 1800 | 2500 |
| Hold time (s) | 2 | 2 | 2 | 2 | 2 |

* Program for steps 1-3 is the same for all elements

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ethanol:water (7:3, V/V), 24 h at room temperature. After filtration, filtrate was evaporated until dry. The residues were dissolved in 96% ethanol to obtained solution with concentration 0.1 g ml\(^{-1}\).

**Reagent and standards.** The standards of quercetin and BHA (butyl hydroxyl anisole) were purchased from Extrasynthese, Lyon, France. All other chemicals were of reagent grade and were used without further purification.

**Assessment of the free radical scavenging activity in 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay.** The antioxidant activity using the DPPH assay was assessed by the method of Tagashira and Ohtake (1998). A test sample solution (plant extract) (200 µl) was added to 4 ml of 100 mmol l\(^{-1}\) ethanolic DPPH. After vortexing, the mixture was incubated for 10 minutes at room temperature and the absorbance was measured at 517 nm. The differences in absorbance between a test sample and a control (ethanol) was considered as an activity. The activity was shown as \(IC_{50}\) value. Quercetin and BHA (100 mg ml\(^{-1}\) in ethanol) were used as reference substances. All values are shown as a mean value of the three measurements.

**Assessment of the hydroxyl radical scavenging activity.** Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the plant extracts for hydroxyl radicals generated from the Fe\(^{3+}\)/ascorbate/EDTA/H\(_2\)O\(_2\) system. The attack of the hydroxyl radical on deoxyribose leads to TBARS (thiobarbituric acid-reactive substances) formation (Haliwell and Gutteridge, 1981). The extracts were added to the reaction mixture containing 2.8 mmol l\(^{-1}\) deoxyribose, 100 mmol l\(^{-1}\) FeCl\(_3\), 104 mmol l\(^{-1}\) EDTA, 100 mmol l\(^{-1}\) ascorbic acid, 1 mmol l\(^{-1}\) H\(_2\)O\(_2\), and 230 mmol l\(^{-1}\) phosphate buffer (pH 7.4), making up a final volume of 1.0 ml. In the series of control experiments reference substances, such as quercetin and BHA (100 mg ml\(^{-1}\) in phosphate buffer-pH 7.4), were used instead of the extracts. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by the method given by Ohkawa et al. (1979). One ml of thiobarbituric acid TBA (1%) and 1.0 ml trichloroacetic acid (TCA 2.8%) were added to the test tube and were incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate.

**Assessment of antioxidant activity.** The antioxidant activity of the plant extracts was evaluated using β-carotene-bleaching assay (Wanasundara et al. 1994). A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 ml chloroform. One ml of this solution was pipetted into a round-bottom flask. When chloroform was rotary evaporated at 40 °C under vacuum, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier and 50 ml of distilled water were added to the flask with vigorous shaking. Aliquots (5 ml) of this emulsion were transferred into a series of tubes containing 2 mg of each plant extract or 2 mg of BHA (butylated hydroxyanisole) or 2 mg of quercetin, for comparison. Aliquots (5 ml) of emulsion without any further additions were used as control. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 10-min intervals by keeping the sample in a water bath at 50 °C until the visual color of β-carotene in the control sample had disappeared (about 120 min).

**Assessment of the capacity of inhibition of spontaneous lipid peroxidation (LP).** The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in liver homogenate using the method of Ohkawa et al. (1979). The amount of formed malondialdehyde (MDA) was quantitated by reaction with thiobarbituric acid and used as a measure of lipid peroxidation. The results were expressed as nmol MDA.mg\(^{-1}\) protein. The content of protein was determined according to the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

**Preparation of liver homogenate.** The rat livers were exposed, dissected free from extraneous tissues, rinsed with chilled 1.15% KCl solution (pH 7.0) and 50% homogenate was prepared in 0.15 mol l\(^{-1}\) sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 3500 g for 10 minutes at 4 °C and the supernatant was used for the estimation of lipid peroxidation level.

**Preparation of control:** to 0.5 ml of liver homogenate supernatant (LHS), 10 µl H\(_2\)O and 4.5 ml extractive solvent (10 ml 10 % HClO\(_4\) saturated with TBA and 30 ml 20% TCA) were added and heated on 95 °C for 20 minutes. After cooling, the mixture was centrifuged at 3500 g, 10 minute. Absorbance of transparent supernatant was measured on 532 nm.

