Evaluation of individual phenolic compounds and antioxidant properties of black, green, herbal and fruit tea infusions consumed in Serbia: spectrophotometrical and electrochemical approaches

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Summary

The aim of this study was evaluation of individual phenolic compounds and antioxidant activity of commercially consumed black, green, fruit and herbal tea infusions in Serbia in order to characterize the quantity and quality of teas. The most abundant compound was gallic acid, followed by caffeic acid, rutin, (+)-catechin and (–)-epicatechin. The main procyanidin was procyanidin B1. The antioxidant activity was measured using five in vitro methods: determination of 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical cation-scavenging activity (ABTS), ferric reducing antioxidant power (FRAP), reduction power (RP) Fe(III) to Fe(II) and cyclic voltammetry (CV). Obtained results of FRAP and of the Fe(III)/Fe(III) method correlated strongly with the total phenolics content ($R^2 = 0.92246$, $R^2 = 0.88084$, $p < 0.0001$). Antioxidant power of green tea and bearberry tea was considerably higher than that of black tea. Raspberry and cherry showed the highest antioxidant power among fruit tea infusions. Contribution of phenolic compounds to tea antioxidant activity was also quantified in this study. Stepwise linear regression demonstrated that quantification of different phenolic compounds responsible for tea antioxidant activity was dependent on the method used. Gallic acid, caffeic acid (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, procyanidin B1, procyanidin B2 together made up 43.6–99.9% of the antioxidant activity of tea.

Keywords

tea infusions; phenolic compounds; antioxidant activity; cyclic voltammetry; HPLC; stepwise linear regression

Tea is one of the most consumed beverages in the world, after water. In recent years, tea has been largely studied because it is recognized as a dietary source of polyphenolic compounds, such as catechins, theaflavins, flavonol glycosides, flavone glycosides, caffeine, gallic acid, and proanthocyanidins. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic [1–3] as well as ability to modify the gene expression [4, 5]. The health benefits of tea and therapeutic effects in the treatment of many disorders [6] are generally attributed to the antioxidant properties of the major flavonoids, such as catechins, gallocatechins, catechingalate esters and theaflavins. Both in in vivo and in vitro studies, these compounds act as free radical scavengers, which remove endogenously generated superoxide, peroxyl and hydroxyl radicals.

At present, a variety of tea infusions is produced and sold. In previous decades, most Serbian consumers made their own teas due to the widespread availability of various herbs and flowers. Nowadays, tea saw significant expansion in Serbia, due to domestic and foreign producers increasing their product offerings and improving their distribution networks.

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Tea and herbal infusions contribute to the major source of phenolic compounds in the human diet [7]. Lot of studies were conducted for the presence and the activity of antioxidants in the three major forms of tea – green tea, semi-fermented oolong tea and fermented black tea [8]. Several studies were carried out on the antioxidant activity and phenolic content of commercial black, green, herbal and fruit tea infusions [3, 9–15]. However, these researches focused on the relationship between the antioxidant activity and total phenolic contents, while a limited amount of data is available on phenolic profiles and their contribution to antioxidant activity for commercial teas. Also, the correlation between different antioxidant activity evaluation methods, and contents of individual compounds in teas has not been fully investigated yet.

Besides the extensive evidence on the strong biological activity of phenolic compounds in tea, and the fact that individual phenolic compounds may reflect the antioxidant activity that is of significance for practical tea production, the aim of the current study was evaluation of total phenolic and flavonoid contents and antioxidant activities, as well as investigation of the relationship between the antioxidant activity and individual phenolic compounds and, finally, quantification of the contribution of individual phenolic compounds to tea antioxidant activity. Such systematic and scientific data on tea marketed in Serbia was not available so far. This research also contributes to the quality assessment of teas in Serbia.

MATERIALS AND METHODS

Chemicals

The compound 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Acros Organics (Morris Plains, New Jersey, USA); 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS), 2,2-diphenyl-1-picrylhidrazyl (DPPH), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), gallic acid, protocatechuic acid, caffeic acid, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, (–)-epigallocatechin gallate, rutin, morin, quercetin, procyanidin B1, B2, and B3 were purchased from Sigma Aldrich (Steinheim, Germany). Folin Ciocalteu's phenol reagent, potassium peroxodisulfate, ammonium iron(II) sulfate hexahydrate, iron(III) chloride, potassium hexacyanoferrate(III), sodium hydroxide, sodium acetate, sodium nitrite, sodium carbonate, sodium sulphate, aluminum chloride hexahydrate, sodium dihydrogen phosphate, sodium hydrogen phosphate, ascorbic acid, three chloro acetic acid, hydrochloric acid, acetic acid, formic acid and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Ethanol (96% by vol.) and methanol (HPLC grade) were from J.T. Baker (Deventer, The Netherlands).

