Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2015

45

Print ISSN: 0367-4444

UDC: _____

Online ISSN: 2232-7266

Original Scientific Article

9-12

Antioxidant potential of selected traditional plant-based beverages in Bosnia and Herzegovina

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Article info
Received: 11/06/2015
Accepted: 03/12/2015

Keywords:
Antioxidants
Elder
Blackberry
Pomegranate
Boza
Juniper

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Abstract: The main aim of our work was to determine antioxidant capacity of some traditional non-alcoholic beverages in Bosnia and Herzegovina. Eight samples of traditionally prepared beverages were tested by DPPH and FRAP assay. Total phenolic content was determined by Folin-Ciocalteau method and anthocyanidines by Vanilin-HCl method. Total phenolic content was in range of 74.31 mg TEA/L (elder juice with lemon) to 3 365.35 mg TEA/L (pomegranate juice). Anthocyanidines content ranged from 125.27 mg/L (elder juice without lemon) to 1899.08 mg/L (traditionally prepared blackberry juice). Pomegranate juice exhibited the strongest activity against DPPH radicals (75.29% inhibition). The DPPH determined antioxidant capacity showed positive correlation with total phenolic content as well as with flavonoids content. FRAP assay showed stronger antioxidant capacity for most of the samples, compared to ascorbic acid standard. The analyzed traditionally prepared beverages showed strong antioxidant capacity which was even more pronounced than in the commercial juice.

INTRODUCTION

Oxidative stress is deemed to play important role in many acute and chronic diseases (Polimeni *et al.*, 2015; Cobb and Cole, 2015). The burden of chronic diseases like cardiovascular, neurodegenerative diseases and cancer is constantly rising, and so does the research in the field of antioxidants (Pisoschi and Pop, 2015). Antioxidants and functional food containing antioxidants gain rising popularity among consumers (Lobo *et al.*, 2010). Consequently, the market of the functional food with these compounds is rapidly growing. On the other hand, some traditional food is also high in antioxidants, but their use is deprived by the rising number of commercial products. This trend can affect the dietary habits since the consumers are oriented to processed instead of homemade food. The health professionals and authorities should be more devoted to the research of traditional, non-processed food, its functional properties and promotion of its consumption. The main aim of our work was to determine antioxidant capacity of some traditional non-alcoholic beverages in BiH.

EXPERIMENTAL

Eight samples of traditionally prepared beverages (elder juice, elder and lemon juice, juniper berries juice, juniper berries and lemon juice, blackberry juice, pomegranate juice, and boza) were obtained from the home production (home-made products) and from the local market (commercial products).

The antioxidative effects of fruit juices were determined using the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) assay as previously described (Huang *et al.*, 2005; Gorjanović *et al.*, 2013). An aliquot (50 μL) of samples diluted in destiled water (1:9 v/v) was mixed with 2 mL of freshly prepared DPPH methanolic solution (20 mg/L). The absorbance was measured at 517 nm after 16 minutes. The percent of

inhibition was calculated according to Yen and Duh (1994) using Equation 1.

$$\% \text{ inhibition} = A_{k.r(os)} - A_{uz(16\ min)} / A_{k.r(os)} \times 100 \quad (1)$$

$A_{k.r(os)}$= absorbance of control (blank) measured at 0 minutes
$A_{uz(16\ min)}$= absorbance of sample measured at 16 minutes

The IC50 was also calculated and results were expressed as v/v% in comparison to the DPPH. The antioxidant capacity was also measured by ferric reducing antioxidant power (FRAP) assay. The Fe(III)-TPTZ (2,4,6-tripyridyl-s-triazine) complex is used as an oxidant, which reduces to Fe(II)-TPTZ in the presence of antioxidant compound. An aliquot (50 μL) of samples were mixed with 1,5 mL of freshly prepared FRAP reagent (0.3 mol/L acetic buffer pH 3.6, 0.01 mol/L TPTZ and 0.02 mol/L $FeCl_3$ in ratio 10:1:1). The resulting blue colour was measured at 593 nm at 0 and 4 minutes, and the mean absorbance was recorded. The results were expressed as FRAP values (mmol/l Fe^{+2}) and compared to the standard antioxidants (Benzie and Strain, 1996). Pure compounds (cathechine, ascorbic acid and trolox) were used as control standards in both assays.
In addition, total phenolic content was determined by Folin-Ciocalteau method described previously by Slinkard and Singleton (1999). Sample aliquot (20 μL) was added to the portion of FC reagent (100 μL) and 1.58 mL of distilled water, after 8 minutes 300 μl 20% Na_2CO_3 aqueous solution was added and incubated at 40^0C for 30 minutes. The absorbance was measured at 765 nm and used for calculation of total phenolic content.
The PVPP bound phenolic compounds (flavonoids) were determined according to Makkar *et al.* (1993). Two milliliters of samples were mixed with 100 mg of insoluble, crosslinked PVPP. The tubes were vortexed and left for 15 min at 4°C. The non-adsorbed phenolics were determined in the aliquots of supernatant by the same procedure used for total phenolics. The content of PVPP bound phenolics was calculated as the difference between total and non-adsorbed phenolics. The results were expressed as mg tannic acid equivalents (TAE) per liter. Anthocyanidines content was determined by Vanilin-HCl method based on reaction of vaniline with meta-substituted A ring of flavanols resulting in formation of chromophore (Sun *et al.*, 1998). The absorption was measured at 500 nm and results were calculated as catehine content (mg/L). The anthocyanidines absorbance was calculated by Equation 2.