**Preparation of test solution:** to 0.5 ml of liver homogenate supernatant (LHS), 10 µl of plant extract (or reference substance solution) and 4.5 ml extractive solvent were added and heated on 95 °C for 20 minutes. After cooling, the mixture was centrifuged at 3500 x g, 10 minute. Absorbance of transparent supernatant was measured on 532 nm.

The inhibition of lipid peroxidation (%) was calculated using following equation:

$$I\% = \frac{A_0 - A_u}{A_0} \times 100$$

\(A_0\) - absorbance of control; \(A_u\) - absorbance in presence of extract (reference substance).

The amounts of formed malondialdehyde (nmol MDA mg protein\(^{-1}\)) were calculated using following equation:

$$cMDA = \frac{A}{\varepsilon} \times \frac{V}{V_i} \times \frac{10^{-3}}{R}$$

\(A\) - absorbance; \(\varepsilon\) - 156 000 dm\(^3\)/mol; \(V\) - volume before centrifugation; \(V_i\) - volume of LHS; \(R\) - dissolution rate.
Results and discussion

Basic chemical parameters

The basic parameters important for assessment of quality of dried aerial part of *Eryngium campestre* from Kosovo (*Eryngii herba*) and roots (*Eryngii radix*) included determination of total ash, ash insoluble in hydrochloric acid and loss on drying (Table 3). The values of loss on drying ranged from 7.0 to 9.5% in herba and from 7.0–9.9% in radix. The amounts of total ash were lower in samples of herba (5.8–7.3%) in comparison to those of radix (9.6–13.3%). Samples of *Eryngii herba* had lower percentage of water extract (18.3–24.5%) in comparison to the samples of *Eryngii radix* (29.6–36.6%). Obtained results showed presence of larger amounts of possible active components in *Eryngii radix*. The amount of 8.41% total ash from the leaves of *E. foetidisima* are reported previously (Borah et al. 2009).

Flavonoid content

The results of determination of total flavonoid content in *Eryngii herba* are presented in Table 4. The percentages of total flavonoids (0.12 - 0.14%) were expressed as total quercetin. Compared with literature data, the contents of total flavonoids were lower than those previously reported by other researchers. Thus, in dried aerial parts of *E. platanum*, *E. campestre* and *E. maritimum* from Romania, total of 0.32 – 0.56% of flavonoids expressed as rutin were found (Suciu et al. 2006).

Hemolytic activity

The results of the evaluation of hemolytic activity of *Eryngii radix* are presented in Table 5. Obtained values were lower in comparison to the HA of some saponin-containing plants such as *Saponaria officinalis* (radix) (50 SU/g), *Herniaria glabra* (herba) (30 SU/g) or *Primula ver-

### Table 3. The levels of basic chemical parameters for assessment of quality of *Eryngium herba* and *Eryngium campestre* from Kosovo (%)

| Sample | Loss on drying | Ash | Ash insoluble in hydrochloric acid | Water extract |
|--------|----------------|-----|----------------------------------|---------------|
| EH1/02 | 7.5            | 6.6 | 1.9                              | 24.2          |
| EH1/03 | 7.6            | 5.8 | 1.7                              | 22.5          |
| EH2/02 | 6.6            | 6.6 | 2.0                              | 18.8          |
| EH2/03 | 9.1            | 7.3 | 2.3                              | 21.1          |
| EH3/02 | 7.7            | 5.8 | 1.5                              | 21.8          |
| EH3/03 | 9.5            | 5.8 | 1.8                              | 18.3          |
| ER1/02 | 8.9            | 10.7| 1.9                              | 36.6          |
| ER1/03 | 9.8            | 11.9| 3.0                              | 33.6          |
| ER2/02 | 7.0            | 13.0| 2.5                              | 31.6          |
| ER2/03 | 9.0            | 12.0| 3.6                              | 29.6          |
| ER3/02 | 9.9            | 13.3| 4.8                              | 34.7          |
| ER3/03 | 9.3            | 9.6 | 5.3                              | 33.8          |

EH – samples of *Eryngii herba*, ER – samples of *Eryngii radix*, n = 3.

