

Total polyphenol content and antioxidant activity of wild and cultivated asparagus in Croatia

Sadržaj ukupnih polifenola i antioksidacijska aktivnost samonikle i kultivirane šparoge u Hrvatskoj

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TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF WILD AND CULTIVATED ASPARAGUS IN CROATIA

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SUMMARY

*Asparagus is an interesting vegetable as a part of a healthy diet and has been widely studied due to the high nutritional value and potential positive effects on human health. Wild asparagus (*Asparagus acutifolius* L.) grows along the Adriatic coast and islands, while the cultivated asparagus (*A. officinalis* L.) is mostly grown in the eastern region of Croatia. The aim of this study was to determine total polyphenol content (measured spectrophotometrically) and antioxidant activity (DPPH) in wild and cultivated asparagus. No significant differences in total polyphenol content were found between wild and cultivated asparagus. The highest content of polyphenols in wild asparagus was 6.20 [mg GAE/g fresh weight] and 49.60 [mg GAE/g dry weight], and in cultivated asparagus it was 4.52 [mg GAE/g fresh weight] and 50.93 [mg GAE/g dry weight].*

Antioxidant activity measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) method showed statistically significant difference. Higher antioxidant activity was measured in cultivated than in wild asparagus. The results also indicate that increased polyphenol content leads to higher antioxidant activity, but more so in the cultivated asparagus. Based on the results, the cultivated asparagus is an excellent alternative to wild asparagus that should be protected from over-harvesting, thus preventing devastation of plant diversity of islands and coast.

Key-words: *Asparagus acutifolius* L., *Asparagus officinalis* L., DPPH, total polyphenol, antioxidant activity.

INTRODUCTION

Diet rich in fruits and vegetables has a protective effect on human health that is primarily attributed to their polyphenolic content (Liu et al., 2014). Phenolic compounds constitute a major part of antioxidants in plants (Duthie and Crozier, 2000) and their contribution to the antioxidant activity is greater than that of vitamin C (Liu et al., 2014). Most of the natural antioxidants proved to be safer for health with better antioxidant activity, unlike the synthetic antioxidants that have been questioned due to their health risks (Krishnaiah et al., 2007).

Asparagus is vegetable that contains flavonoids (mainly rutin) and other phenolic compounds, which possess strong antioxidant properties (Makris and Rossiter, 2001; Guillén et al., 2008; Lee et al., 2014; Solana et al., 2015). The potential health benefits of

antioxidants in asparagus include reducing the risk of cancer, cardiovascular diseases, cerebrovascular diseases, and other diseases (García Giménez et al., 2012; Wang et al., 2013; Palfi et al., 2014) and are therefore subject of a number of studies on their effects on health (Hafizur et al., 2012; Nishimura et al., 2013; Zhong et al., 2015). Due to the rich source of antioxidants, asparagus and asparagus by-products can be used as bioactive components in foods and are potential functional food ingredient in the food industry (Di Maro et al., 2013; Agudelo Cadavid et al., 2015).

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Wild asparagus plays an important role in the culinary tradition of the Adriatic coast and islands' population (Žaper, 2004). Growing cultivated asparagus in Croatia is in its initial stages and is significantly present only in the eastern parts of the country.

The aim of this study was to determine differences in the total phenol content and antioxidant capacity of wild asparagus (*A. acutifolius* L.) of the island of Pag and cultivated asparagus (*A. officinalis* L.) grown in Croatia near Vinkovci.

Table 1. Location of the tested asparagus

Tablica 1. Lokacija ispitivanih šparoga

Plant species <i>Biljna vrsta</i>	Location <i>Lokacija</i>	Location coordinates <i>Koordinate lokacija</i> (Google Earth)
<i>Asparagus acutifolius</i> L.	Ražanac	44°16'56.08"S 15°20'52.71"1
<i>Asparagus acutifolius</i> L.	Šimuni	44°28'04.63"S 14°57'38.94"1
<i>Asparagus acutifolius</i> L.	Dabovi Stani	44°36'06.97"S 14°50'26.45"1
<i>Asparagus acutifolius</i> L.	Lun	44°41'10.87"S 14°45'01.89"1
<i>Asparagus officinalis</i> L.	Rokovci 1	45°14'33.06"S 18°45'35.51"1
<i>Asparagus officinalis</i> L.	Rokovci 2	45°13'50.08"S 18°44'32.77"1
<i>Asparagus officinalis</i> L.	Andrijaševci	45°13'33.99"S 18°44'19.34"1
<i>Asparagus officinalis</i> L.	Ivankovo	45°17'18.64"S 18°44'08.80"1

Each sample of asparagus (wild and cultivated) contained 20 shoots of 17-20 cm length. After harvest, spears were wrapped in plastic and placed immediately on ice for transport to Agrochemical laboratory of the Agricultural Institute Osijek where the analyses were performed.

