

ANTIOXIDANT ACTIVITY AND CONTENT OF SELECTED MICRONUTRIENTS IN THE FRONDS OF *Asplenium ceterach*

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Abstract

This study aimed to evaluate the total phenolic compound content and antioxidant capacity of the ethanolic extract of *Asplenium ceterach* fronds, as well as the content of selected micronutrients (Fe, Cu, Zn, and Mn) in fronds collected in the Republic of Srpska (Bosnia and Herzegovina). The extract's total phenolic compound (TPC) concentration was 16.08 ± 0.08 mg GAE/gDW. It demonstrated DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity with an IC_{50} value of 29.28 ± 2.20 mg/mL. The hydrogen peroxide scavenging capacity was significantly lower ($IC_{50} = 1420.56 \pm 35.72$ μ g/mL), indicating moderate effectiveness against this reactive oxygen species. The extract also showed hydroxyl radical scavenging ability, with $56.26 \pm 1.05\%$ residual \cdot OH radicals under the tested conditions. Reducing power assays revealed that at 500 μ g/mL, the extract reduced Fe^{3+} to 1420.20 ± 53.67 mmol/L Fe^{2+} equivalents, while its Cu^{2+} reducing capacity was 423.14 ± 29.20 μ g Trolox/mL at a TPC concentration of 300 μ g/mL. The micronutrient levels in *A. ceterach* were as follows: Fe (164.57 ± 4.08 mg/kg) > Zn (15.61 ± 0.43 mg/kg) > Mn (12.39 ± 0.19 mg/kg) > Cu (1.62 ± 0.12 mg/kg). Our *in vitro* findings suggest that the ethanolic extract of *A. ceterach* could serve as a potential natural source of antioxidant compounds.

Key words: DPPH scavenging activity, reducing power, hydroxyl radical scavenging activity, H_2O_2 scavenging activity

INTRODUCTION

Oxidative stress is a state characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses. When ROS, including superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and especially the hydroxyl radical (\cdot OH),

accumulate excessively, they cause oxidative damage to lipids, proteins, and nucleic acids (Halliwell and Gutteridge, 2015). Among these, the hydroxyl radical is considered the most harmful due to its extremely high reactivity and lack of enzymatic detoxification systems. It is mainly produced through Fenton and Haber–Weiss reactions catalyzed by transition metals such as Fe^{2+} and Cu^+ . Oxidative stress plays a key role in the development of numerous chronic diseases (e.g. type 2 diabetes, Alzheimer's disease, inflammation (Reuter *et al.*, 2010; Phaniendra *et al.*, 2015)). Therefore, identifying and characterizing natural antioxidants that can scavenge ROS, especially $\cdot\text{OH}$ radicals, remains a significant focus of scientific and therapeutic research.

Plants are a rich source of bioactive compounds with strong antioxidant properties, with phenolic compounds being particularly important. Phenolic compounds, including phenolic acids, flavonoids, and tannins, exert antioxidant effects mainly through hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms. These compounds can directly neutralize free radicals, halt chain reactions, and chelate transition metals involved in Fenton reactions, thereby reducing $\cdot\text{OH}$ radical formation (Rice-Evans *et al.*, 1997; Shahidi and Ambigaipalan, 2015; Tatipamula and Kukavica, 2021). The structural features of phenolics, especially the number and position of OH groups, largely influence their ability to scavenge radicals.

Alongside organic antioxidants, essential micronutrients such as copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) play important roles in maintaining cellular redox balance. These elements serve as cofactors for key antioxidant enzymes, including CuZn-superoxide dismutase, Mn-superoxide dismutase, and catalase (Wróblewski *et al.*, 2024). Although Fe and Cu are vital for enzymatic activity, their redox-active nature can also promote Fenton reactions and increase $\cdot\text{OH}$ production if they are present in excess (Valko *et al.*, 2005). Additionally, redox-inactive metals can impair the antioxidant defense system by damaging the electron transport chain and inducing lipid peroxidation (Jomova *et al.*, 2012). Therefore, measuring their concentrations is important both from a nutritional standpoint and considering their potential pro-oxidant effects.