### Table 4. The content of total flavonoids in *Eryngii herba*, *Eryngium campestre*

| Sample | 0.12±0.01 | 0.14±0.01 | 0.13±0.01 | 0.13±0.03 | 0.13±0.04 | 0.14±0.03 |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|

n = 3; Sd – standard deviation; EH – samples of *Eryngii herba*

### Table 5. The values of hemolytic activity of *Eryngii radix*, *Eryngium campestre* (saponin units per g plant material, SU/g)

| Sample | 14.4 | 15.3 | 15.8 | 14.9 | 16.3 | 14.08 |
|--------|------|------|------|------|------|-------|

ER – samples of *Eryngii radix*, n = 3.
is (radix) (120 SU/g), (Ph. Jug. 5). Data of hemolytic activity of Eryngii radix are not available in the literature. Among information about the presence of triterpene saponins in Eryngium campestre and other Eryngium species, data of chemical structure of saponins could be found (Kartal et al. 2005; 2006). Romanian researchers investigated the chemical composition of indigenous Eryngium species in Romania (E. planum, E. campestre and E. maritimum) and found that triterpene saponins (determined by gravimetric method) were presented in the aerial part of the plants, ranging from 3.7-10.1% (Suciu et al. 2006).

Mineral content

Determination of mineral content included determination of eight microelements (Cd, Co, Cu, Fe, Mn, Pb and Zn) and two macroelements (Na and K). The results obtained for the samples of Eryngii herba and Eryngii radix are presented in Table 6. Obtained results differ a lot depending on the plant organ or the origin of the sample. In some cases the content of investigated minerals in Eryngii herba was relatively constant such as that of Zn (15.14-20.39 mg kg⁻¹), Cr (0.7-1.57 mg/kg) or Cu (8.17-11.17 mg kg⁻¹) while for the other minerals higher variability occurs (Fe from 69.93-196.77 mg kg⁻¹; Mn 31.46-47.68 mg kg⁻¹). The content of toxic elements was very low, from 0.17-1.33 mg kg⁻¹ and from 0.05-0.12 mg kg⁻¹ for Pb and Cd, respectively. The content of K in Eryngii herba was very high, from 1765.10-2538.08 mg kg⁻¹ while the content of Na ranged from 43.65-94.7 mg kg⁻¹.

Similar results were obtained for the content of minerals in Eryngii radix (Table 6). The content of Mn was almost the same as it was found in herba, from 25.19-41.19 mg kg⁻¹, the content of Zn ranged from 15.55-25.29 mg kg⁻¹, and for Cu from 9.16-12.45 mg kg⁻¹. Great variability in the content of Fe was occurred again and significantly larger amounts were found in Eryngii radix ranging from 198.9-325.7 mg kg⁻¹. The content of Na in Eryngii radix was higher than in Eryngii herba, ranging from 170.2-590.9 mg kg⁻¹, while the content of K in Eryngii radix was also very high, from 743.7-961.8 mg kg⁻¹ but significantly lower than in Eryngii herba. The content of toxic elements was very low, from 0.10-1.76 mg kg⁻¹ and from 0.04-0.16 mg kg⁻¹ for Pb and Cd, respectively.

Generally, Eryngii herba accumulates larger amounts of K while Eryngii radix accumulates larger amounts of Na and Fe. Comparing with the literature data, the leaves of E. billardieri contain higher amount of N, K, Ca and Mg and lower amount of P, S and Na than some common vegetables, while the content of Fe, Mn, Zn and Cu were at the same level (Turan et al. 2003). In leaves of E. foetidissima, 24.26 mg g⁻¹ Fe and 11.26 mg g⁻¹ K were found (Borah et al. 2009).