Preparation of tea infusions

Twenty-six kinds of commercially available black (BT), green (GT), herbal (HT) and fruit (FT) tea in teabags were purchased at local markets in Serbia. All samples of tea were manufactured in the northern part of Serbia (Vojvodina) in 2010 by two national producers. The kinds of teas used for the study are presented in Tab. 1. Tea infusions were prepared according to the instructions provided on the packaging. Briefly, 2.0000 g ± 0.0001 g of each tea sample (1 tea bag) was weighed and infused in 200 ml deionized water heated to 95 °C for 10 min. Literature report [16] states that there was no apparent difference in the antioxidant potential of tea infusions with and without bags after being infused at 90 °C for 10 min. The solutions were filtered through cotton wool and then the residue was washed with deionized water, cooled to room temperature and finally diluted to 250 ml with deionized water.

Instruments

An Agilent 8453 UV/VIS spectrophotometer (Agilent Technologies, Santa Clara, California, USA) was used for absorbance measurements and spectra recording, using optical cuvettes of 1 cm optical path. The pH measurements were made with Hanna instrument pH-meter (Hanna Instruments, Smithfield, Rhode Island, USA) equipped with glass electrode. A model 1200 (Agilent Technologies) was used for HPLC analysis. The analytical column was C_{18} Zorbax Eclipse XDB-C18, 5μm, 4.6 × 150 mm (Agilent Technologies). Cyclic voltammograms were recorded on a CHI760B instrument (CHInstruments, Austin, Texas, USA). The cell was equipped with GC electrode, an accessory platinum electrode of larger area (Model CHI221, cell top including Pt wire counter electrode) and an Ag/AgCl reference electrode (Model CHI111). All measurements were taken at ambient temperature.

Total polyphenolic content (TP)

Total polyphenols were measured spectrophotometrically at 760 nm after the reaction with Folin-Ciocalteu's (FC) phenol reagent, according to the methods described by SINGLETON et al. [17] and STRATIL et al. [18]. The measurement was compared to a standard calibration curve of gallic

acid (GA) solution (1–10 $\mu g \cdot ml^{-1}$), and the results were expressed as grams of gallic acid equivalents (GAE) per kilogram of tea sample. Gallic acid stock solution was prepared in ethanol at a concentration of 5 $mg \cdot ml^{-1}$. All measurements were performed in triplicate.

Total flavonoid content (TF)

The total flavonoid content was measured by the aluminium chloride spectrophotometric method described by ZHISHEN et al. [19] and YANG et al. [20] with minor modifications. A volume of 0.25 ml of tea infusion was added to 10 ml volumetric flask containing 3 ml of deionized water. Then, 0.3 ml of 5% $NaNO_2$ was added. After incubation at room temperature for 5 min, 1.5 ml 2% aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) was added. Again, the flask was kept at room temperature for 5 min and then 2 ml of 1 $mol \cdot l^{-1}$ sodium hydroxide (NaOH) was added. The flask was filled with deionized water to the mark. Absorbance of reaction mixture was measured against the reagent blank at 510 nm. Catechin was used as a standard and the results expressed as gram of catechin equivalents (CE) per kilogram of tea sample. The levels of total flavonoid contents in teas were determined in triplicate.

Antioxidative assays

For DPPH method [21], which was slightly modified, a solution of DPPH (1×10^{-4} $mol \cdot l^{-1}$) was prepared in methanol. A volume of 5.0 ml of this solution and 100 μl of tea infusion were mixed in 10 ml volumetric flask and filled with methanol to the mark. The discoloration of the DPPH radical was measured at 520 nm, 30 min after the start of the reaction. The Trolox calibration curve was plotted as a function of the decrease in absorbance.

$$\Delta A = A_{\text{blank}} - A_{\text{sample}} \quad (1)$$

The final results were expressed as moles of Trolox equivalents (TE) per kilogram of tea sample.