$$A = (A_S - A_b) - (A_c - A_0) \quad (2)$$

A_S – absorbance of test sample
A_b- absorbance of sample containing 0 mg of catechin
A_c – absorbance of control solution
A_0 – absorbance of control solution containing 0 mg of catechin

The calculated absorbances were used for determination of the anthocyanidines content from the external calibration plot. All the reagents were of analytical grade and supplied from Fluka and Sigma Aldrich.

RESULTS AND DISCUSSION

The calibration curves were linear in range of 50-1000 mg/L TAE (R^2=0.999) and in range 20-300 mg/L of catechin (R^2=0.998) (Table 1).
Total phenolic content was in range of 74.31 mg TAE/L (elder juice) to 3365.35 mg TAE/L (pomegranate juice) (Table 2). The presented phenolics content respondes to one liter of native sample, which is used as purchased in case of samples 6 (commercial blackberry juice), 8 and 9 (two boza samples), while the rest of sampes are usually diluted with water (1:10) prior to consumption. Taking this factor in count, the highest total phenolic content (820.53 mg TAE/L) was in sample 6 (commercial blackberry juice), and the lowest (7.43 mg TAE/L) in sample 1 (elder and lemone juice). The PVPP bound phenolics were dominant in elder juices (samples 1 and 2), commercial blackberry juice (sample 6) and pomegranate juice (sample 7). The oposite case was found in boza samples (samples 8 and 9). Anthocyanidines content ranged from 125.27 mg/L (elder and lemon juice) to 1899.08 mg/L (traditionally prepared blackberry juice) in native samples (Table 2). Taking into account the dilution factor, the highest antocyanidine content was found in boza samples (samples 8 and 9) and commercial blackberry juice (sample 6).

Table 1: Absorbances of tannic acid (total phenolic content) and catechin (anthocyanidines) series of standards

	Total phenolic content		Anthocyanidines	
Standard	mg TAE/L	A_{765nm}	mg/L catechin	A_{500nm}
1	50	0.16	20	0.007
2	100	0.19	50	0.009
3	150	0.20	100	0.014
4	250	0.25	200	0.022
5	500	0.37	250	0.027
6	1000	0.58	300	0.032

Table 2: Content of total phenolic compounds (TP), PVPP bound phenolics (PVPP b), PVPP non-adsorbed phenolics (PVPP nb) and anthocyanidines (AN) in samples

Sampl.	TP (mg TAE/L)	PVPP b (mg TAE/L)	PVPP nb (mg TAE/L)	AN (mg/L catechin)
1	74.31	58.09	16.22	147.72
2	136.87	91.60	45.27	125.27
3	360.28	180.97	179.31	237.53
4	317.84	194.37	123.47	293.67
5	2505.19	1172.95	1332.24	1899.08
6	820.53	547.37	273.16	540.65
7	3365.35	2223.01	1142.34	1322.98
8	273.15	98.30	174.85	956.04
9	210.59	51.39	159.20	1539.83

1 – Elder; 2 - Elder and lemon; 3 - Juniper berries; 4 - Juniper berries and lemon; 5 - Blackberry homemade; 6 - Blackberry commercial;
7 – Pomegranate; 8 - Boza 1; 9 - Boza 2.

Pomegranate juice exhibited the strongest activity against DPPH radicals (75.29 % inhibition), followed by traditionally prepared blackberry juice (42.86 % of inhibition) with their IC50 values of 5.5 % (v/v) and 6.4 % (v/v), respectively (Figure 1).

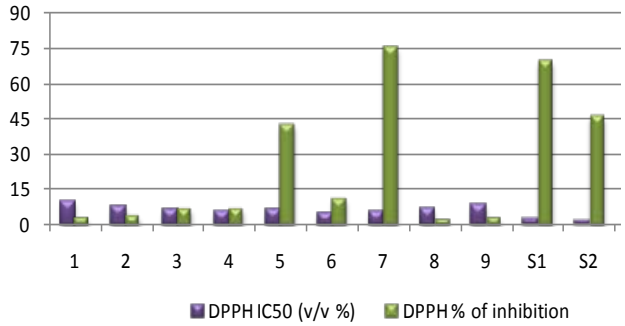

Figure 1: Antioxidant activity by DPPH assay (1 – Elder; 2 - Elder and lemon; 3 - Juniper berries; 4 - Juniper berries and lemon; 5 - Blackberry homemade; 6 - Blackberry commercial; 7 – Pomegranate; 8 - Boza 1; 9 - Boza 2; S1 – Ascorbic acid; S2 – Catechin; S3 – Trolox)

The DPPH antioxidative activity of sample 7 (pomegranate juice) was higher than for the ascorbic acid and catechine pure compounds, and slightly lower than for Trolox. The samples 1 (elder juice) and 2 (elder and lemon juice), as well as two boza samples (samples 8 and 9) showed lowest DPPH% of inhibition and highest IC50 values. FRAP assay showed stronger antioxidant capacity for most of the samples, compared to ascorbic acid standard (Figure 2).