Radical scavenging and antioxidant activity

DPPH free radical scavenging activity

Free radical scavenging activity of Eryngium extracts was determined by comparing the activity with that of referent substances (quercetin and BHA), which possess known antioxidant potential. The values obtained in DPPH assay for plant extracts are shown together with that of referent substances (Table 7). The highest scavenging effect was obtained with quercetin (IC₅₀=0.06 mg ml⁻¹) and the lowest one with the root extract of E. campestre (IC₅₀=1.14 mg ml⁻¹). The results suggest that E. campestre extracts act as non-specific donators for hydrogen atoms or electrons in the DPPH-assay. When compared to the reference substances, the Eryngium extracts were found to be less efficient. Nevertheless, the existing scavenging effects of the

Table 6. The content of minerals in Eryngii herba and Eryngii radix after mineralization in micro-wave digestion system (MW) (w/mg/kg)

| Sample     | Cd  | Co  | Cr  | Cu  | Fe   | K    | Mn  | Na  | Pb  | Zn  |
|------------|-----|-----|-----|-----|------|------|-----|-----|-----|-----|
| **Eryngii herba** |     |     |     |     |      |      |     |     |     |     |
| EH1/02     | 0.11| 0.08| 0.70| 8.17| 69.9 | 1963 | 33.78| 43.66| 0.17| 17.19|
| EH2/02     | 0.14| 0.10| 1.57| 9.90| 119.7| 2167 | 47.68| 81.50| 0.75| 19.17|
| EH3/02     | 0.11| 0.08| 1.58| 8.26| 114.5| 2228 | 44.67| 56.74| 0.28| 20.40|
| EH1/03     | 0.067| 0.09| 1.55| 11.17| 148.6| 1774 | 32.51| 46.15| 0.18| 15.99|
| EH2/03     | 0.06| 0.13| 1.36| 10.39| 196.8| 1765 | 31.46| 62.87| 0.97| 15.93|
| EH3/03     | 0.10| 0.10| 1.23| 10.74| 162.5| 1797 | 32.86| 94.70| 1.33| 17.47|
| **Eryngii radix** |     |     |     |     |      |      |     |     |     |     |
| ER1/02     | 0.16| 0.50| 3.19| 12.45| 213.7| 934  | 26.11| 590.91| 0.14| 25.29|
| ER2/02     | 0.12| 0.44| 4.33| 11.29| 209.7| 849  | 25.19| 551.85| 0.36| 22.63|
| ER3/02     | 0.10| 0.49| 3.82| 10.64| 325.7| 744  | 26.02| 492.99| 0.10| 20.95|
| ER1/03     | 0.04| 0.24| 2.53| 9.16| 198.9| 932  | 41.41| 170.70| 0.46| 16.57|
| ER2/03     | 0.09| 0.18| 2.45| 9.52| 208.7| 962  | 41.18| 172.38| 1.76| 16.68|
| ER3/03     | 0.06| 0.19| 2.03| 10.43| 183.3| 910  | 41.19| 218.67| 0.62| 15.56|

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examined extracts probably could be attributed to the presence of flavonoids, but also could be resulted of the activity of other secondary biomolecules present in the extracts.

Compared to literature data, different Eryngium species manifest different radical scavenging activity. Thus, the methanol extracts of leaves and inflorescence of Eryngium caucasicum at flowering stage show remarkable activity with IC$_{50}$ = 0.15±0.01 mg ml$^{-1}$ for leaves and 0.39±0.02 mg ml$^{-1}$ for inflorescence (Ebrahimzadeh et al. 2009; Nabavi et al. 2009). Radical scavenging activity of $E$. maritimum methanol extract was investigated, as well, revealing IC$_{50}$ = 0.28 mg ml$^{-1}$ in the ABTS assay (Meot-Duros et al. 2008).

Table 7. Free radical scavenging activity of the extracts of Eryngium campestre in DPPH assay and inhibition of OH$^-$ radical production (%)

| Sample           | DPPH assay (IC$_{50}$, mg/ml) | OH$^-$ (% of inhibition) |
|------------------|-------------------------------|--------------------------|
| Eryngii radix    | 0.72                          | 45.00                    |
| Eryngii herba    | 1.14                          | 50.11                    |
| Quercetin        | 0.06                          | 42.05                    |
| BHA              | 0.15                          | 52.09                    |

