Trace elements and antioxidants in *Astragalus onobrychis* L. subsp. *chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova

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Abstract

Leaves of wild-growing species *Astragalus onobrychis* L. subsp. *chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova were collected during the different stages of growth and analyzed for iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), soluble proteins, reduced glutathione (GSH), total flavonoid and total carotenoid contents. Iron, copper and manganese have shown similar seasonal patterns of increase of metal content in leaves during the vegetative period. The highest content values of the examined elements occured in the seed forming stage, except zinc. The highest value of this element was recorded in the initial stage of vegetation. Antioxidant compounds have the highest values in the blooming stage of vegetation. The results from the present study suggest that researched plant species represent a good source of trace elements and antioxidant compounds.

Keywords: *Astragalus onobrychis* • Trace element • Protein • Total flavonoids • Total carotenoids • Antioxidants • GSH

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It is known that the element concentrations in plant tissue vary widely between species, stage of maturity and differences in soil fertility. Differences in the internal storage of elements contribute to variations within species [1]. Plants synthesize primary and secondary metabolites starting with simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts. The complex organic compounds, produced in plants leaves, include antioxidant compounds as a protection against oxidative damage [2].

Herbs do not only provide us chemicals of medicinal value, but also trace elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health [3]. Molecular evidence suggests that trace elements and antioxidants in plants lower risks of cancer and cardiovascular diseases through mechanisms modulating free radical attack on nucleic acids, proteins and polyunsaturated fatty acids [4].

Astragalus L. (Fabaceae) is generally considered the largest genus of vascular plants with an estimated number of 2500–3000 species [5]. Many species of *Astragalus* are used to restore overgrazed range, control erosion and provide useful sources for producing important drugs [6].

This research was designed to study mineral and antioxidant composition of *A. onobrychis* L. var. *Chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova leaves during the active vegetative period. We determined trace element contents (Fe, Cu, Zn and Mn), quantities of soluble proteins, GSH and total flavonoids and carotenoids contents.

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EXPERIMENTAL

Plant material

The leaves of *A. onobrychis* L. var. *chlorocarpus* were collected in 2008 during the active vegetative period (April to October) from Seličevica mountain, southeastern Serbia. The plant material was collected at three stages of growth (SG) as follows:

1st SG – the initial vegetation stage (April 2008),
2nd SG – the blooming stage (June 2008) and
3rd SG – the seed forming stage (October 2008)

Botanical identification was provided by the Botany Department, Faculty of Science, University of Niš, Serbia, where a voucher specimen is deposited.

METHODS

Trace element analysis

Air-dried samples were digested using concentrated $HNO_3/HClO_4$ acid mixture in a Teflon beaker, with heating to 130–140 °C for 1 h until a clear solution was obtained. After wet digestion, levels of metals in samples were determined by AAS, using Perkin Elmer 1100 atomic absorption spectrophotometer with graphite furnace. The accuracy of the analysis was monitored by inclusion of international reference samples in the analytical program: SO-1, SO-2, SO-3 and CRM 189 [7].

Antioxidants analysis

One gram of plant material was ground with quartz sand in cold mortar. The ground material was suspended in 5 cm^3 0.1 mol/dm^3 phosphate buffer (K_2HPO_4/KH_2PO_4, pH 7) with a plant material to medium ratio of 1:5, centrifuged for 10 min at 15000 rpm. After the centrifugation the supernatant was evaluated for GSH determination according to Ellman [8], and protein by Folin reagents [9]. Total flavonoids were estimated according to Marckam [10]. Carotenoids were extracted with acetone and determined spectrometrically using molar extinction coefficients according to Wettstein [11].

The experimental results were expressed as mean ± standard deviation of three replicates.

RESULTS AND DISCUSSION

Elemental concentrations found in leaves of *A. onobrychis* L. var. *chlorocarpus* are presented in Figure 1.

Stage of growth affected the concentration of the various elements in the leaves. Copper and zinc content did not vary greatly between different stages of growth and the values ranged from 4.20–6.84 mg kg^{-1} (Cu) and 13.84–19.54 mg kg^{-1} (Zn). Concentration of another two examined elements, iron and manganese differed significantly, especially in the seed forming stage. Table 1 shows the antioxidant compounds composition of *A. onobrychis* L. var. *chlorocarpus* leaves.

It can be seen in Table 1 that the highest values of all investigated parameters were found in the blooming vegetation stage, and the lowest values were recorded in the seed forming stage.

The occurrence of oxygen in atmosphere caused the enveloping of live organism protection mechanism, which makes oxygen penetration impossible from outside into a cell. It is well known that oxygen can be toxic in the following forms: reduced, activated, sometimes even in molecular form. Oxygen toxic forms have bad influence on different plant tissue, especially on chloroplasts [12]. For protection, plants produce organic detoxification molecules in which iron, copper, zinc and manganese feature as essential structural components. Plants therefore are primary sources of trace elements and antioxidant compounds, such as GSH, flavonoids and carotenoids. All of these molecules function in enzymatic and/ or non-enzymatic mediated plant as defence against environmental oxidative stress [13].