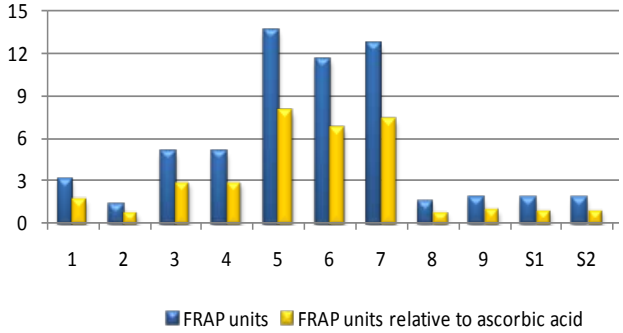

Figure 2: Antioxidant activity by FRAP assay (1 – Elder; 2 - Elder and lemon; 3 - Juniper berries; 4 - Juniper berries and lemon; 5 - Blackberry homemade; 6 - Blackberry commercial; 7 – Pomegranate; 8 - Boza 1; 9 - Boza 2; S1 – Ascorbic acid; S2 – Catechin; S3 – Trolox)

The FRAP antioxidative activity of samples 5 (blackberry homemade juice), 6 (blackberry commercial juice) and 7 (pomegranate juice) was approximately five times higher than for the pure standard compounds. The lowest antioxidative capacity was recorded for samples 1 (elder juice) and 2 (elder and lemon juice), as well as for the two boza samples (samples 8 and 9). This is in good agreement with the DPPH assay results.

The DPPH determined antioxidant capacity showed positive correlation with total phenolic content as well as with flavonoids content (Figure 3).

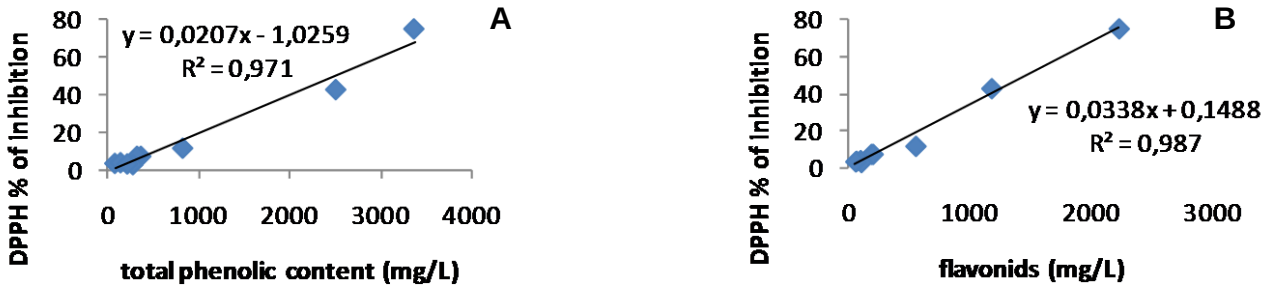

Figure 3: Correlation of DPPH antioxidant activity with total phenolic content (A) and flavonoids content (B).

CONCLUSION

The analyzed traditionally prepared beverages showed strong antioxidant capacity which was even more pronounced than in the commercial juice. The PVPP bound phenolics were predominant in commercial blackberry juice and homemade pomegranate juice, which also showed the highest antioxidative activity in both DPPH and FRAP assays. Health promotion of potential benefits of such homemade products should be enhanced.

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Summary/Sažetak

Cilj rada je bio odrediti antioksidativni kapacitet nekih tradicionalnih bezalkoholnih napitaka u BiH. Testirano je osam uzoraka pripremljenih prema tradicionalnoj recepturi primjenom DPPH i FRAP eseja. Sadržaj ukupnih fenola je određen primjenom Folin-Ciocalteau metode, a antocijanidini su određeni vanillin-HCl metodom. Sadržaj ukupnih fenola je bio u rasponu od 74.31 mg TAE/L (sok od zove s limunom) do 3365.35 mg TAE/L (sok od nara). Sadržaj antocijanidina je bio u rasponu od 125.27 mg/L katehina (sok od zove) do 1899.08 mg/L katehina (sok od kupine). Najveću aktivnost u DPPH testu pokazao je sok od nara (%inhibicije = 75.29). Antioksidativni kapacitet utvrđen DPPH testom pokazao je pozitivnu korelaciju sa sadržajem ukupnih, kao i PVPP vezanih fenola. Rezultati FRAP testa pokazali su jači antioksidativni kapacitet za većinu ispitivanih uzoraka u odnosu na standard askorbinske kiseline. Analizirani tradicionalni napici pokazali su antioksidativni kapacitet koji je bio čak i veći nego u komercijalnim napicima.