Asplenium ceterach L. (Aspleniaceae) is a widely distributed fern species whose native range extends from Europe through northwestern Africa to western and central Asia, reaching as far as Tibet (POWO, 2026). It is primarily associated with temperate biomes. The species typically inhabits rock crevices, limestone outcrops, rocky slopes, dry stone walls, and old masonry structures, where it grows under conditions of limited substrate depth and restricted water availability. *Asplenium ceterach* commonly inhabits open, sun-exposed habitats, where it is subjected to pronounced temperature fluctuations and periodic drought stress (Škondrić, 2019). It is mentioned in several studies for its medicinal properties, including antidiuretic, antihelmenthic, and as a remedy for skin and respiratory problems (Malamas and Marselos, 1992), haemorrhoids, hypertension (Froissard *et al.*, 2015; Živković *et al.*, 2022), as a kidney “stone breaker” (Leporatti and Guarrera, 2007; Güneş *et al.*, 2017; Tomou and Skaltsa, 2018), and for diarrhea, neuralgia, prostate issues (Karadeniz *et al.*, 2015), as well as inflammatory bacterial, and fungal diseases (Mir *et al.*, 2013). These medicinal properties are attributed to the presence of mineral salts, mucilage, tannins, flavonoids, caffeic acid, and chlorogenic acid (Di Sanzo *et al.*, 2013). Despite its wide distribution in Bosnia and Herzegovina, this fern

species has been the subject of only a limited number of ethnobotanical studies. However, this species is mentioned in the traditional medicine of Konjuh mountains as a treatment for spleen diseases, as a diuretic, to promote sweating, and to cleanse the blood (Huseinović *et al.*, 2024), while in Serbia there are reports of its use as an anxiolytic (Živković *et al.*, 2017, 2022; Matejić *et al.*, 2020). According to one study from Bosnia and Herzegovina, only the fronds of this plant are used in traditional medicine (Redzic, 2010), while a study from Croatia showed that most people use it in the form of tea (Dolina and Luczaj, 2014).

Our study aimed to evaluate the total phenolic content and antioxidant potential of *Asplenium ceterach* using DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (*Ferric reducing antioxidant power assay*), CUPRAC (*Cupric reducing antioxidant capacity*), and H₂O₂ scavenging assays, as well as hydroxyl radical scavenging capacity assessed by EPR (*Electron Paramagnetic Resonance*) spectroscopy. Furthermore, the content of essential micronutrients (Cu, Fe, Zn, and Mn) was determined to provide a comprehensive insight into the antioxidant and nutritional potential of this insufficiently explored fern species.

MATERIALS AND METHODS

Plant material

For this study, fronds (leaf organs of ferns) of *Asplenium ceterach* were collected (Figure 1). The samples were collected in June 2020 at Kovačina, located on the karst rim of Popovo Polje (Trebinje, the Republic of Srpska, Bosnia and Herzegovina).



Figure 1. *Asplenium ceterach* L.

(Photo by S. Škondrić)

The plants were growing on rocky terrain along a rural road. One frond was taken from each individual plant, so the total sample comprised fronds from a large number of individuals. Species identification was performed according to Nikolić (2020), and the nomenclature was aligned with Euro+Med (2026). Herbarium material was deposited in the Herbarium of the

Department of Botany, Faculty of Natural Sciences and Mathematics, University of Banja Luka.

Preparation of plant extracts

The fronds were dried at room temperature in the shade in an airy place. After drying, the plant material was ground into a powder using an electric grinder. The plant powder (10 g) was homogenized with 200 mL of 80% ethanol (w:v = 1:20). The homogenate was sonicated for 5 minutes and mixed for 30 minutes using a magnetic stirrer before filtration through filter paper (ALBET, 73 g/m²). The filtrate was designated as extract 1 (E1). The residue remaining on the filter paper was re-extracted with 100 mL of 80% ethanol as described above. After filtration, extract 2 (E2) was separated and combined with extract 1 (E1). The combined extract was stored at -18 °C until use and centrifuged for 10 minutes at 10,000 rpm before analysis.