Hydroxyl radical scavenging activity

The effect of Eryngium extracts on the inhibition of hydroxyl radical production (IOH) was assessed by the iron (II)–dependent deoxyribose damage assay. It is well known that the Fenton reaction generates hydroxyl radicals (OH$^-$), which degrade deoxyribose, using Fe$^{2+}$ salts as an important catalytic component (Halwill and Gutteridge, 1981). Oxygen radicals may attack sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose, causes degradation of sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBA). Table 7 presents the results of the effects of examined Eryngium extracts as well as that of reference substances on OH$^-$ radical production. They show that both extracts of Eryngium campestre and reference substances inhibited the production of OH$^-$ radicals. The strongest inhibitory activity was exhibited by BHA (52%). Both extracts of Eryngium campestre exhibited significant inhibitory effect, 45% and 50%, respectively, higher than the percentage of inhibition obtained by quercetin (42%). Previously, it has been shown that quercetin and its glycosides exert inhibitory activity against lipid peroxidation (Cook and Samman, 1996; Dangles, 2000).

Antioxidant activity

The antioxidant activity of Eryngium campestre extracts was weak in β-carotene/linoleic acid system. From the results mentioned above, it was shown that Eryngium campestre extracts exhibited strong free radical scavenging activity against DPPH and OH$^-$ radicals, comparable to the activity of quercetin and BHA. In the β-carotene/linoleic acid system Eryngii extracts have shown modest antioxidant effects, which was comparable with the activity of quercetin for the period of the first 60 minutes (Table 8). According to the literature data, antioxidant activity of Eryngium species is an interesting topic for the science. It was found that antioxidant index of leaf extract of E. foetidum determined by measuring the coupled oxidation of carotene and linoleic acid had a value of 5.65±0.46. The antioxidant index is the ratio of bleaching rate of control (system with no added test compounds) to the bleaching rate when a test compounds was added into the system (Chanwitheesuk et al. 2005). It was reported that vitamins C and E, carotenes, xanthophylls, tannins and total phenolics may be responsible for the activity, as they were present in leaves of E. foetidum (11.4; 0.0069; 1.92; 1.60; 17.7 and 98.4 (mg%), respectively). Accounting that the bleaching rate of β-carotene was determined by the differences in the spectral absorbance reading between the initial and last reading of bleaching that remained essentially linear, divided by time, antioxidant index was estimated for the investigated samples of Eryngium campestre. It was obtained that antioxidant index of Eryngii radix had a value of 0.54 while for Eryngii herba it was 0.69. Both values were significantly under the antioxidant activity of E. foetidisima.

Table 8. Effect of ethanol extracts of Eryngium campestre in comparison to BHA and quercetin on the oxidation of β-carotene-bleaching assay

| Sample      | A$_{270}$ (% of initial value) |
|-------------|-------------------------------|
|             | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| Eryngii radix | 100 | 80 | 60 | 52 | 42 | 40 | 38 | 37 | 36 |
| Eryngii herba | 100 | 81 | 63 | 54 | 46 | 43 | 41 | 40 | 39 |
| Quercetin   | 100 | 89 | 82 | 62 | 46 | 34 | 26 | 20 | 16 |
| BHA         | 100 | 81 | 70 | 63 | 57 | 51 | 48 | 45 | 42 |
| Control     | 100 | 61 | 57 | 47 | 40 | 38 | 36 | 32 | 30 |
Inhibition of lipid peroxidation (LP)

Antioxidant activity of ethanol extracts of the aerial part and root of *E. campestre* was examined over the inhibition of spontaneous lipid peroxidation in rat liver homogenate as well, measuring the content of formed MDA by TBA assay (TBARS). The results given in Table 9 showed that the content of formed MDA was lower when the extract of root or aerial part were added into the system (3.67 ± 0.04 and 4.05±0.07 nmol ml⁻¹, respectively), but still the levels of MDA were significantly higher than those obtained when quercetin was used as an antioxidant (3.12 ± 0.04 nmol ml⁻¹).

