JOURNAL OF MEDICINAL FOOD
***J Med Food* 12 (3) 2009, 1–7**

DOI: 10.1089/jmf.2008.0081

JMF-2008-0081-Piljac-Zegarac_1P
Type: full-communication

Antioxidant Capacity and Polyphenolic Content of Blueberry (*Vaccinium corymbosum* L.) Leaf Infusions

J. Piljac-Žegarac,[1] A. Belščak,[1] and A. Piljac[2]

[1]Department of Molecular Biology, Institute Ruder Bošković; and [2]University Clinic for Diabetes, Endocrinology and Metabolic Disorders, Vuk Vrhovac, Zagreb, Croatia

ABSTRACT Antioxidant capacity and polyphenolic content of leaf infusions prepared from six highbush blueberry cultivars (*Vaccinium corymbosum* L.), one wild lowbush blueberry cultivar (*Vaccinium myrtillus* L.), and one commercially available mix of genotypes were determined. In order to simulate household tea preparation conditions, infusions were prepared in water heated to 95°C. The dynamics of extraction of polyphenolic antioxidants were monitored over the course of 30 minutes. Extraction efficiency, quantified in terms of the total phenol (TP) content, and antioxidant capacity of infusions, evaluated by the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, were compared with cultivar type and extraction time. The 30-minute infusions exhibited the highest TP content and antioxidant capacity according to all three assays. Wild blueberry infusion had the highest TP content (1,879 mg/L gallic acid equivalents [GAE]) and FRAP values (20,050 μM). The range of TP values for 30-minute infusions was 394–1,879 mg/L GAE with a mean of 986 mg/L GAE across cultivars; FRAP values fell between 3,015 and 20,050 μM with a mean of 11,234 μM across cultivars. All 30-minute infusions exhibited significant scavenging capacity for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, comparable to different concentrations of catechin, gallic acid, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid. Overall, tested infusions showed significant reducing capacity as well as radical scavenging potential, which places blueberry leaf tea high on the list of dietary sources of antioxidants.

KEY WORDS: • ***antioxidant capacity*** • ***2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)*** • ***blueberry*** • ***2,2-diphenyl-1-picrylhydrazyl*** • ***ferric reducing antioxidant power*** • ***infusions*** • ***total phenol content***

INTRODUCTION

A WIDE ARRAY OF POSITIVE health effects has been ascribed to phytochemicals present in blueberry fruit, such as their ability to protect against cancer,[1,2] stroke,[3] and urinary tract disease.[4] Results of a study conducted by scientists from Tufts University have shown that a diet rich in blueberry extract improves short-term memory loss and reverses some loss of balance and coordination in aging rats.[5] The substances considered to be directly responsible for antioxidant capacity of blueberries and their positive health effects are polyphenolic compounds capable of neutralizing free radicals generated by the body. More specifically, the following phenolics are considered to be the major contributors to antioxidant capacity of blueberry fruits: anthocyanins, quercetin, kaempferol, myricetin, and chlorogenic acid.[6,7] Although significant attention has been focused on the antioxidant capacity of polyphenols present in blueberry fruit,[8–11] limited information is available on the phenolic antioxidant content in leaf tissues of only a few highbush blueberry cultivars.[12] Therefore, the aim of our study was to determine the total phenol (TP) content and antioxidant properties of blueberry leaf infusions and monitor the influence of extraction duration on these parameters.

For this purpose, leaves were collected from six different highbush blueberry cultivars (*Vaccinium corymbosum* L.) and one wild lowbush blueberry cultivar (*Vaccinium myrtillus* L.). In addition, a commercially available mixture of leaves from several blueberry genotypes (mix) was purchased. Leaf infusions were prepared in water. We wanted to monitor the extraction dynamics of polyphenolic antioxidants in water, over the course of 30 minutes, and compare the extraction efficiency and antioxidant capacity of infusions evaluated by ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays, with cultivar type and extraction duration. Considering the fact that the antioxidant capacity and phenolic content of blueberries can be influenced by factors other

Manuscript received 21 March 2008. Revision accepted 20 September 2008.