Determination of the content of total phenolic compounds

The content of total phenolic compounds (TPC) was determined according to Singleton and Rossi (1965) by measuring the absorbance at 724 nm. The reaction mixture contained 100 µL of the plant extract and 450 µL of Folin-Ciocalteu reagent (diluted with distilled water in a ratio 1:3). After 5 minutes of incubation at room temperature, 450 µL of 1M Na₂CO₃ was added to the mixture. The mixture was vortexed well and then incubated in the dark for 60 minutes. The quantification of TPC was calculated based on the calibration curve for gallic acid and expressed as gallic acid equivalent (GAE) per g of DW.

Determination of the ability to remove DPPH radicals

The ability to remove DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) was determined according to the method described by Liyana-Pathiranana and Shahidi (2005). The absorbance was measured at 517 nm.

The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

The IC₅₀ was determined using an inhibition curve constructed from the percentage inhibition versus phenolic compound concentration in the extract.

Ability to scavenge H₂O₂

The ability of the extract to scavenge hydrogen peroxide was determined according to the work of Ebrahimzadeh *et al.* (2010). A hydrogen peroxide solution (0.5 M) was prepared in a 0.067 M Na-phosphate buffer (pH 6.4). The sample contained 920 µL of Na-phosphate buffer 0.067 M, 30 µL of 0.5 M H₂O₂, and 50 µL of plant extract in different concentrations. The samples were mixed and incubated at room temperature in the dark for 30 minutes. After incubation, the absorbance of the sample (A_{sample}) and control (A_{control}) was measured at 230 nm. The control contained 970 µL of Na-phosphate buffer 0.067 M pH 6.4, and 30 µL of 0.5 M H₂O₂. The blank sample (Sb) contained 50 µL of extract at different concentrations and 950 µL of Na-phosphate buffer. The percentage of H₂O₂ removal was determined for each extract concentration according to the following equation:

$$\% \text{ scavenging of H}_2\text{O}_2 = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

The IC₅₀ was determined using an inhibition curve constructed from the percentage inhibition versus phenolic compound concentration in the extract.

Ability to scavenge ·OH radicals

To test the ability of the extracts to scavenge hydroxyl radicals, a solution containing the extract was used in combination with the Fenton reaction and the spin trap 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) (Milutinović *et al.*, 2023). The reaction mixture (total volume 29 µL) consisted of the extract (1 µL, previously diluted 1000-fold in water), deionized water (25 µL), H₂O₂ (2 µL, final concentration 0.35 mM), and DEPMPO (1 µL, final concentration 3.5 mM). This mixture was placed in a gas-permeable Teflon tube, and FeSO₄ (1 µL, final concentration 0.15 mM) was added immediately. The EPR spectra were recorded 2 minutes later at room temperature using a Bruker Biospin Elexsys II 540 spectrometer. The following operating parameters were used for the experimental setup: center field 3500 G, microwave power 10 mW, microwave frequency 9.85 GHz, modulation frequency 100 kHz, modulation amplitude 1 G. In the control experiments, the extract was replaced by an equivalent amount of solvent. The anti-·OH activity (AA) of the extracts was calculated using the following equation:

$$AA(\%) = ((I_c - I_a) / I_c) * 100$$

where I_c and I_a are the double integral values for the control and sample, respectively, derived from the EPR spectra (using Xepr software).

Determination of the Fe chelating capacity

The Fe chelating capacity was determined according to the method described by Carter (1971) with some modifications. The reaction mixture contained 937.5 µL of ethanol, 12.5 µL of 1 mM FeSO₄, and 50 µL of plant extract with different concentrations of phenolic compounds. After vortexing and incubation for 10 min, 50 µL of 2 mM ferrozine (disodium;4-[3-pyridin-2-yl-6-(4-sulfonatophenyl)-1,2,4-triazin-5-yl]benzenesulfonate) was added, and the mixture was incubated for an additional 10 min in the dark. Ferrozine reacts with the remaining Fe²⁺ to form a pink complex, and the absorbance was measured at 562 nm using a Shimadzu UV-1800 spectrophotometer. The control contained ethanol instead of plant extract, with all other components identical. The percentage of Fe chelation was calculated using the following equation:

$$\% \text{ chelating} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

Determination of the Fe-reduction capacity

FRAP (ferric reducing antioxidant power) was performed according to the method developed by Benzie and Strain (1996). FRAP solution (10:1:1) consists of 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM FeCl₃. 1.5 mL of FRAP solution was homogenized with 50 µL of plant extract and 150 µL dH₂O and incubated for 4 minutes at 37°C. The absorbance was measured at 593 nm. The capacity of the samples to reduce Fe³⁺ was expressed in mmol/L Fe²⁺.