The ABTS radical-scavenging activity was measured using the methods of RE et al. [22] and ARTS et al. [23]. An aliquot of tea infusion (100 μl) was mixed with 3.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the reduction in absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the decrease in absorbance (Eq. 1). The final results were expressed as moles of TE per kilogram of tea samples.

Ferric reducing-antioxidant power (FRAP) assay was performed as previously described by BENZIE and STRAIN [24]. In the FRAP assay, antioxidants in the sample reduce Fe^{3+}-TPTZ complex to the ferrous form at low pH 3.6 with an increase in absorbance at 595 nm. Briefly, 3.0 ml of freshly prepared FRAP reagent was mixed with 20 μl of tea infusion along with 380 μl of water (total volume was 3.4 ml). The absorbance at 595 nm was recorded after 5 min incubation at 37 °C. FRAP values were expressed as moles of Fe^{2+} equivalents (FE) per kilogram of tea sample.

Reducing power (RP) assay Fe(III) to Fe(II) was determined as described by OYAIZU [25]. Reducing power was expressed in relation to the reducing power of ascorbic acid as a positive control (Ascorbate Equivalent Antioxidant Capacity, *AEAC*). A volume of 100 μl of tea infusion was mixed with 1.5 ml of phosphate buffer (0.2 $mol \cdot l^{-1}$, pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 20 min. A volume of 1.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1 500 × g. The upper layer of the solution (1.5 ml) was mixed with 1.5 ml of distilled water and 0.3 ml of 0.1% $FeCl_3$. Absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

RP values were expressed as moles of ascorbic acid equivalents (AAE) per kilogram of tea sample.

Electrochemical determination of antioxidant capacity

Prior to each run, the surface of the glassy carbon electrode was freshly abraded with 1.0, 0.3 and 0.05 μm alumina powder, rinsed with redistilled water and degreased in ethanol in ultrasonic bath. The scan was taken in the potential range between 0 mV and 800 mV with a scan rate 100 $mV \cdot s^{-1}$. Cyclic voltammograms were also recorded for Trolox, vitamin E equivalent, in the concentration range (2–80 $\mu mol \cdot l^{-1}$) [3]. A calibration curve of the area below the major voltammetric anodic peak (Q_{600}) versus concentration (c) obtained for this standard was used to calculate Trolox Equivalent Antioxidant Capacity (*TEAC*) of studied teas.

Antioxidant composite index (*ACI*)

Antioxidant potency composite index was calculated for each sample as score according to Eq. 2 [26]:

$$\text{Score} = \text{Sample score/Best score} \times 100 \quad (2)$$

An index value of 100 was assigned to the best score for each test, and then calculated an index

score for all other samples within the test. The average of index scores obtained for all tests for antioxidant capacities was defined as its antioxidant potency composite index (*ACI*). All assays were given equal weight, and an overall mean index value was calculated on a normalized basis for each sample.

Separation of individual phenolic compounds in tea infusions

An Agilent chromatograph equipped with autosampler and photodiode-array and fluorescence detector (1200 Series) was used for the HPLC analysis. The separation was performed with a Zorbax Eclipse C_{18} column kept at 25 °C, at a flow rate of 0.8 ml·min^{-1}, and an injection volume of 20 μl. Detection was performed by scanning from 260 nm to 400 nm. For the gradient elution, the following programme was used: solvent A (acetonitrile) and solvent B (0.1% formic acid in water) as follows: 10% A in 0 min, then 10% A for 15 min, followed by 35 min at 30% A (slightly modified from [27]). The individual phenolic compounds were separated within 50 min. Identification was carried out by comparing the retention times and spectral data with those of standards. Quantitative determination of individual phenolic compounds in tea infusions was calculated using calibration lines.

Statistical analysis

Data are presented as mean ± standard deviation (*SD*) for triplicate determinations. Statistical analysis was performed by paired Student *t*-test, using a statistical package running on a computer (Statistica 8.0; StatSoft, Tulsa, Oklahoma, USA). A probability of $p < 0.05$ was considered to be statistically significant [28]. Moreover, stepwise regression (Statistica 8.0) was used to evaluate how much variability could be explained by each independent variable (phenolic compounds) for the dependent variable (antioxidant activity).