**Table 9.** The content of MDA (nmol mg⁻¹) and the inhibition of lipid peroxidation (%) in rat liver homogenate by ethanol extracts of aerial part and root of *E. campestre*

| Sample          | TBARS – homogenate (nmol MDA mg protein⁻¹) | Inhibition of LP (%) |
|-----------------|------------------------------------------|----------------------|
| Control (ethanol) | 4.60 ± 0.02                              | 0.0                  |
| *Eryngii radix*  | 3.67 ± 0.04                              | 20.0                 |
| *Eryngii herba*  | 4.05 ± 0.07                              | 12.2                 |
| Quercetin       | 3.12 ± 0.04                              | 36.9                 |

The range of inhibition of lipid peroxidation in the rat liver homogenate was 20% and 12% for the extract of root and aerial part of *E. campestre*, respectively. Obtained values were lower than that of quercetin (36.9%). Compared to the recently published data on the activity of some other wild plants such as *Calamintha nepeta*, *Calamintha gradiflora* and *Micromeria crista*, higher inhibition activity of ethanol extracts on lipid peroxidation in the same biological system was exhibited (20.35%, 25.6% and 29.6% of inhibition, respectively) (Kadifkova Panovska 2004). Besides evident free radical scavenging activity against DPPH and hydroxyl (OH⁻) radical, antioxidant activity of *Eryngium campestre* in both non-biological (β-carotene/linoleic acid) and biological (rat liver homogenate) system manifested a low capacity.

**Conclusion**

Analysis of the chemical composition of the aerial part and root of *Eryngium campestre* from Kosovo showed that the content of total ash, ash insoluble in hydrochloric acid, loss on drying and the content of water extract were relatively constant regards the differences in the characteristics of the locations and year of collection. Samples of *Eryngii herba* gave lower percentage of water extract (18.3-24.5%) in comparison to the samples of *Eryngii radix* (29.6-36.6%). The analysis of mineral content (Zn, Fe, Cu, Mn, Ni, K, Co, Pb and Cr) showed that different part of *E. campestre* accumulate different amount of investigated minerals. Evaluation of radical scavenging and antioxidant activity showed that higher radical-scavenging activity against DPPH radical has been presented by the ethanol extract of root of *E. campestre* (IC₅₀ = 0.72 mg ml⁻¹) compared to the aerial part of the plant (IC₅₀ = 1.14 mg ml⁻¹). Furthermore, the inhibition capacity on the production of hydroxyl radical in deoxyribose system was found to be strong (50% and 45% for aerial part and root ethanol extract, respectively). However, both ethanol extracts of *E. campestre* from aerial part and root, exhibited low antioxidant activity in β-carotene/linoleic acid system as well as low capacity for inhibition of spontaneous lipid peroxidation in rat liver homogenate.

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Резиме

Хемиска карактеризација и антиоксидативна активност на Eryngium campestre L., Apiaceae од Косово

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Ключни зборови: Eryngium campestre, флавоноиди, минерали, DPPH тест, антиоксидативна активност, β-каротен-избелувачки тест, TBA тест.

Оваа студија се однесува на дефинирање на хемиски состав и утврдување на антиоксидативна активност in vitro на екстракти подготвени од надземниот дел и од коренот на Eryngium campestre L. (Apiaceae) од Косово. Анализата на хемискиот состав вклучува определување на вкупен пепел, пепел нерастворлив во хлороводородна киселина, губиток со сушење и содржина на воден екстракт, како и определување на содржина на вкупни флавоноиди во надземниот дел и хемолитична активност на коренот. Составот на минералите (Zn, Fe, Mn, Ni, K, Co, Pb, Cd и Cr) во надземниот дел и во коренот се определени со користење на атомска апсорпциона спектроскопија (AAS и ETAAS). Утврдено е дека различни делови од E. campestre акумулираат различни количества од испитуваните минерали. Антиоксидативната активност е испитувана со четири методи: DPPH тест, инхибиција на продукција на хидроксил радикал, β-каротен-избелувачки тест и инхибиција на липидна пероксидација (TBA тест). Во DPPH системот етанолниот екстракт од коренот покажува подобна радикал-фаќачка активност (IC_50 = 0.72 mg ml⁻¹) во споредба со соодветните екстракти од надземниот дел (IC_50 = 1.14 mg ml⁻¹). Од друга страна, етанолниот екстракт од надземниот дел покажува поголем инхибирачки капацитет врз продукцијата на хидроксил радикалот во системот од дезоксирибоаза во споредба со екстрактот од коренот (50% и 45%, соодветно). Двата етанолни екстракти од E. campestre покажуваат ниска антиоксидативна активност во β-каротен-избелувачки тест, како и низок капацитет за инхибирање на спонтана липидна пероксидација во хомогенат од црн дроб од стаорец.