Determination of Cu reduction capacity

The capacity of ethanol extracts to reduce Cu^{2+} was determined according to the method proposed by Apak *et al.* (2004) with some modifications. Working solutions were prepared by mixing 0.3 mL of 0.01 M CuCl_2 , 0.3 mL of 10 mM acetate buffer (pH 7), 0.3 mL of a 7.5 mM ethanolic neocuprine solution, and 0.05 mL of ethanolic extract. The mixture was then homogenized with a vortex and incubated for 30 minutes at room temperature. After incubation, the absorbance was measured at 450 nm. A blank sample was prepared in the same way, with 0.05 mL of ethanol added instead of the plant extract. Trolox was used as a standard in the concentration range of 20-300 $\mu\text{g mL}^{-1}$, and the results are expressed in mg of Trolox equivalents per mL of extract.

Determination of the content of essential elements in plants

The quantification of the content of elements (Fe, Cu, Zn, Mn and Ni) in the studied plants was carried out in the extracts obtained after the destruction of the plant tissue with concentrated nitric acid supplemented with 30% H_2O_2 and 70% HClO_4 (Pequerul *et al.*, 1993) by the atomic absorption spectrophotometry method (AAAnalyst 400, Perkin Elmer, USA). The selected wavelengths (nm) of the elements to be determined were as follows: Fe: 248.33, Cu: 324.75, Zn: 213.70, Mn: 279.48, and Ni: 232.00. A stock solution for a single element (1000 mg/L, Perkin Elmer, USA) and an appropriate diluent extractant were used to prepare the standard solutions. All analytical procedures were performed in triplicate using glassware prewashed with 10% HNO_3 . Quality control of acid digestion and element quantification was carried out by analyzing certified reference material (ERM[®]-CD218, rye grass) and recovery values in the acceptable range (85-103%) for each element.

RESULTS AND DISCUSSION

Phenolic compounds and antioxidant properties

Phenolic compounds, especially flavonoids, possess antioxidant activity (Simonetti *et al.*, 2001). Their role in antioxidant processes can be explained in three ways: direct reactions with ROS, being substrates for enzymes (POX), and participating in the chelation of transition metals and the Fenton reaction (Veljović-Jovanović *et al.*, 2018).

Table 1. Content of total phenolic compounds in ethanol extract of *A. ceterach* fronds and its biological activity measured by different methods

TPC	DPPH scavenging	H_2O_2 scavenging	$\cdot\text{OH}$ scavenging	Fe^{3+} reduction	Cu^{2+} reduction	Fe chelating
mgGEA/g DW	IC_{50} (mg/mL)	IC_{50} (mg/mL)	(% of remaining $\cdot\text{OH}$)	(mmol/L Fe^{2+})	(mgTrolox/mL)	(%)
16.08±0.79	29.28±2.25	1420.56±34.72	56.26±1.05	1420.2±53.67	423.14±29.2	36.46±3.95

In the present study, the concentration of phenolic compounds in the ethanol extract of *A. ceterach* was 16.08 ± 0.08 mg GEA/g DW (Table 1). In study Sidhoum *et al.* (2020), the

methanol extract of *A. ceterach* from Algeria showed a total phenolic content (TPC) of 3926.19 $\mu\text{g/g}$ DW, with chlorogenic acid being the most abundant, while spectrophotometric measurement of TPC using the Folin-Ciocalteu assay in the same samples gave 7466.67 ± 148.49 $\mu\text{g/g}$ DW, suggesting that the differences in results could be due to the measurement methods. The TPC content in the methanol extract of *A. ceterach* ranged from 43.10 ± 6.10 mg/mL to 110.62 ± 10.70 mg/mL across 22 samples from Turkey (Pekgöz and Çinbilgel, 2019). The authors concluded that altitude itself is not a decisive factor that determines the chemical composition of plants. However, it may be a factor in the occurrence of other ecological factors, such as soil properties, water, humidity, light, and temperature. In the study by Živković *et al.* (2017), the TPC in the methanol extract of *A. ceterach* sporophyte was 232 mg GAE/g FW, while for the gametophyte it was 51 mg GAE/g FW. In the sporophyte and gametophyte of *A. ceterach*, compounds included phenolic acids, flavonoids, and their derivatives, as well as xanthenes, with chlorogenic acid detected in the highest concentration (Živković *et al.*, 2017).