Address reprint requests to: Dr. J. Piljac-Žegarac, Department of Molecular Biology, Institute Ruder Bošković, Bijenička c. 54, P.O. Box 180,10000 Zagreb, Croatia, E-mail: jpiljac@irb.hr

than the genotype, such as growing season and location,[9,13] maturity,[9,14] and storage conditions,[15,16] care was taken to eliminate most of these parameters by sampling leaves from 3-year-old plants maintained in the same field-grown collection and storing them immediately after sampling at −80°C. The commonly used Folin-Ciocalteau test was employed for the detection of total phenolics, FRAP was used to determine the reducing potential of leaf infusions, and DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging assays were chosen for determination of antioxidant capacity. The choice of methods was based on their accuracy, reproducibility, and efficiency and the fact that a lot of literature data is available for easy comparison with the results of the screening of medicinal plant extracts previously performed by other authors.[17,18]

MATERIALS AND METHODS

Chemicals and instruments

Except for the Folin-Ciocalteu reagent (Fluka, Buchs, Switzerland) and $FeSO_4 \cdot 7H_2O$ (Kemika, Zagreb, Croatia), all the chemicals and reagents used in this study were of analytical grade and supplied by Sigma Chemical Co. (St. Louis, MO). Spectrophotometric measurements were performed on a double-beam ultraviolet-visible spectrophotometer (Bio-Spec-1601, (Shimadzu Corp., Kyoto, Japan).

Plant material and sampling

Young leaves from six different highbush blueberry cultivars of the Pacific Northwest (Jersey, Bluetta, Bluecrop, Berkeley, Burlington, and Coville) and from one wild lowbush blueberry cultivar were hand-picked from a field-grown collection maintained by the Center for Agriculture of Mountainous Regions located in Gorski Kotar (Croatia) in late summer. All plants were 3 years old and were grown under the same climate and soil conditions. After collection, the leaves were placed on ice and frozen at −80°C within a couple of hours. Leaf lyophilisates were prepared the next day by 24-hour lyophilization run on a Lyovac GT 2 (STERIS GmbH, Hürth, Germany). Leaf infusions were prepared on the day the measurements were performed by adding 500 mg of lyophilized leaf powder to 30 mL of deionized water heated to 95°C. The extraction proceeded in a closed plastic vial, shaken at 55 rpm on a Cole-Parmer (Vernon Hills, IL) rocking platform. Leaf powder was packaged in a fine cloth of 12–25 μm pore size (Miracloth, Merck GmbH, Darmstadt, Germany) during extraction; therefore, it was not necessary to filter the infusions. The initial temperature of added water was 95°C because Perva-Uzunalić *et al.*[19] determined that maximum achieved extraction efficiency of catechins with water is obtained at 95°C after 10–20 minutes of extraction. Extraction duration was prolonged to 30 minutes, without additional heating, and 500-μL portions of infusions were collected each 5 minutes for testing of TP content and antioxidant capacity. Sampling of infusions was performed in 5-minute intervals in order to monitor the interdependence between extraction duration and polyphenolic composition/reducing potential/radical scavenging capacity.

TP content

The TP content of blueberry leaf infusions was determined using the Folin-Ciocalteu colorimetric method,[20] with gallic acid as the standard. The TP content was expressed as mg/L gallic acid equivalents (GAE). The 1:2 dilution of infusions of wild blueberry, Bluetta, Burlington, Berkeley, and the commercial mix was taken into account in calculation of GAE.

Antioxidant capacity

FRAP assay. The FRAP assay was used to estimate the antioxidant potential of tested infusions, according to the original method of Benzie and Strain.[21] Absorbance readings of the reagent/sample mixture were taken after 4 minutes, at 593 nm, along with the absorbance reading of the reagent blank of distilled water. The results, obtained from triplicate analyses, were expressed as μM $FeSO_4 \cdot 7H_2O$ and derived from a calibration curve determined for this standard (100–1,000 μM).

DPPH$^{\bullet}$ radical scavenging assay. Antioxidant capacity of infusions was determined using the DPPH$^{\bullet}$ radical scavenging assay described by Brand-Williams *et al.*[22] In brief, 5 μL (for dark-colored extracts of wild blueberry, Bluetta, Burlington, Berkeley, and mix) or 20 μL (for light-colored extracts of Jersey, Bluecrop, and Coville) of infusion was added to a volume of 0.094 mM DPPH$^{\bullet}$ radical solution in methanol made up to 1 mL. The reaction was carried out in closed Eppendorf tubes shaken at 20°C. The scavenging capacity was evaluated by measuring the absorbance at 515 nm after 60 minutes of reaction at 20°C in a spectrophotometer. The results were expressed as mM 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC).