RESULTS AND DISCUSSION

The results on total phenolic (TP) and total flavonoid (TF) contents in the tea infusions are presented in Tab. 1. Tea is known to have a high content of polyphenolics, about 36% polyphenols on a dry weight basis [7]. Total phenolic contents varied from 26.16 g·kg^{-1} to 240.74 g·kg^{-1} among the tested tea infusions. Nettle (HT6) had the lowest content, while green tea (GT) had the highest content of total phenols.

The highest TP content was found in green tea, GT (240.74 g·kg^{-1}), followed by black tea, BT, (164.65 g·kg^{-1}) bearberry, HT1 (160.78 g·kg^{-1}), St. John's wort HT2 (84.93 g·kg^{-1}) and thyme, HT4 (83.77 g·kg^{-1}). Nettle, HT6 (26.16 g·kg^{-1}), milfoil, HT12 (33.83 g·kg^{-1}) and chamomile, HT13 (36.39 g·kg^{-1}) had the lowest TP content.

Among fruit tea infusions, the highest TP content had raspberry, FT3 (223.86 g·kg^{-1}) and cherry, FT1 (148.39 g·kg^{-1}), while pineapple, FT10 (45.84 g·kg^{-1}) had a low content of TP.

The results for total flavonoid content clearly indicate the richest flavonoid sources: green tea, GT (84.94 g·kg^{-1}), bearberry, HT1 (75.15 g·kg^{-1})

Tab. 1. Total phenolic and flavonoid contents in black, green, herbal and fruit tea infusions.

Tea and designation		Total phenols [g·kg^{-1}]	Total flavonoids [g·kg^{-1}]
Black	BT	164.65 ± 0.42^{n}	63.34 ± 0.0^{l}
Green	GT	240.73 ± 1.29^{p}	84.94 ± 0.85^{n}
Bearberry	HT1	160.78 ± 2.84^{n}	75.15 ± 1.42^{m}
St. John's wort	HT2	84.93 ± 2.97^{l}	42.03 ± 0.85^{j}
Hibiscus	HT3	49.90 ± 2.32^{f}	16.08 ± 0.57^{c}
Thyme	HT4	83.77 ± 3.87^{l}	39.32 ± 0.94^{j}
Rtanj tea	HT5	74.25 ± 7.11^{j}	32.73 ± 0.0^{i}
Nettle	HT6	26.16 ± 0.52^{a}	12.54 ± 0.29^{a}
Rose hips	HT7	73.99 ± 2.58^{i}	44.56 ± 1.28^{k}
Elder	HT8	42.67 ± 0.0^{d}	29.07 ± 0.99^{g}
Lime	HT9	59.21 ± 2.46^{h}	21.20 ± 0.29^{d}
Sage	HT10	50.43 ± 4.52^{f}	24.71 ± 1.72^{e}
Mint	HT11	79.34 ± 0.13^{k}	32.97 ± 2.13^{h}
Milfoil	HT12	33.83 ± 0.13^{b}	14.53 ± 1.28^{b}
Chamomile	HT13	36.39 ± 1.94^{c}	11.79 ± 0.14^{a}
Cherry	FT1	148.39 ± 0.11^{m}	29.95 ± 1.43^{h}
Strawberry	FT2	61.34 ± 1.62^{h}	20.22 ± 0.71^{d}
Raspberry	FT3	223.86 ± 0.27^{o}	31.65 ± 1.14^{h}
Forest fruits	FT4	50.95 ± 2.19^{f}	16.49 ± 0.28^{c}
Apricot	FT5	55.31 ± 1.55^{g}	20.94 ± 0.28^{d}
Sweet cherry	FT6	60.13 ± 1.82^{h}	20.31 ± 0.0^{d}
Blueberry	FT7	54.08 ± 0.42^{g}	21.29 ± 0.71^{d}
Apple	FT8	62.21 ± 0.91^{h}	20.75 ± 0.58^{d}
Pomegranate	FT9	81.91 ± 1.29^{l}	25.02 ± 0.57^{f}
Pineapple	FT10	45.84 ± 3.82^{e}	25.35 ± 0.57^{f}
Exotic fruits	FT11	58.12 ± 1.16^{h}	17.76 ± 0.0^{c}

Values are mean ± *SD* ($n = 3$). Values with different letters within columns are statistically different at $p < 0.05$ by paired Student *t*-test.
Total phenols are expressed as grams of gallic acid equivalents per kilogram of tea sample. Total flavonoids are expresed as grams of catechin equivalents per kilogram of tea sample.

and black tea, BT (63.34 g·kg^{-1}); raspberry, FT3 (31.65 g·kg^{-1}) and cherry, FT1 (29.95 g·kg^{-1}) were the richest among fruit tea infusions.

