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Total Phenolic Content and Antioxidant Capacity in Infusions of Various Herbal Teas

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Abstract: Among numerous organic compounds, herbal teas contain various phenolic compounds that may affect the physiological and antioxidant activity of the tea. The aim of this work was to evaluate total phenolic content (TPC) and antioxidant capacity (AC) against peroxide free radicals in the infusions of various herbal teas.
TPC was determined using the spectrophotometric method with Folin-Ciocalteu's reagent, where Me(VI) ions are reduced with phenolics from tea samples to blue-coloured Me(V)-oxides with absorption maximum at 743 nm. Gallic acid, was used as a standard.
AC against peroxyl free radicals, was determined by using the manual spectrofluorimetric method with fluorescein. Trolox (T), which is a hydrosoluble synthetic analogue of natural vitamin E, was used as a standard. TPC varied from 488.8 mg GAE/100 mL (for bearberry tea) to 13.7 mg GAE/100 mL (for sage). Obtained values for AC were in a range 4076.3 μmol TE/100 mL (for bearberry tea) to 251.4 μmol TE/100 mL (for tea against gastritis).

INTRODUCTION

Total phenol content (TPC), and the identification of individual phenolic compounds in plant extracts have been extensively studied since the last decade of the 20th century mainly due to their antioxidant activity (Bors et al., 1984; Cotelle et al., 1996; Zheng and Wang, 2001; Chukarina et al., 2007; Borowska et al., 2009; Orčić et al., 2011), its anti-inflammatory action (Ferrándiz and Alcaraz, 1991), genetic effects (Brown, 1980), and prooxidant action - in the presence of transition metal ions (Ahmad et al., 1992). Today, there are a number of definitions of antioxidants, but none have been generally accepted. Antioxidants are a group of different natural compounds in the human body which are important for protection against the harmful effects of free radicals. There are two sources of antioxidants available. The first refers to the ability of our body to produce antioxidants in the reactions of vitamins and minerals, while second is external source of antioxidants found in various foods (Denisov and Afanas'ev, 2005). It is assumed that the oldest known source of antioxidants is a green tea. The natural world has a lot of antioxidants, and most common are vitamin C and E, phenols and flavonoids.

The aim of this study was to quantify the total phenolic content (TPC) and the antioxidant capacity (AC) of various herbal teas. Twenty samples of herbal teas were analyzed: sage (*Salvia officinalis* L.), green and black tea (*Camellia sinensis* L.), heather (*Calluna vulgaris* L.), bearberry (*Arctostaphylos uva-ursi* L.), peppermint (*Mentha piperita* L.), cranberry (*Vaccinium vitis idaea* L.), mint (*Mentha crispa* L.), chamomile (*Matricaria chamomilla* L.), commercial tea mixture used against gastritis, rosemary (*Rosmarinus officinalis* L.), savory (*Satureja montana* L.), comfrey (*Symphytum officinale*

L.), thyme (*Thymus vulgaris* L.), St. John's wort (*Hypericum perforatum* L.), Turkey malva (*Herniaria glabra* L.), artichoke (*Cynara scolymus* L.), buckwheat (*Fagopyrum esculentum* Moench), tarragon (*Artemisia dracunculus* L.), and a mixture of tarragon and buckwheat. Also, two samples of black tea produced by different manufacturers, and the samples of sage, heather and buckwheat taken from different locations were analyzed.

EXPERIMENTAL

Sample

Twenty five samples of different herbal teas were analyzed for TPC and twenty one for AC.

Sample preparation

One gram of each sample of dried leaves or herb was soaked in 100 ml of boiling, high purity water, left to stand for 10 min, and then filtered into flask of 100 ml. 1.00 ml of this solution was transferred to the flask of 25 ml. Aliquots of 1.5 ml were transferred to centrifugal tubes and centrifuged for 20 minutes at 10 000 rpm at 4 °C. Supernatants were stored in a freezer until analysis.