For comparison, the DPPH$^{\bullet}$ antiradical capacity was determined for 30-minute infusions and the common polyphenolic standards, catechin, gallic acid, and Trolox. A 0.1-mL aliquot of infusion (diluted 1:10) or methanol solution containing different standard concentrations (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM) was added to 3.9 mL of 0.025 g/L DPPH$^{\bullet}$ in methanol. The reaction mixtures were shaken for 1 hour in the dark, and absorbance values were recorded at 515 nm.

The results were presented as percentage quenching (Q), defined by the formula of Yen and Duh[23]:

$$Q = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \quad (1)$$

where A_0 is the initial absorbance of the control at $t = 0$ minutes and $A_{A(t)}$ is the absorbance of the antioxidant at $t = 60$ minutes. The DPPH$^{\bullet}$ radical stock solution was prepared daily, and special care was taken to minimize the loss of free radical capacity of the stock solution during the course of sample preparation.[24]

$ABTS^{\cdot+}$ radical scavenging assay. The TEAC of infusions was also estimated by the $ABTS^{\bullet+}$ radical cation decolorization assay.[25] On the day of analysis, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. A 20-μL aliquot of the tested blueberry leaf infusion (Jersey, Bluecrop, and Coville undiluted; wild, Bluetta, Burlington, Berkeley, and mix diluted 1:5) was added to 2.0 mL of the diluted $ABTS^{\bullet+}$ solution, and the absorbance readings were taken after exactly 6 minutes. The reagent blank was prepared by adding 20 mL of ethanol instead of the sample. All measurements were performed in triplicate. Trolox, a water-soluble vitamin E analogue, was used as a standard. Different solutions (0–2 m*M*) of Trolox were prepared in 96% ethanol and assayed under the same procedure as the samples. The TEAC and the *Q* value for 30-minute infusions were calculated based on the decrease of absorbance exerted by standard solutions and appropriately diluted tested infusions after 6 minutes.

Statistical analysis

All presented numeric values are averages of three measurements ± standard deviation (SD). The SD calculated for triplicate measurements amounted to less than 15%.

RESULTS AND DISCUSSION

TP content and reducing capacity of blueberry leaf infusions

T1 ▶ Table 1 presents the TP content in mg/L GAE and FRAP values (in μ*M*) determined for eight tested blueberry leaf infusions and different times of extraction. There is a general increasing trend in both TP and FRAP values with prolonged extraction time. The highest TP and FRAP values for all infusions were observed for a 30-minute extraction time. The observed range of TP values for 30-minute infusions is 394–1,879 mg/L GAE with a mean of 986 mg/L GAE across cultivars; that of FRAP values falls between 3,015 and 20,050 μ*M* with a mean of 11,234 μ*M* across cultivars. This represents a 4.8-fold difference in TP between wild blueberry with the highest TP (1,879 mg/L GAE) and Bluecrop with the smallest TP (394 mg/L GAE). In a study of blueberry fruit extracts obtained from different cultivars, Howard *et al.*[26] observed 2.9-fold and 2.4-fold differences in the TP content from 2000 and 2001 harvest years, respectively.

The FRAP results for 30-minute infusions were again the highest for wild blueberry (20,050 μ*M*) and the lowest for Bluecrop (3,015 μ*M*), yielding a 6.7-fold difference between the two. The sequence of 30-minute infusions based on decreasing order of TP is wild > Berkeley > Burlington > mix > Coville > Bluetta > Jersey > Bluecrop. The sequence of 30-minute infusions based on decreasing order of FRAP values is slightly different, as follows: wild > Burlington > Bluetta > mix > Berkeley > Coville > Jersey > Bluecrop. The results of both assays indicate that wild blueberry infusions possess the highest reducing capacity. Interestingly, Moyer *et al.*[14] also observed the highest antioxidant capacity (oxygen radical absorbance capacity and FRAP assays) in wild plants

Table 1. TP Content and FRAP Values for Blueberry Leaf Infusions at Different Extraction Times