Among herbal tea infusions, chamomile, HT13 (11.79 g·kg^{-1}) had a low content of total flavonoids, as well as nettle, HT6 (12.54 g·kg^{-1}) and milfoil, HT12 (14.53 g·kg^{-1}). Forest fruits, FT4 (16.49 g·kg^{-1}) and exotic fruits, FT11 (17.76 g·kg^{-1}) among fruit teas had the lowest contents of TP.

Since the Folin-Ciocalteu reaction is based on chemical reduction of the reagent, many other substances with reducing activity may have influence on it. Thus, the results obtained by this method for total phenolic content of tea infusions might not only reflect the levels of phenolic compounds, but also contents of Maillard reaction products [29]. Therefore, identification of individual phenolic compounds is of importance to reveal the real phenolic profiles and antioxidant activities of tea infusion samples.

Because of the limitation of Folin-Ciocalteu method for phenolic content determination, individual phenolic compounds including gallic acid, caffeic acid, rutin, morin, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin gallate, quercetin, procyanidin B1, procyanidin B2, procyanidin B3, (–)-epigallocatechin and protocatechuic acid were determined in tea infusion samples and the results were summarized in Tab. 2A and Tab. 2B. The typical HPLC profile of the polyphenols in tea infusion is given in Fig. 1. Obtained results showed that tea infusions samples contained catechins, rutin, morin, quercetin and procyanidins, and a number of well-known non-flavonoid polyphenols, such as gallic acid and protochatechuic acid, as well as a number of chlorogenic acids such as caffeic acid. Relatively high levels of gallic acid, caffeic acid and rutin were found, while the values were lower for catechins and procyanidins. Also, variations were found in phenolic profiles among different tea infusions. In all samples, the levels of gallic acid, caffeic acid, rutin, morin, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin gallate, quercetin, procyanidin B1, procyanidin B2, procyanidin B3, (–)-epigallocatechin and protocatechuic acid were in the range of 0.05–1.98 g·kg^{-1}, not detected (ND)–1.13 g·kg^{-1}, ND–4.93 g·kg^{-1}, ND–1.23 g·kg^{-1}, 0.028–1.47 g·kg^{-1}, 0.016–2.64 g·kg^{-1}, ND–1.96 g·kg^{-1}, ND–1.726 g·kg^{-1}, 0.022–1.12 g·kg^{-1}, 0.015–1.12 g·kg^{-1}, ND–0.130 g·kg^{-1}, ND–1.60 g·kg^{-1}, ND–0.223 g·kg^{-1}, respectively. The major catechins (+)-catechin, (–)-epicatechin, (–)-epigallocatechin gallate and (–)-epigallocatechin, and gallic acid were detected at the contents of 7.11 g·kg^{-1} in green tea (GT), 5.16 g·kg^{-1} in bearberry (HT1), 2.447 g·kg^{-1} in black tea (BT), 1.957 g·kg^{-1} in rose hips (HT7), 0.617 g·kg^{-1} in St. John's wort (HT2) and 0.282 g·kg^{-1} in thyme (HT4). Fruit tea infusions, cherry, FT1 (4.41 g·kg^{-1}) and raspberry, FT3 (3.704 g·kg^{-1}), had the highest contents of major catechins and gallic acid.

Tea catechins are subject to polymerization due to the manufacturing process [30]. Among the catechin polymers, procyanidins B1, B2 were detected in all tested tea infusions, while procyanidin B3 was detected in St. John's wort, HT2 (0.130 g·kg^{-1}), green tea, GT (0.030 g·kg^{-1}), black tea, BT (0.023 g·kg^{-1}) and sage, HT10