Standard preparation for total phenolic content (TPC)

Fifty milligrams of the pure gallic acid (GA) was dissolved in distilled water in a 100 mL flask, so that the concentration of GA was 500 mg/L. Stability of this solution is 2 days at 4 °C. This primary standard solution was used for preparation of series of working solutions (10, 25, 50, 75, 100, 150 and 200 mg/L), which in the 0.2 mL aliquots were added in a total volume of 2 ml of the reaction mixture, so that the final concentrations of GA were 1, 2.5, 5, 7.5, 15, and 20 mg/L.

Determination of total phenolic content

The total phenolic content (TPC) was determined using the spectrophotometric method (on PerkinElmer UV/Vis spectrometer Lambda 25) by using Folin-Ciocalteu's reagent, which Me(VI) ions are reduced with phenolics from samples of teas to blue-coloured Me(V)-oxides, with absorption maximum at 743 nm (Singleton and Rossi, 1965; Slinkard and Singleton, 1977). Briefly, 0.2 mL of the supernatant of diluted infusions of tea or working standard was added to 1 ml of 1/10 diluted Folin-Ciocalteu reagent. This mixture was left to stand for 10 minutes. Next, 0.8 ml of 7.5%. Na_2CO_3, was added to the Folin-Ciocalteu reagent. After 30 minutes of incubation at room temperature, the absorbance of solutions was read at 743 nm. TPC was expressed as GA equivalents (mg) per volume of tea infusion (100 ml).

Oxygen Radical Absorbance Capacity (ORAC) Assay

Antioxidant capacity (AC) against peroxyl free radicals, was determined using the manual spectrofluorimetric method with fluorescein as a fluorescent probe. Trolox (T), which is a hydrosoluble synthetic analogue of natural vitamin E, was used as a standard.

Standard preparation for Oxygen Radical Absorbance Capacity Assay

One milligram of trolox was weighed on the analytical balance then dissolved in 0.1 mL of ethanol and supplemented with 3.9 mL of water (initial concentration c_1 = 1 mmol/L). Such solution is stable for 7 days at 4 °C. An aliquot of this solution (40 μL) was supplemented with 1960 mL water giving a solution whose concentration was 20 μmol/L. To prepare the calibration curve, final concentrations of 0.1, 0.25, 0.5, 0.75 and 1 μmol/L of trolox were measured.

Determination of antioxidant capacity

Two analysis of centrifuged sample reacting with peroxide radicals generated from 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) were carried out using the Perkin-Elmer luminiscence spectrometer LS 55, with λ_{ex} 485 nm and λ_{em} 520 nm. Fluorescein was used as the target for free radicals attack. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical (Cao, and Prior, 1999). Standard was freshly prepared trolox (T), having a final concentration of 1 μmol/L. The total volume of reaction mixture was 2 mL. The mixture contained sample/standard (100 μL), fluorescein (50 μL, final concentration of 8 nM), AAPH (200 μL, final concentration of 64 mM) and water (1650 μL). Mixture containing the sample, fluorescein solution and water was incubated at 37 °C for 15 min. After incubation, AAPH was rapidly added to the mixture to start the reaction. The relative fluorescence intensity (RIF) was measured in batches every 5 minutes after the addition of AAPH. Measurement is repeated until the complete fluorescence quenching (RIF = 0). The samples were termostated at 37 °C during the analysis. The final ORAC values were calculated using a linear equation from calibrated curve. ORAC values were expressed as μmol trolox equivalents (TE) per volume of tea infusion (100 mL).

All measurements were performed in duplicate and results are expressed as mean ± s.d.

RESULTS AND DISCUSSION

Total phenol content (TPC) in herbal tea infusions was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method using gallic acid (GA) as the standard. Maximum wavelength for blue colored complex was 743 nm. After determination of the λ_{max} of colored complex, the absorbances of seven different concentrations of GA solutions were taken to construct the calibration curve (Keskin-Šašić et al., 2012, Fig. 1).