Cultivar, extraction time (minutes)	*TP (mg/L GAE)*	*FRAP (μmol/L)*
Wild		
5	1,040 ± 104	13,718 ± 256
10	1,252 ± 102	15,795 ± 359
15	1,539 ± 29	16,381 ± 154
20	1,672 ± 134	18,670 ± 272
25	1,752 ± 133	19,535 ± 278
30	1,879 ± 187	20,050 ± 854
Burlington		
5	635 ± 58	13,645 ± 498
10	958 ± 77	15,598 ± 100
15	1,188 ± 124	15,443 ± 298
20	1,321 ± 78	17,462 ± 173
25	1,628 ± 114	19,339 ± 314
30	1,738 ± 150	19,793 ± 754
Berkeley		
5	852 ± 98	3,706 ± 395
10	1,260 ± 116	5,531 ± 223
15	1,382 ± 104	8,607 ± 91
20	1,401 ± 57	9,241 ± 104
25	1,656 ± 153	11,231 ± 186
30	1,785 ± 29	11,642 ± 709
Mix		
5	222 ± 26	5,162 ± 204
10	355 ± 28	6,053 ± 269
15	466 ± 56	8,242 ± 698
20	497 ± 23	8,532 ± 224
25	536 ± 37	11,125 ± 814
30	561 ± 19	13,206 ± 924
Coville		
5	200 ± 11	2,296 ± 153
10	278 ± 25	2,788 ± 59
15	381 ± 21	3,662 ± 282
20	439 ± 37	3,644 ± 156
25	477 ± 50	3,299 ± 117
30	537 ± 24	4,051 ± 238
Bluetta		
5	221 ± 10	5,347 ± 428
10	365 ± 33	5,921 ± 178
15	487 ± 9	6,433 ± 228
20	459 ± 40	10,465 ± 837
25	522 ± 30	13,202 ± 660
30	522 ± 11	14,326 ± 573
Jersey		
5	232 ± 13	1,840 ± 68
10	281 ± 26	2,395 ± 145
15	370 ± 29	2,632 ± 240
20	401 ± 28	3,187 ± 85
25	430 ± 44	3,618 ± 191
30	475 ± 7	3,791 ± 272
Bluecrop		
5	198 ± 17	1,975 ± 66
10	287 ± 26	2,372 ± 210
15	328 ± 79	2,463 ± 147
20	367 ± 28	2,750 ± 99
25	379 ± 10	2,832 ± 203
30	394 ± 19	3,015 ± 195

and seedlings (rabbiteye blueberry) from the *Vaccinium* population and not the cultivars.

Radical scavenging capacity of blueberry leaf infusions

One of the prerequisites of an antioxidant is its ability to neutralize the radical-induced oxidative stress. Thus, the antioxidant substance should be reactive towards the radical and the resultant antioxidant radicals unreactive towards biomolecules. In order to test the radical scavenging of blueberry leaf infusions, reactions of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals with the extracts were performed. In the presence of blueberry leaf infusions, the absorption of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ decreased, indicating their radical neutralizing nature.

F1 ▶ Figure 1 shows $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging capacities of blueberry leaf infusions, expressed as m*M* TEAC, as a function of extraction time. The 30-minute wild blueberry leaf infusion is the most efficient $DPPH^{\bullet}$ radical scavenger (7.67 m*M* TEAC), followed by Burlington (7.08 m*M* TEAC). Bluecrop and Coville infusions exhibited the poorest $DPPH^{\bullet}$ radical scavenging capacity: 1.72 and 1.61 m*M* TEAC, respectively. It is evident that the infusions are divided into two groups, with wild, Burlington, and Berkeley having high TEAC values and mix, Bluetta, Jersey, Bluecrop, and Coville having moderate to low TEAC values. There is a 4.8-fold difference between infusions with the highest (wild) and lowest (Coville) $DPPH^{\bullet}$ antiradical capacity. The same difference was previously noted in TP values between these two infusions.