Table 1. Contents of protein, GSH, total flavonoids and carotenoids in leaves of A. onobrychis L. var. chlorocarpus (Griseb.) S. Kozuharov et D.K. Pavlova

SG	Protein mg/g	GSH μmol/mg prot.	Flavonoids mg/g dray	Carotenoids mg/g
1st	7.96±0.53	0.86±0.07	0.50±0.05	0.39±0.05
2nd	9.98±0.81	0.95±0.10	0.70±0.09	0.43±0.08
3rd	4.92±0.60	0.72±0.04	0.31±0.06	0.29±0.06

The role of iron in a growing plant is associated with enzyme systems, especially in production of chlorophyll. It also serves as an activator for biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation [14]. On the base normal levels of iron in plants (50–300 mg kg^{-1}) [15], it can be said that the examined plant accumulates sufficient content of iron in all stages of growth. The Fe content in examined species increased during vegetation and showed maximum in the seed forming stage of growth, 343.02 mg kg^{-1}. In Texas loco (*Astragalus mollissimus*) content of Fe varies slightly during vegetation and showed maximum in February, 400 mg kg^{-1} [16]. Sheded *et al.* reported that the range of Fe in their study was 261––1239 mg kg^{-1} in selected medicinal plants in Egypt [17].

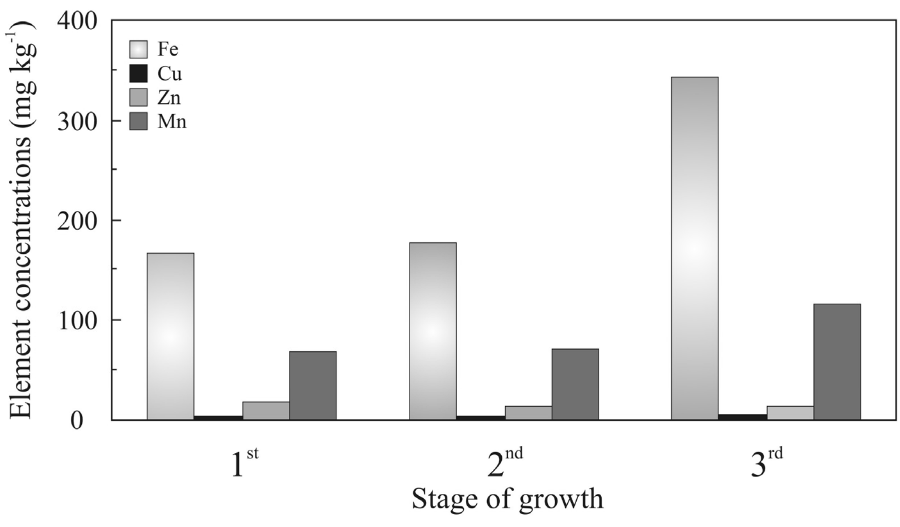

Figure 1. Trace element concentrations in leaves of A. onobrychis L. var. chlorocarpus (Griseb.) S. Kozuharov et D.K. Pavlova during the active vegetative period.

Normal copper contain in plants ranged from 5 to 20 mg kg^{-1} [15]. In our study Cu content is related to increasing plant maturity (4.20–6.84 mg kg^{-1}). The permissible limit set by FAO/WHO in edible plants was 3 mg kg^{-1} [18]. According to Khan *et al.* the range of Cu content in different grasses was 10–21.6 mg kg^{-1} [19].

Zinc is an essential constituent of several enzyme systems in plants, particularly protein synthesis [20]. Content of Zn in our investigation ranged between 13.87 mg kg^{-1} in blooming vegetation stage and 19.54 mg kg^{-1} in initial vegetation stage. Optimal levels of zinc in plants are (15–75 mg kg^{-1}) [15]. According to Bowen, the range of Zn in agricultural products should be between 15 to 200 mg kg^{-1} [21].

Manganese is present in physiologically active plant parts, especially in the stem and shoots. Its functional role is usually linked with chloroplast membrane systems. Also, it serves as an activator for enzymes and growth processes [14]. The range of Mn varied between 69.34 mg kg^{-1} in initial stage of vegetation and 115.91 mg kg^{-1} in seed forming stage. Optimal levels of manganese in plants are 25–250 mg kg^{-1} [15]. The permissible limit set in edible plants was 2 mg kg^{-1} [18]. After comparison, it is found that examined plant in all stage of vegetation accumulate Mn above this limit. Sheded *et al.* reported that the range of Mn in their study was between 44.6 and 339 mg kg^{-1} in selected medicinal plant of Egypt [17].