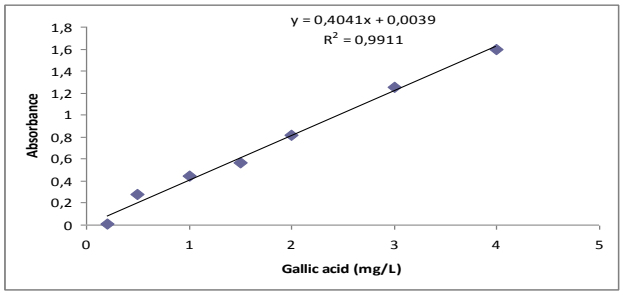

Figure 1: Calibration curve for gallic acid

TPC was determined in 25 different tea samples and mixtures of tea. As shown in Table 1, the values for TPC varied from 488.8 mg GAE/100 mL (bearberry tea) to 13.7 mg GAE/100 mL (sage).

TPC are different for the same tea samples taken from different locations or from different manufacturers. The values were following: sample of black tea I 250.7 mg GAE/100mL, black tea II, 187.1, heather I 79.6, and heather II 25.10, sage I 72.6, sage II 25.11, and sage III 13.7, buckwheat I 231.8, buckwheat II 89.6 mg GAE/100mL.

Table 1: TPC in investigated tea's infusions

Sample	TPC (mg GAE/100mL)
Uva ursi	488.8 ± 28.58
St. John's wort	274.5 ± 7.69
Black tea I	250.7 ± 10.04
Tarragon+Buckwheat mixture	231.8 ± 12.39
Black tea II	187.1 ± 13.90
Tarragon	156.6 ± 44.14
Cranberry	146.3 ± 4.32
Green tea	145.1 ± 15.97
Peppermint	137.1 ± 0.33
Thyme	134.3 ± 6.18
Chamomile	132.9 ± 18.51
Mint	117.2 ± 9.98
Buckwheat I	105.4 ± 13.01
Comfrey	102.4 ± 9.43
Buckwheat II	89.6 ± 19.29
Tea against gastritis	87.9 ± 7.17
Heather I	79.6 ± 11.44
Savory	78.5 ± 23.49
Sage I	72.6 ± 18.37
Artichoke	68.1 ± 6.25
Turkish malva	46.8 ± 6.41
Sage II	25.11 ± 1.65
Heather II	25.10 ± 6.99
$Rosemary_{(Suban)}$	18.9 ± 1.44
Sage III	13.7 ± 2.16

I, II and III: different manufacturer or location

Measurement of antioxidant capacity (AC) was performed by manual ORAC method (Cao and Prior, 1999). Maximum of excitation (λ_{max}=485 nm) and emission (λ_{max}=520 nm) wavelengths were determined using trolox (T) as a standard. After determination of excitation and emission wavelengths, relative fluorescence intensity of five different concentrations of T solutions was used to construct the calibration curve (Fig. 2).

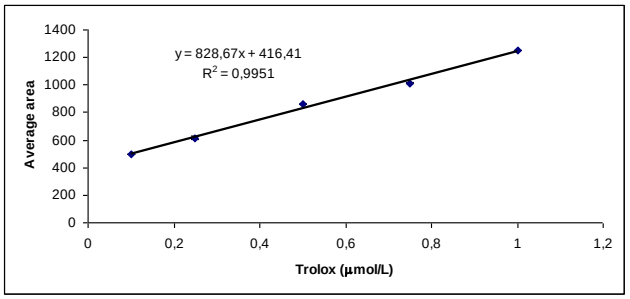

Figure 2: Calibration curve for trolox

The values for AC against peroxyl free radicals in 21 different tea samples are shown in Table 2. As shown in Table 2, there is a big difference in AC values between selected samples. The AC values varied from 4076.3 µmol TE/100 mL (bearberry tea) to 251.4 µmol TE/100 mL (tea mixtures used against gastritis).