In the case of $ABTS^{\bullet+}$ radical scavenging properties, the grouping of tested infusions is again evident, with wild, Burlington, and Berkeley exhibiting high TEAC values and mix, Bluetta, Jersey, Bluecrop, and Coville exhibiting low TEAC values. In this assay, the 30-minute Burlington infusion showed the best $ABTS^{\bullet+}$ radical scavenging properties (9.29 m*M* TEAC), slightly better than wild (9.00 m*M* TEAC), although for all other extraction times wild had higher values than Burlington. Just as in the case of $DPPH^{\bullet}$ radical scavenging, Bluecrop showed the poorest $ABTS^{\bullet+}$ scavenging efficiency (1.10 m*M* TEAC). The observed difference between the highest and lowest TEAC values for the ABTS assay is 8.1-fold. The TEAC values obtained in the ABTS assay for wild, Burlington, and Berkeley infusions are greater than those obtained in the DPPH assay, which may be explained by the fact that $DPPH^{\bullet}$ reacts only with lipophilic antioxidants, while $ABTS^{\bullet+}$ reacts with both hydrophilic and lipophilic antioxidants.[27]

Influence of extraction time on TP content and antioxidant capacity of blueberry leaf infusions

All blueberry leaf infusions exhibited increasing phenolic content, reducing power (according to FRAP), and radical scavenging capacity (according to DPPH and ABTS assays) as a function of extraction time. In the case of ABTS assay results, the relationship was almost linear for certain cultivars (Fig. 1b). These findings are logical because prolonged extraction results in more concentrated phenolics in the infusions. Consequently, a linear or nearly linear relationship between the TP content and antioxidant potential may be expected. According to the findings of Perva-Uzunalić *et al.*,[19] extraction at 95°C for 10–20 minutes will ensure optimal extraction efficiency of catechins, which are highly concentrated in most plant leaves. In this study, the prolonged extraction time of 120 minutes for green tea caused a

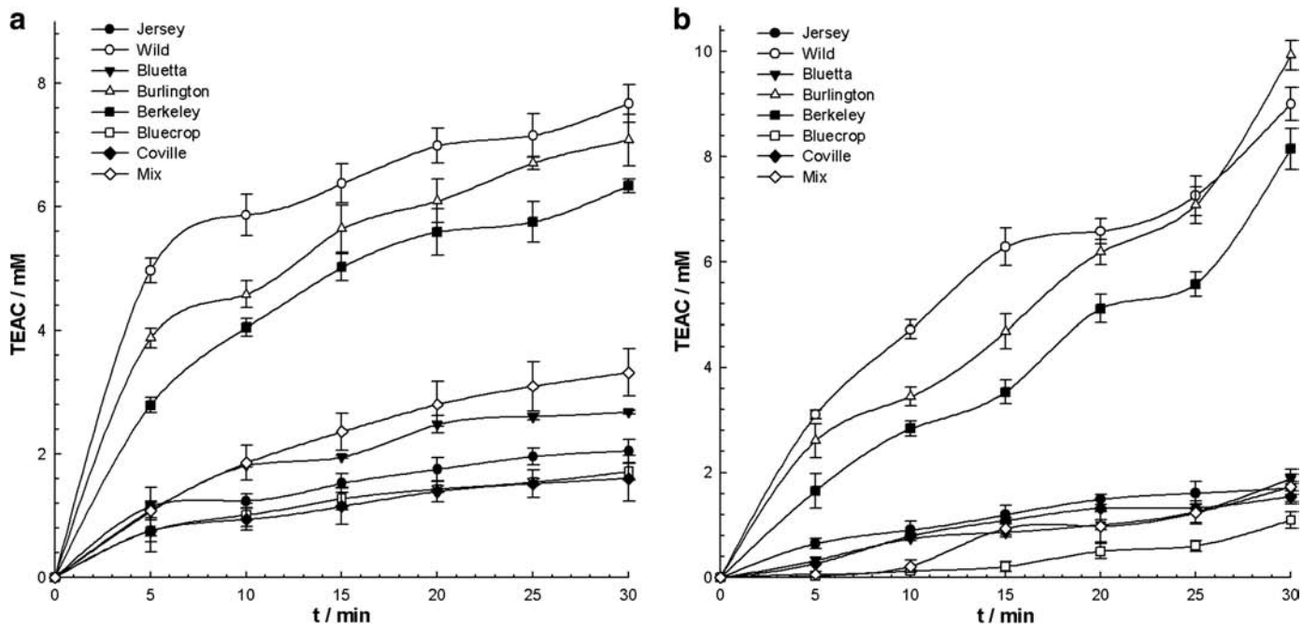

FIG. 1. TEAC of blueberry leaf infusions determined by (**a**) DPPH and (**b**) ABTS assays with respect to extraction time.

significant decrease (420 g/kg) of major catechins from the maximum value of 525–500 g/kg after 20 minutes, due to their degradation. We did not observe a decrease in TP even after prolonging extraction time to 30 minutes.