There are no significant differences between Cu and Zn concentrations in the examined stages of vegetation. Larger differences in Fe and Mn concentrations were found between the first and last stages of vegetation. The element composition of older tissue fluctuates more and has higher concentrations than younger plant material. Accumulation of elements in plants follows the same general trend with season of growth and development. The specific accumulation rates in plants differ because of genetics and hereditary traits among and within species and varieties. Since genetic differences in plants exist, differences can be expected in concentration of elements within them. The results of the present study suggest that the leaves of wild *A. onobrychis* L. var. *chlorocarpus* should be considered as an important source of iron, copper, zinc and manganese.

Soluble proteins vary greatly between different stages of growth and the values ranged from 4.92 mg/g to 9.98 mg/g, respectively (Table 1). The lowest level was recorded in the seed forming stage. Similar results are published for *A. mollissimus*, in the seed maturity stage of vegetation when the protein content in leaves was the lowest [16]. In our study, protein contents in leaves were significantly higher in blooming stage than in other SG. Different protein variation over seasons had been observed by Bogdanović in *Picea omorika* (Panč.) Purkinye [22].

The tripeptide glutathione, is involved in quenching oxygen free radicals through the ascorbate/GSH cycle [23]. GSH quantity in our research was highest in leaves in the blooming stage (0.92 μmol/mg prot., Table 1). The investigated plant leaves contained lower quantity of GSH, in comparison with literature data [24].

Flavonoids are another class of phytochemicals that contribute considerably to the total antioxidant capacity [25]. Flavonoid-rich plants reportedly show protection against atherogenesis by inhibiting oxidation of low density lipoproteins in endothelial cells and macrophages [26]. The highest value of flavonoid content was in leaves in blooming stage (0.70 mg/g dray, Table 1). Leaves of the studied plant contained higher amount of flavonoids than that measured in *Allium n*utans L. [27], but much lower in comparison with plants from South Africa [28].

Carotenoids, pigment molecules responsible for the colour of many fruits and vegetables, have important functions in photosynthesis and are abundant in plant leaves. On the other hand, carotenoids have important biological functions and those consumed through from plant foods can be biologically transformed to provitamin A. Carotenoids also exhibit considerable antioxidant capacity based on their symmetrical linear 40-carbon tetraterpene structure, which features alternating double and single carbon-carbon bonds [29]. The level of carotenoids accumulation in leaves of *A. onobrychis* L. var. *chlorocarpus* during the active vegetative period are shown in Table 1. The highest value of carotenoid content was found in blooming vegetation stage (0.43 mg/g, Table 1). According to De Pee and Bloem the bioavailability of carotenoids in DGLVs is reduced by the leaf matrix [30]. Based on the results of antioxidants analysis reported in the present study and the radical scavenging activities of *A. onobrychis* L. var. *chlorocarpus* it seems likely that the investigated plant species is rich in dietary antioxidants.

CONCLUSION

This study revealed that *Astragalus onobrychis* L. subsp. *chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova from Serbia is a good source of Fe, Cu, Zn, Mn and antioxidant compounds. Iron, copper and manganese have shown similar seasonal pattern, the increase the metal content in leaves during the vegetative period. The investigated antioxidant compounds exhibit the highest contents in blooming stage of vegetation. This vegetation stage is the best period for minerals and antioxidants exploitation.

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IZVOD

ELEMENTI U TRAGOVIMA I ANTIOKSIDANTI U *Astragalus onobrychis* L. SUBSP. *chlorocarpus* (GRISEB.) S. KOZUHAROV *ET* D.K. PAVLOVA

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(Naučni rad)

U radu je proučavan sadržaj elemenata u tragovima, proteina i antioksidanata u listu biljne vrste *Astragalus onobrychis* L. subsp. *chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova (stanište na planini Seličevica), tokom aktivnog vegetacionog perioda 2008. godine. Sadržaj elemenata: Fe, Cu, Zn i Mn je određen tehnikom atomske apsorpcione spektroskopije (AAS). Sadržaj proteina i antioksidantnih jedinjenja: redukovanog glutationa, ukupnih flavonoida i ukupnih karotenoida je određen tehnikom UV–Vis spektroskopije. Elementi Fe, Cu i Mn ispoljavaju sličnu tendenciju akumulacije u listu, povećanje sadržaja tokom vegetacionog perioda, sa maksimumom akumulacije u fazi formiranja semena. Najviši sadržaj cinka u listu zabeležen je u inicijalnoj fazi vegetacije. Sadržaj proteina i antioksidantnih jedinjenja je najviši u fazi cvetanja. Na osnovu rezultata ovog istraživanja može se zaključiti da je list biljne vrste *Astragalus onobrychis* L. subsp. *chlorocarpus* (Griseb.) S. Kozuharov *et* D.K. Pavlova dobar izvor elemenata u tragovima i antioksidantnih jedinjenja.

Ključne reči: *Astragalus onobrychis* • Elementi u tragovima • Proteini • Ukupni flavonoidi • Ukupni karotenoidi • Antioksidanti • GSH