Table 2: AC in investigated samples

Sample	AC (µmol TE/100 mL)
Uva ursi	4076.3 ± 227.97
Green tea	3070.9 ± 32.44
Tarragon+Buckwheat mixture	1877.5 ± 209.42
Black tea II	1763.5 ± 123.09
Tarragon	1454.9 ± 77.08
Black tea I	1439.8 ± 141.80
Chamomile	1402.2 ± 26.96
St. John's wort	1366.9 ± 8.21
Comfrey	1328.4 ± 240.17
Peppermint	1322.5 ± 198.53
Artichoke	992.4 ± 41.47
Heather	811.6 ± 48.36
Mint	789.8 ± 205.55
Savory	632.6 ± 223.02
Sage	538.4 ± 7.30
Turkish malva	515.8 ± 86.88
Buckwheat II	359.0 ± 92.60
Buckwheat I	328.2 ± 79.43
Cranberry	323.6 ± 132.04
Thyme	303.6 ± 166.83
Tea against gastritis	251.4 ± 186.13

I, II and III: different manufacturer or location

In summary, although bearberry tea showed the highest values for both analyzed parameters (TPC and AC), the correlation between these values for all tested samples of

herbal teas (taking into account bearberry tea) is present only to a certain extent ($r^2 = 0.6303$).

CONCLUSION

The highest TPC was detected in bearberry tea 488.8 mg GAE/100 $mL_{inf.}$, followed by St. John's wort 274.5, black tea I 250.7, tarragon + buckwheat mixture 231.8, black tea II 187.1, tarragon 156.6, cranberry 146.3, green tea 145.1, peppermint 137,1, thyme 134.3, chamomile 132.9, mint 117,2, buckwheat I 105.4, comfrey 102.4, buckwheat II 89.6, tea against gastritis 87.9, heather I 79.6, savory 78.5, sage I 72.6, artichoke 68,1, Turkish malva 46.8, sage II 25.11, heather II 25.10, rosemary 18.9 and sage III 13.7 mg GAE/100 $mL_{inf.}$.

The TPC for the same types of herbal teas but from different locations and different manufacturer, are different. The possible reasons for these differences could be different ways of tea cultivation and period of harvesting, differenences in agronomic procedures, processing, fermentation time and other processes specific for company's production.

The highest AC values against peroxyl free radicals was in bearberry tea (4076.3 μmol TE/100$ml_{inf.}$), followed by green tea 3070.9, tarragon + buckwheat mixture 1877.5, black tea II 1763.5, tarragon 1454.9, black tea I 1439.8, chamomile 1402.2, St. John's wort 1366.9, comfrey 1328.4, peppermint 1322.5, artichoke 992.4, heather 811.6, mint 789.8, savory 632.6, sage 538.4, Turkish malva 515.8, buckwheat II 359.0, buckwheat I 328,2, cranberry 323.6, thyme 303.6, and tea against gastritis 251.4 μmolTE/100$ml_{inf.}$.

Although bearberry tea showed the highest both TPC and AC values, the correlation between these two parameters for all investigated herbal teas (bearberry tea included) was insignificant (r^2=0,6303).

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Summary/Sažetak

Biljni čajevi sadrže brojne organske spojeve, među kojima i različite fenolske spojeve koji mogu uticati na fiziološku i antioksidativnu aktivnost samog čaja. Cilj ovog rada je određivanje sadržaja ukupnih fenola (TPC) i antioksidativnog kapaciteta (AC) protiv peroksidnih slobodnih radikala u infuzama nekih biljnih čajeva. Sadržaj TPC određivan je spektrofotometrijskom metodom koristeći se Folin-Ciocalteu-ovim reagensom, čiji se metalni joni sa fenolima iz uzoraka reduciraju u odgovarajuće plavo obojene okside sa maksimumom apsorpcije na 743 nm, a kao standard korištena je galna kiselina.

AC protiv peroksidnih slobodnih radikala određivan je upotrebom manuelne spektrofluorimetrijske metode sa fluoresceinom, a kao standard korišten je troloks (T), koji je hidrosolubilni sintetski analog prirodnog vitamina E.

Vrijednosti TPC kretale su se u području od 488.8 mg GAE/100 mL (za uvin čaj) do 13.7 mg GAE/100 mL (za čaj od kadulje). Dobijene vrijednosti za AC bile su u području 4076.3 μmol TE/100 mL (za uvin čaj) do 251.4 μmol TE/100 mL (za čaj protiv gastritisa).