An average 2.3-fold increase in the TP content was observed when extraction time was prolonged from 5 to 30 minutes. The reducing capacity according to FRAP also increased, on the average, 2.2-fold when the extraction time was prolonged from 5 to 30 minutes. Finally, the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging capacities also significantly increased for the 30-minute extraction time as opposed to the 5-minute extraction time: 2.2-fold and 4.3-fold (because of discrepancies, the values for mix and Bluecrop infusions were not included in this calculation), respectively. These findings are in line with those of Belaya *et al.*,[28] who observed a 1.5-fold increase in antioxidant activity of bearberry (*Arctostaphylos adans*) leaf extracts when extraction time was prolonged from 5 minutes to 30 minutes.

Antioxidant capacity of blueberry leaf infusions in comparison with medicinal plant extracts

The extraction procedure (solvent, extraction time, temperature) exerts a significant influence on the final content of antioxidants in the infusion. Because of a myriad of extraction conditions reported in the literature, it is often difficult to compare the results of antioxidant capacity studies performed by various authors. Therefore, we chose to compare our results to the results obtained in those studies that have reported similar extraction conditions.

Kiselova *et al.*[18] studied the water-phase antioxidant activity of extracts from 23 Bulgarian medicinal plants in relation to their polyphenol content and compared it to the values of commonly consumed teas (mate, green and black tea, honeybush, and rooibos). The extracts were prepared by incubating dried plant material in boiling water for 10 minutes. An aqueous extract of *Alchemilla vulgaris* L. showed the highest ABTS$^{\bullet+}$ radical scavenging activity (4.79 m*M* TEAC), greater than the activity of the most potent tea, mate (3.50 m*M* TEAC). The value obtained for *A. vulgaris* L. is almost identical to our TEAC for the 10-minute wild blueberry leaf infusion (4.72 m*M*). This makes our wild blueberry leaf infusion a more potent ABTS$^{\bullet+}$ radical scavenger than 22 tested Bulgarian herb extracts. In our study, three out of eight 10-minute blueberry leaf infusions exhibited greater TEAC than the honeybush infusion (1.46 m*M* TEAC): wild (4.72 m*M* TEAC), Burlington (3.45 m*M* TEAC), and Berkeley (2.84 m*M* TEAC).

Katalinic *et al.*[17] determined the TP content and antioxidant capacity of 70 medicinal plant infusions, extracted for 30 minutes in water heated to 95°C. In comparison to the values reported in this study, the FRAP values for our 30-minute blueberry leaf infusions rank close to the very top of the list, with wild blueberry (20,050 μM) and Burlington (19,793 μM) coming very close to *Melissae folium* infusion (25,234 μM), which exhibited the highest antioxidant capacity among 70 selected medicinal plants. In addition, the FRAP values for Bluetta (14,326 μM) and mix (13,206 μM) come very close to the second best on the top 70 list, the *Spiraea herba* infusion (15,256 μM). In terms of DPPH$^{\bullet}$ radical scavenging capacity, the 30-minute *M. folium* infusion exhibited an almost identical quenching potential (85.6%) as our wild blueberry infusion (84.9%). The results of the ABTS assay indicate that the Burlington leaf infusion (89.2%) is a more potent ABTS$^{\bullet+}$ radical quencher than the *M. folium* infusion (83.6%). This leads to the conclusion that blueberry leaf infusions show significant reducing capacity as well as radical scavenging potential, which places them high on the list of dietary sources of antioxidants and justifies the recommended consumption of blueberry leaf tea.