Evaluation of antioxidant activity requires the application of *in vitro* assays based on different reaction mechanisms. The DPPH assay assesses the ability of antioxidants to scavenge a stable free radical via hydrogen or electron donation. The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+} , reflecting reducing potential (Benzie and Strain, 1996), while the CUPRAC method evaluates the reduction of Cu^{2+} to Cu^+ (Apak *et al.*, 2004). Determination of H_2O_2 -scavenging capacity provides insight into the potential for preventing hydroxyl radical formation via Fenton chemistry. Importantly, EPR spectroscopy enables direct and specific detection of short-lived radical species, offering a reliable approach for assessing $\bullet\text{OH}$ radical scavenging activity in biological samples.

The ethanolic extract of *A. ceterach* fronds showed an ability to inhibit DPPH radicals, with an IC_{50} value of 29.28 ± 2.25 mg/mL (Table 1). In the methanolic extract of *A. ceterach* sampled in Turkey, collected at different altitudes, the DPPH value ranged from 47.91 ± 15.03 $\mu\text{g/mL}$ to 294.66 ± 3.40 $\mu\text{g/mL}$ (Pekgöz and Çinbilgel, 2019), and the sample with the highest TPC did not exhibit the highest antioxidant activity as measured by its ability to remove DPPH radicals. The methanolic extract of *A. ceterach* from Bulgaria demonstrated DPPH scavenging ability with an IC_{50} value of 14 μg (Petkov *et al.*, 2022). In the study by Živković *et al.* (2017), for methanolic extracts of *A. ceterach* sporophytes and gametophytes, the ability to remove DPPH radicals was shown to be 0.46 ± 0.02 mg/mL and 30.47 ± 3.26 mg/mL, respectively. The authors concluded that the higher content of phenolic compounds in the sporophyte extract contributes to stronger antioxidant activity. Hydrogen peroxide with Fe generates hydroxyl radicals as a product through the Fenton reaction. The superoxide anion radical can also react with Fe^{3+} , regenerating Fe^{2+} , which can then participate in the Fenton reaction. The hydroxyl radical is one of the strongest ROS in biological systems. It reacts with the polyunsaturated fatty acids of cell membranes and causes damage and "leakage" in the membranes. The hydroxyl radical is capable of causing oxidative damage to all molecules within biological systems and contributes to carcinogenesis, mutagenesis, and cytotoxicity (Akanni *et al.*, 2014). The mutagenic capacity of hydroxyl radicals is a consequence of their interactions with DNA and therefore plays an important role in the development of cancer (Akanni *et al.*, 2014). It is known that the reducing properties of an extract can serve as a measure of its antioxidant activity by donating hydrogen atoms to break the chain of free radicals. The ethanol extract of

A. ceterach showed the ability to remove H₂O₂ with an IC₅₀ of 1420.56±35.72 mg/mL (Table 1). Additionally, the extract of *A. ceterach* showed the ability to remove ·OH radicals with 56.26±1.05% of ·OH radicals remaining (Table 1), compared to 13.6±0.87% for 2 mM Trolox. From the obtained results, it follows that the extract of *A. ceterach* can remove H₂O₂ and hydroxyl radicals and thus prevent the occurrence of oxidative DNA damage and lipid peroxidation. The determination of the antioxidant potential of the plant extract based on its ability to reduce Fe³⁺ is based on the theoretical assumption that any good reducing agent is also a good antioxidant. Fe³⁺ reduction is used as an indicator of the electron-donating capacity of plant extracts (Petkov *et al.*, 2022). The ability of plants to reduce Fe³⁺ ions varies greatly between species, as highlighted in the review by Nwozo *et al.* (2023). The authors showed that the range of values for the FRAP method is very wide (3.25-1225 µg/mL). At a concentration of 500 µg/mL, the extract of *A. ceterach* showed the ability to reduce Fe³⁺ with a value of 1420.20±53.67 mmol/L Fe²⁺ (Table 1). Aqueous extract of *A. ceterach* from India showed the ability to reduce Fe³⁺ with a value of 29.36±0.32 mM Fe²⁺/100gDW (Fatima *et al.*, 2020). The extract of *A. ceterach* also showed the ability to reduce Cu²⁺ with a value of 423.14±29.20 µg Trolox/mL at a TPC concentration of 300 µg/mL (Table 1). At a concentration of phenolic compounds of 300 µg/mL, the *A. ceterach* extract could chelate Fe with a value of 36.46±3.95 % (Table 1).