Reagents and Chemicals

Chemicals including high performance liquid chromatography (HPLC)-grade methanol, were purchased from J.T.Baker Inc. (New Jersey, USA); 2,2-diphenyl-picrylhydrazyl, $\leq 100\%$ and Gallic acid were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA), Folin-Ciocalteu 2M phenol reagent was purchased from Kemika (Zagreb, Croatia). All chemicals used in this study were of analytical grade (Gallic acid, Folin-Ciocalteu 2 M phenol reagent, sodium carbonate).

Extraction procedure

24 h after harvesting, spears of fresh green asparagus were flash-frozen in liquid nitrogen and stored at -20°C . Frozen samples were homogenized in blender, weighed (~ 2 g), and total polyphenols were extracted with 5 mL of acidified methanol (1% hydrochloric acid) for 60 min in an ultrasonic bath (Sonorex RH 510 H, Bandelin electronics, Berlin, Germany) and reextracted with 5 mL of acidified methanol (1% hydrochloric acid) for 30 min. After centrifugation (LC-320, Tehnica, Slovenija) at 4°C and 9000 rpm for 10 min, supernatant was used for spectrophotometrical determination of total polyphenols (Escarpa and Gonzales, 2000).

Total polyphenol content

Total polyphenols were determined by Folin-Ciocalteu method (Slinkard and Singleton, 1977) modi-

MATERIAL AND METHOD

Plant material

Samples of wild asparagus (*A. acutifolius* L.) were collected in early April 2014 at four locations on the island of Pag and the surrounding area, while samples of cultivated green asparagus (*A. officinalis* L.) were collected at the same time at four locations around Vinkovci, Croatia (Table 1). Identification of plant material was made at the Faculty of Agriculture in Osijek.

fied in micro method as follows. An aliquot (100 μL) was mixed with 2000 μL of sodium carbonate solution (2% w/v) and 100 μL of Folin-Ciocalteu reagent. After incubation at room temperature for 30 min in dark, the absorbance was read against the blank at 765 nm (Specord 200, Analytic Jena, Germany). Total polyphenols were expressed as mg of gallic acid equivalents (GAE) g^{-1} for fresh weight (FW), and dry weight (DW) of the plant respectively (Waterhouse, 2002). The measurement was carried out in three replicates and data are showed as a mean value.

The DPPH radical scavenging activity - The percentage of antioxidant activity (aa%)

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time.

The percentage of antioxidant activity (aa%) was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. (1995). The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 0.2 mL of sample, 2 mL of methanol and 1 mL of DPPH radical solution 0.5 mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes

in colour were read [absorbance (abs)] at 517 nm after 100 min of reaction using a UV-Vis spectrophotometer (Specord 200, Analytic Jena, Germany). The mixture of methanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing methanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (aa%) was determined according to Mensor et al. (2001). The measurement of antioxidant activity was carried out in three replicates and data were presented in two ways. The first one is aa%, which is calculated according to free radical inhibition, the concentration of extract needed for inhibition of 50% DPPH radicals. The second one is calculated according to standard curve with gallic acid as standard. Antioxidant activity is given expressed as the equivalent of gallic acid needed for reducing initial concentration of free radicals.

Statistical analysis

Comparison of total polyphenols, as well as the comparison of antioxidant activity between wild and cultivated samples was carried out by using a multivariate permutation test, a modern version of the Hotelling t-test. The correlation between the content of total poly-

phenols and antioxidant effect was carried out by using a non-parametric correlation (Pearson's linear regression (r) and p value for correlation) (Bonnini et al., 2014). Statistical analyses were carried out in the programs PAST (v. 2.17) (Hammer et al., 2001) and GraphPad Prism for Windows (v. 5) (GraphPad Software, 2013). Results were considered statistically significant at $p < 0.05$. All applied tests were two-tailed.

RESULTS AND DISCUSSION

Asparagus is recognized as a good source of polyphenolic compounds with significant antioxidant capacity. According to recent findings, the wild asparagus from the island of Pag, Croatia, and the surrounding area, haven't been analysed and compared yet with the cultivated asparagus. The analysis of total polyphenol content and antioxidant capacity was done and their mutual correlation was examined.