Radical scavenging capacity of blueberry leaf infusions in comparison to antioxidant standards

DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging/quenching capacities of 30-minute blueberry infusions (diluted 1:10 for

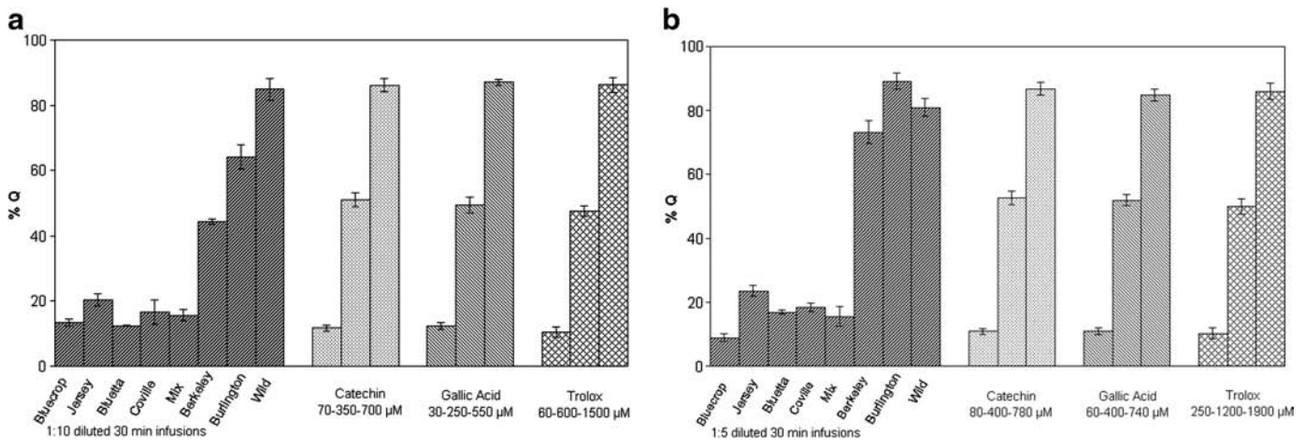

FIG. 2. Quenching capacity of (**a**) 1:10 diluted 30-minute blueberry leaf infusions with respect to DPPH$^{\bullet}$ radical and (**b**) 1:5 diluted 30-minute blueberry leaf infusions with respect to ABTS$^{\bullet+}$ radical in comparison to different concentrations of the pure standards catechin, gallic acid, and Trolox.

the DPPH assay and 1:5 for the ABTS assay) were compared with the radical scavenging capacity of different concentrations of the pure phenolics gallic acid, catechin, and Trolox, which are often used as standards in antioxidant research. Figure 2 presents the results in terms of percentage Q values of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. Catechin, gallic acid, and Trolox showed a dose-dependent $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ quenching capacity. Catechin and gallic acid were more efficient radical quenchers than Trolox; at 1 mM concentrations of catechin and gallic acid, the bleaching of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ was complete within the allocated reaction time.

In comparison to pure standards, the 1:10 dilutions of 30-minute blueberry infusions showed significant $DPPH^{\bullet}$ quenching capacity. Again, wild, Burlington, and Berkeley infusions were more efficient than the remaining infusions, with wild having a Q value of 84.9%, which is comparable to Q values for 700 μM catechin, 550 μM gallic acid, and 1.5 mM Trolox concentrations. The lowest Q values were observed for 1:10 dilutions of 30-minute Bluetta (12.4%) and Bluecrop (13.3%) infusions and were comparable to 70 μM catechin, 30 μM gallic acid, and 60 μM Trolox concentrations.

In comparison to $DPPH^{\bullet}$ radical quenching capacity, the $ABTS^{\bullet+}$ radical quenching capacity was greater for all 1:5 dilutions of 30-minute infusions, except wild, Bluecrop, and mix. For the mix infusion, the Q value was identical with respect to $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ and amounted to 15.6%. Wild blueberry infusion was a slightly less efficient $ABTS^{\bullet+}$ radical scavenger (80.9%) than Burlington (89.2%), whose quenching capacity is comparable to 780 μM catechin, 740 μM gallic acid, and 1.9 mM Trolox concentrations. As in the case of the DPPH assay, the lowest Q value was observed for 1:10 dilutions of 30-minute Bluetta and Bluecrop infusions, amounting to 16.9% and 8.9%, respectively.