The micronutrient levels

The micronutrient levels in the examined fronds were as follows: Fe 164.57 ± 4.08 mg/kg; Zn 15.61 ± 0.43 mg/kg, Mn 12.39 ± 0.19 mg/kg, and Cu 1.62 ± 0.12 mg/kg (Table 2).

Table 2. Content of micronutrients: iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) in fronds of *A. ceterach*

Fe	Cu	Mn	Zn
(mg/kgDW)			
164.57±4.081	1.618±0.124	12.386±0.191	15.605±0.432

The micronutrient content in *A. ceterach* samples from Bulgaria was 151.3 ± 4.5 mg/kg for Fe, 76.0 ± 3.3 mg/kg for Zn, and 6.4 ± 0.3 mg/kg for Cu (Petkov *et al.*, 2022). Compared to our findings, the Fe content was slightly lower, while the Cu and Zn levels were significantly higher in Bulgarian samples. Variability in micronutrient concentrations among fern populations can be linked to plant genotype and environmental conditions. Soil pH, organic matter content, and redox potential influence the availability and uptake of metal ions (Kabata Pendias, 2011). In addition to soil chemistry, abiotic stress such as drought, temperature fluctuations, and light intensity can modulate micronutrient assimilation and partitioning within tissues (Broadley *et al.*, 2007). Moreover, anthropogenic influences, including atmospheric deposition from industrial emissions and agricultural inputs (fertilizers, pesticides), can locally elevate or suppress micronutrient levels in soil and vegetation (Zhou *et al.*, 2025). Ferns, including *A. ceterach*, may employ efficient metal transporters and chelation

mechanisms that influence the internal distribution of trace elements (Clemens, 2006). For example, the synthesis of metal-binding ligands such as organic acids and phytochelatins can affect the sequestration and storage of micronutrients in fronds (Cobbett and Goldsbrough, 2002).

Iron, manganese, zinc, and copper are essential trace metals in the human body. They are considered vital components in metabolic pathways, oxygen transport, immune and antioxidant activity, and DNA synthesis, and serve as enzyme cofactors (Szabo *et al.*, 2021). The recommended daily intake (RDA) for iron, according to the WHO, is 8–27 mg/day for adults, but it also depends on age, health, and gender. The upper limit (UL) is 40–45 mg/day (Institute of Medicine, 2001). Although the human body requires iron for the synthesis of oxygen transport proteins such as hemoglobin and myoglobin, and for the formation of heme enzymes involved in oxidation-reduction reactions, excessive iron uptake can cause several health problems, ranging from diarrhea, vomiting and abdominal pain to serious damage, such as intestinal necrosis, blood loss, organ failure, coma and even death (Hurrell, 1997). On the other hand, iron deficiency can cause anemia, as well as sepsis, mortality, or low birth weight in infants during pregnancy (Abbaspour *et al.*, 2014). The RDA for Cu is 0.9 mg/day for adults, while UL is 10 mg/day (Institute of Medicine, 2001). Cu is a cofactor for enzymes involved in iron metabolism, as well as the regulation of gene expression and the immune system. Furthermore, Cu is vital in oxidative stress, as it is a component of enzymes such as superoxide dismutase (Owen, 1982). Foods rich in copper include fish, nuts, meat, chocolate, and seeds (Ross *et al.*, 2020). Common symptoms of excessive copper intake are gastrointestinal and liver problems, similar to those caused by iron (Institute of Medicine, 2001). The RDA for Zn is 8 to 11 mg/day for adults, while the UL is 40 mg/day. Zinc plays an important role in the catalytic activity of enzymes, cell division, and DNA synthesis. It is also crucial for development and growth. (Institute of Medicine, 2001). The RDA for Mn is 1.8 to 2.3 mg/day for adults, while the UL is 11 mg/day (Institute of Medicine, 2001). Manganese is widely present in metabolic reactions as a cofactor for many enzymes, including manganese superoxide dismutase, arginase, and pyruvate carboxylase. It is important for blood coagulation, bone formation, and liver and kidney function (Ross *et al.*, 2020). Considering all of the above, it can be concluded that the determined concentrations of Fe, Cu, Mn, and Zn in the examined plant extracts in BIH are within permissible values for all four trace metals.