Total polyphenol content

The highest content of polyphenols in wild asparagus was 6.20 [mg GAE/g FW] and 49.60 [mg GAE/g DW], and in cultivated asparagus, it was 4.52 [mg GAE/g FW] and 50.93 [mg GAE/g DW] (Fig. 1).

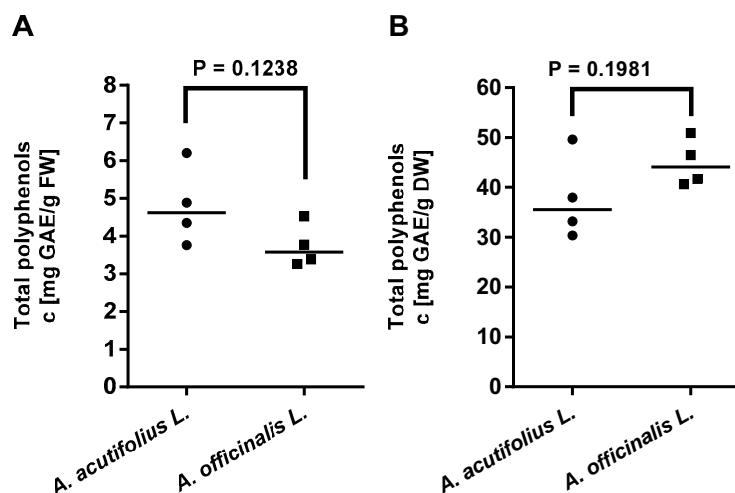


Figure 1. Total polyphenol content in wild (*A. acutifolius* L.) and cultivated (*A. officinalis* L.) asparagus: A-fresh weight, B-dry weight

Grafikon 1. Sadržaj ukupnih polifenola u samonikloj (*A. acutifolius* L.) i kultiviranoj (*A. officinalis* L.) šparogi: A-sveža tvar, B-suha tvar

The difference in total phenol content between wild and cultivated asparagus was not statistically significant. Sun et al. (2007) found that the total phenol content of asparagus (*A. officinalis*) in acetone, methanol and aqueous extract, was 5.0 ± 0.5 , 4.9 ± 0.7 , and 4.9 ± 0.9 [mg catechin equivalent/g dry weight], respectively. The investigation of Istrian wild asparagus showed that the results for the 40% EtOH and 96% EtOH extract of wild asparagus were 793 ± 33 mg GAE/100g DW and 181 ± 26 mg GAE/100 g DW, respectively (Poljuha et al., 2015). Very high content of phenolic compounds was found in the Portuguese wild asparagus:

624 ± 28 mg GAE/g of extract (Martins et al., 2011), but in the Spanish one, it was only 17.60 ± 0.29 mg GAE/g of extract (Morales et al., 2012).

The results of our analysis are not in accordance with the Ferrara et al. (2011), the study which found that the phenolic content of *A. acutifolius* (41.97 ± 1.95 mg/100 g) was higher compared to *A. officinalis* (27.62 ± 1.11 mg/100 g). From the foregoing, it can be concluded that the phenolic profile of populations from different geographical areas varies considerably (Poljuha et al., 2015). However, large differences can

be partly explained by various analysis modifications both in the extraction of polyphenols and in the results. Phenol biosynthesis in plants is dependent on a number of environmental factors, such as UV radiation, soil, biotic or abiotic stress (Cheynier et al., 2013); it is also dependent on the timing of the harvest of asparagus (Lee et al., 2014). Cultivation conditions, especially light conditions, are of great importance for the rutin and polyphenol content (Maeda et al., 2010).

Antioxidant activity

The highest antioxidant activity for cultivated asparagus were 0.26 [mg GAE/g FW] and 2.83 [mg GAE/g DW], and the lowest 0.12 [mg GAE/g FW] and 1.61 [mg GAE/g DW]. The highest antioxidant activity for wild asparagus were 0.09 [mg GAE/g FW] and 0.7 [mg GAE/g DW], and lowest 0.01 [mg GAE/g FW] and 0.1 [mg GAE/g DW]. In contrast to the content of total polyphenols, antioxidant activity showed statistically significant difference when comparing wild to cultivated asparagus (Fig. 2).