CONCLUSIONS

According to the results presented in this study blueberry leaves, prepared and consumed as tea, represent a rich source of potent phenolic antioxidants. The grouping of tested infusions, according to the results of all employed methods, points to Burlington, Berkeley, and wild infusions as very potent radical scavengers with very high TP content and significant reducing capacity according to the FRAP assay. In fact, our wild blueberry leaf infusion was a more potent $ABTS^{\bullet+}$ radical scavenger than 22 tested Bulgarian herb extracts. Based on the results of this *in vitro* study, blueberry leaf tea is a significant source of potent dietary antioxidants.

ACKNOWLEDGMENTS

This research has been financially supported by the TEST program—Technological Research and Implementation Project E38/2005 and Science Project 098-0982913-2829—financed by the Croatian Ministry of Science, Education and Sports. Special thanks to Ivan Salopek, Branka Salopek-Sondi, and Snježana Mihaljević for valuable help in sample collection and preparation of leaf lyophilisates.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Smith SH, Tate PL, Huang G, Magee JB, Meepagala KM, Wedge DE, Larcom LL: Antimutagenic activity of berry extracts. *J Med Food* 2004;7:450–455.
2. Mazur W, Uehara M, Wähälä K, Adlercreutz H: Phytoestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry meal in human subjects. *Br J Nutr* 2000;83:381–387.
3. Wang Y, Chang CF, Chou J, Chen HL, Deng X, Harvey BK, Cadet JL, Bickford PC: Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. *Exp Neurol* 2005;193:75–84.
4. Ofek I, Goldhar J, Sharon N: Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. *Adv Exp Med Biol* 1996;408:179–183.
5. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen J, Bickford PC: Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* 1999;19:8114–8121.
6. Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF: Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J Agric Food Chem* 2001;49:1270–1276.
7. Sellappan S, Akoh CC, Krewer G: Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J Agric Food Chem* 2002;50:2432–2438.
8. Faria A, Oliveira J, Neves P, Gameiro P, Santos-Buelga C, De Freitas V, Mateus N: Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *J Agric Food Chem* 2005;53:6896–6902.
9. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland CM: Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agric Food Chem* 1998;46:2686–2693.
10. Srivastava A, Akoh CC, Yi W, Fischer J, Krewer G: Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. *J Agric Food Chem* 2007;55:2705–2713.
11. Zheng W, Wang SJ: Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem* 2003;51:502–509.
12. Ehlenfeldt KM, Prior RL: Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *J Agric Food Chem* 2001;49:2222–2227.
13. Connor AM, Luby JJ, Tong BS, Finn CE, Hancock JF: Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *J Am Soc Hort Sci* 2002;127:89–97.

14. Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE: Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium, Rubes* and *Ribes. J Agric Food Chem* 2002;50:519–525.
15. Kalt W, Forney CF, Martin A, Prior RL: Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J Agric Food Chem* 1999;47:4638–4644.
16. Connor AM, Luby JJ, Hancock JF, Berkheimer S, Hanson EJ: Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *J Agric Food Chem* 2002;50:893–898.
17. Katalinic V, Milos M, Kulisic T, Jukic M: Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem* 2006;94:550–557.
18. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T: Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian hers. *Phytother Res* 2006;20:961–965.
19. Perva-Uzunalić A, Škerget M, Knez Ž, Weinreich B, Otto F, Grüner S: Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine. *Food Chem* 2006;96:597–605.
20. Singleton VL, Rossi JA Jr: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965;16:144–158.
21. Benzie IF, Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': the FRAP assay. *Anal Biochem* 1996;239:70–76.
22. Brand-Williams W, Cuvelier ME, Berset C: Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* 1995;28:25–30.
23. Yen GC, Duh PD: Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *J Agric Food Chem* 1994;42:629–632.
24. Blois MS: Antioxidant determinations by the use of a stable free radical. *Nature* 1958;181:1199–1200.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26:1231–1237.
26. Howard LR, Clark JR, Brownmiller C: Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J Sci Food Agric* 2003;83:1238–1247.
27. Prior RL, Wu X, Schaich K: Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 2005;53:4290–4302.
28. Belaya NI, Filippenko TA, Bely AV, Gribova NY, Nikolaevskii AN, Biryukova AA: Electric-field-assisted extraction of antioxidants from bearberry (*Arctostaphylos adans*) leaves. *Pharm Chem J* 2006;40:504–506.