CONCLUSION

The obtained results show that the ethanolic extract of *A. ceterach* fronds can be an important source of phenolic compounds with significant antioxidant potential, as confirmed by DPPH, H₂O₂, [•]OH scavenging, and reducing power assays. The presence of essential micronutrients (Fe, Zn, Mn, and Cu) in the fronds further emphasizes the nutritional importance of *A. ceterach*. Overall, the results suggest that *A. ceterach* may represent a valuable natural source of bioactive compounds of interest for nutritional and antioxidant applications.

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ANTIOKSIDATIVNA AKTIVNOST I SADRŽAJ ODABRANIH MIKRONUTRIJENATA U LISTOVIMA VRSTE *Asplenium ceterach*

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Sažetak

Cilj ovog istraživanja je bio ispitivanje sadržaja fenolnih jedinjenja i antioksidativnog kapaciteta etanolnog ekstrakta listova vrste *Asplenium ceterach*, sakupljene u Republici Srpskoj (BiH). Takođe, cilj rada je bio i određivanje koncentracije odabranih mikronutrijenata (Fe, Cu, Zn, Mn) u listovima ove vrste. Dobijeni rezultati su pokazali da je koncentracija ukupnih fenolnih jedinjenja (TPC) u ekstraktu iznosila $16,08 \pm 0,08$ mg GAE/gDW. Ekstrakt je pokazao i sposobnost uklanjanja DPPH (2,2-diphenyl-1-picrylhydrazyl) radikala sa IC_{50} vrijednošću od $29,28 \pm 2,20$ mg/mL. Kapacitet uklanjanja vodonik peroksida bio je značajno niži ($IC_{50} = 1420,56 \pm 35,72$ µg/mL), što ukazuje na umjerenu efikasnost za uklanjanje ove reaktivne vrste kiseonika. Ekstrakt je takođe pokazao sposobnost uklanjanja hidroksilnih radikala, pri čemu je u ispitivanim uslovima izmjereno $56,26 \pm 1,05\%$ preostalog $\cdot OH$ radikala nakon primjene ekstrakta. Pri koncentraciji od 500 µg/mL TPC, ekstrakt je redukovao Fe^{3+} sa

vrijednošću od $1420,20 \pm 53,67$ mmol/L Fe^{2+} ekvivalenta, dok je njegov kapacitet redukcije Cu^{2+} iznosio $423,14 \pm 29,20$ μg Trolox/mL pri TPC koncentraciji od 300 $\mu\text{g}/\text{mL}$. Koncentracije mikronutrijenata u listovima vrste *A. ceterach* bile su sljedeće: Fe ($164,57 \pm 4,08$ mg/kg) > Zn ($15,61 \pm 0,43$ mg/kg) > Mn ($12,39 \pm 0,19$ mg/kg) > Cu ($1,62 \pm 0,12$ mg/kg). Naši *in vitro* rezultati ukazuju na to da bi etanolni ekstrakt listova vrste *A. ceterach* mogao poslužiti kao potencijalni prirodni izvor antioksidativnih jedinjenja.

Ključne riječi: sposobnost uklanjanja DPPH, redukciona sposobnost, sposobnost uklanjanja hidroksilnog radikala, sposobnost uklanjanja H_2O_2

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