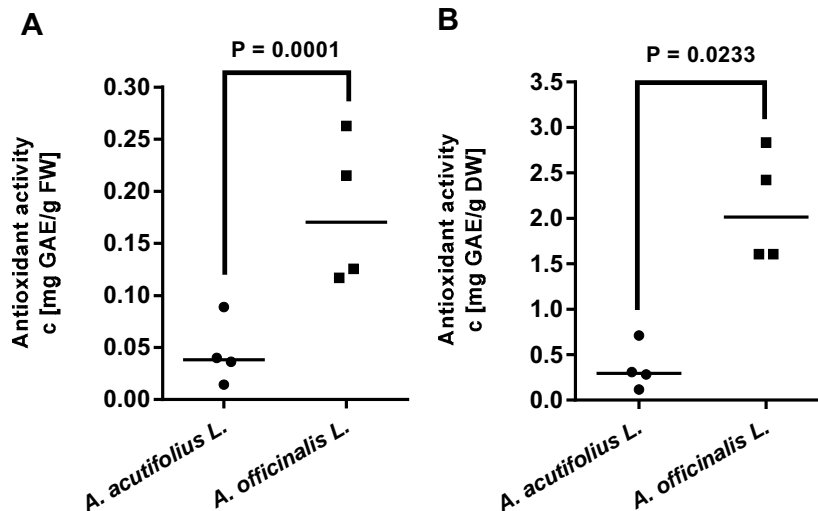


Figure 2. Antioxidant activity in wild (*A. acutifolius L.*) and cultivated (*A. officinalis L.*) asparagus: **A**-fresh weight, **B**-dry weight

Grafikon 2. Antioksidativna aktivnost samonikle (*A. acutifolius L.*) i kultivirane (*A. officinalis L.*) šparoge: **A**-svježa tvar, **B**-suha tvar

Antioxidant capacity of Istrian wild asparagus measured by DPPH method (mmol TE/100 g DW) was 1.1 ± 0.3 in aqueous solution, 0.9 ± 0.3 in 40% EtOH and 0.3 ± 0.1 in 96% EtOH (Poljuha et al., 2015). Using the same method Tiveron et al. (2012) found the value of $15.8 \pm 0.38 \mu\text{mol Trolox/g DW}$ for Brazilian asparagus

(*A. officinalis*), and the results of Solana et al. (2015) were in the range of 1.18 to 2.49 (rutin equivalent mg/g).

The statistics have showed the significant difference between wild and cultivated asparagus, calculating the 50% DPPH (Fig. 3).

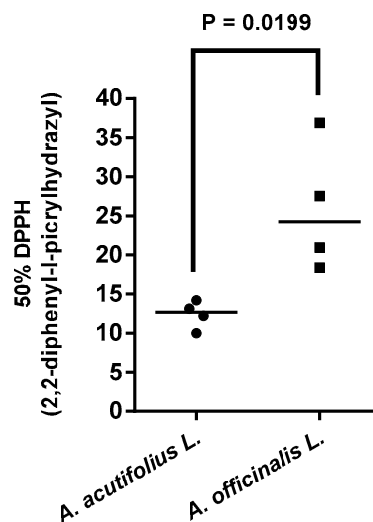


Figure 3. Inhibition 50% DPPH in wild (*A. acutifolius L.*) and cultivated (*A. officinalis L.*) asparagus

Grafikon 3. Inhibicija 50% DPPH samonikle (*A. acutifolius L.*) i kultivirane (*A. officinalis L.*) šparoge

The lowest measured value of 50% DPPH of wild asparagus was 9.99, and the highest 14.20, while for the cultivated one, the lowest amount was 18.38, and the highest 36.91. Ferrara et al. (2011) compared *A. acutifolius* and *A. officinalis* for IC₅₀ DPPH and measured 72.4 and 89.0 µg/mL, respectively. Measuring antioxidant activity by DPPH of Spanish wild asparagus, EC₅₀ mg/mL was 4.87 ± 0.38 (Morales et al., 2012), and for

the Portuguese wild asparagus, the value EC₅₀ µg/mL was 423 ± 24 (Martins et al., 2011).

Non-parametric correlation analysis of our results showed the relations between the total polyphenols and antioxidant activity; it has been proven that the increase of the content of polyphenol increases the antioxidant activity, in both fresh and dry matter, but more in cultivated asparagus (Fig. 4).

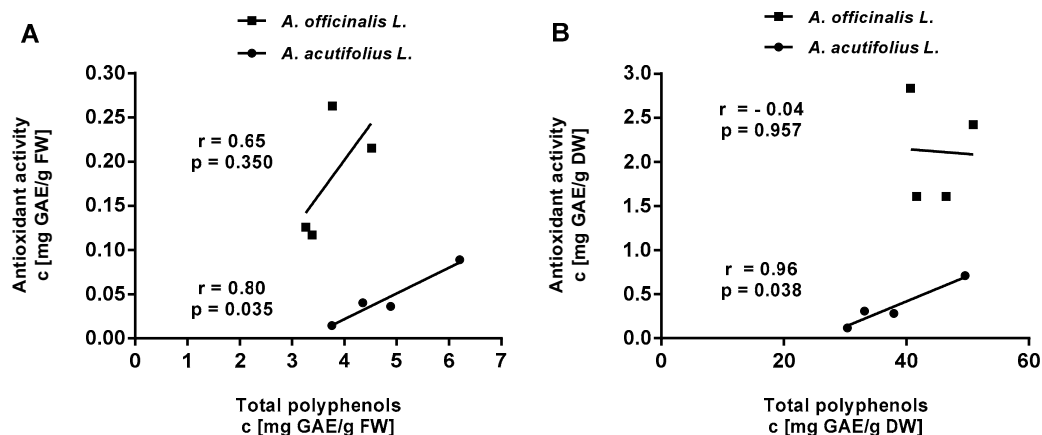


Figure 4. Correlation of antioxidant activity and total polyphenols in wild (*A. acutifolius* L.) and cultivated (*A. officinalis* L.) asparagus: A-fresh weight, B-dry weight

Grafikon 4. Korelacija antioksidativne aktivnosti i ukupnih polifenola u samonikloj (*A. acutifolius* L.) i kultiviranoj (*A. officinalis* L.) šparogji: A-svježa tvar, B-suha tvar

Our results are consistent with the results of studies which have found a positive correlation between antioxidant activity and polyphenol content (Sun et al., 2007; Conforti et al., 2011; Cheng et al., 2013; Poljuha et al., 2015), while Tiveron et al. (2012) found the highest correlations for DPPH and FRAP methods, but did not find correlation for Rancimat method. Different values for antioxidant activity between wild and cultivated asparagus can be attributed, not only to polyphenol content, but other factors such as content of flavonoids, vitamin C and sulphate, production year, and especially the temperature range (Takács-Hájos and Zsombik, 2015) and the time of harvest (Lee et al., 2014).

CONCLUSION

The results of this study showed no statistically significant differences in the content of total polyphenols between wild and cultivated asparagus; however, antioxidant activity of cultivated asparagus is higher than the one of the wild species. Favourable agro-climatic growing conditions in Eastern Slavonia, adequate nutrition and good care of the crops possibly contribute to higher antioxidant activity of cultivated asparagus. Based on the results, we can conclude that the cultivated asparagus is an excellent alternative to wild asparagus that should be protected from over-harvesting, thus preventing the devastation of plant diversity of Croatian islands and coast.

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SADRŽAJ UKUPNIH POLIFENOLA I ANTIOKSIDACIJSKA AKTIVNOST SAMONIKLE I KULTIVIRANE ŠPAROGE U HRVATSKOJ

SAŽETAK

Zbog brojnih nutritivnih vrijednosti i potencijalnoga pozitivnoga učinka na ljudsko zdravlje, šparoga se pokazala kao interesantna kultura za konzumaciju te predmet brojnih znanstvenih ispitivanja. Samonikla šparoga (*Asparagus acutifolius* L.) raste duž Jadranske obale i otoka, dok se kultivirana šparoga (*Asparagus officinalis* L.) uglavnom uzgaja u istočnome dijelu Hrvatske. Ovim istraživanjem određivan je ukupni sadržaj polifenola (spektrofotometrijski) i antioksidativna aktivnost (DPPH) kod samoniklih i kultiviranih šparoga. Rezultati su pokazali da nema statistički značajne razlike u sadržaju ukupnih polifenola između samoniklih i kultiviranih šparoga. Najviša vrijednost ukupnih polifenola samoniklih šparoga iznosila je 6,20 [mg GAE/g svježe tvari], odnosno 49,60 [mg GAE/g suhe tvari], dok je kod kultiviranih šparoga bila 4,52 [mg GAE/g svježe tvari], odnosno 50,93 [mg GAE/g suhe tvari]. Antioksidativna aktivnost mjerena DPPH (2,2-difenil-1-pikrilhidrazil) metodom, pokazala je statistički značajne razlike. Veća je antioksidativna aktivnost izmjerena kod kultiviranih, nego kod samoniklih šparoga. Također je utvrđeno da porastom polifenola raste antioksidativna aktivnost, ali više kod kultiviranih šparoga. Temeljem rezultata, zaključujemo da je kultivirana šparoga odlična alternativa samonikloj, koja bi trebala biti zaštićena od pretjeranog ubiranja, kako bi se zaštitila prirodna raznolikost obale i otoka.

Ključne riječi: *Asparagus acutifolius* L, *Asparagus officinalis* L, DPPH, ukupni polifenoli, antioksidacijska aktivnost

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