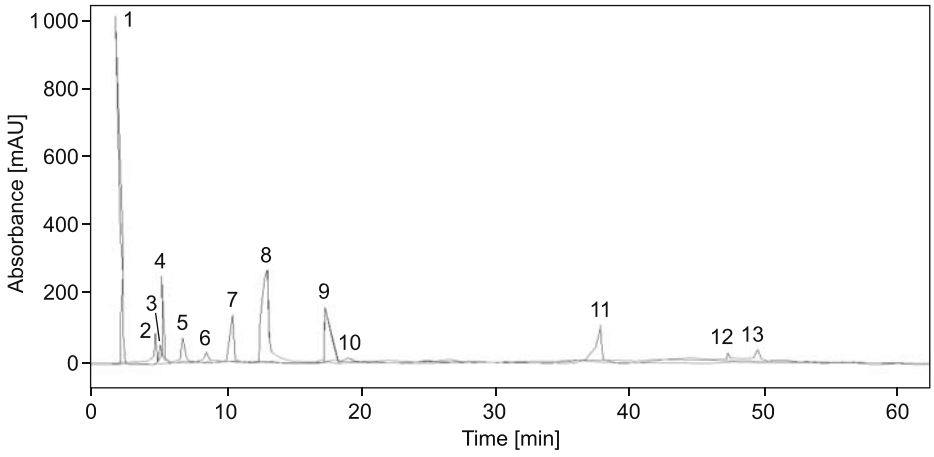

Fig. 1. Chromatogram of a herbal tea infusion.

Recorded at 280 nm: 1 – gallic acid, 4 – protocatechuic acid, 7 – caffeic acid.
Recorded using fluorescence detector: 2 – procyanidin B1, 3 – (–)-epigallocatechin, 5 – (+)-catechin, 6 – procyanidin B2, 8 – (–)-epicatechin, 9 – (–)-epigallocatechin gallate, 10 – procyanidin B3.
Recorded at 360 nm: 11 – rutin, 12 – morin, 13 – quercetin.

Tab. 2A. Contents of individual phenols in black, green, herbal and fruit tea infusions.

Tea	Gallic acid [g·kg^{-1}]	Caffeic acid [g·kg^{-1}]	Rutin [g·kg^{-1}]	Morin [g·kg^{-1}]	(+)-Catechin [g·kg^{-1}]	(–)-Epicatechin [g·kg^{-1}]	(–)-Epigallo catechin gallate [g·kg^{-1}]
BT	1.34 ± 0.22	0.195 ± 0.003	1.485 ± 0.007	ND	0.263 ± 0.008	0.26 ± 0.01	0.374 ± 0.008
GT	1.98 ± 0.33	0.261 ± 0.005	1.091 ± 0.007	0.38 ± 0.01	0.40 ± 0.01	2.64 ± 0.12	1.73 ± 0.08
HT1	1.10 ± 0.18	0.238 ± 0.005	1.99 ± 0.01	ND	0.73 ± 0.02	0.97 ± 0.03	1.29 ± 0.11
HT2	0.12 ± 0.02	0.115 ± 0.002	1.09 ± 0.13	ND	0.132 ± 0.004	0.26 ± 0.01	ND
HT3	0.05 ± 0.01	0.087 ± 0.003	0.846 ± 0.006	0.136 ± 0.006	0.213 ± 0.006	0.047 ± 0.002	ND
HT4	0.18 ± 0.03	0.128 ± 0.003	4.93 ± 0.03	ND	0.041 ± 0.001	0.026 ± 0.001	ND
HT5	0.16 ± 0.03	0.054 ± 0.001	0.509 ± 0.003	1.007 ± 0.004	0.112 ± 0.003	0.048 ± 0.003	0.155 ± 0.004
HT6	0.16 ± 0.03	0.113 ± 0.002	0.218 ± 0.002	ND	0.38 ± 0.01	0.24 ± 0.01	0.178 ± 0.009
HT7	0.96 ± 0.16	0.183 ± 0.004	0.137 ± 0.004	ND	0.072 ± 0.002	0.19 ± 0.01	0.54 ± 0.03
HT8	0.26 ± 0.04	0.132 ± 0.003	1.68 ± 0.01	0.152 ± 0.003	0.103 ± 0.002	0.053 ± 0.003	0.087 ± 0.004
HT9	0.19 ± 0.03	0.074 ± 0.002	0.428 ± 0.002	ND	0.041 ± 0.002	0.23 ± 0.01	0.29 ± 0.01
HT10	0.08 ± 0.01	0.49 ± 0.01	ND	1.23 ± 0.01	0.057 ± 0.002	0.23 ± 0.01	ND
HT11	0.12 ± 0.02	0.049 ± 0.001	4.08 ± 0.03	0.113 ± 0.003	0.086 ± 0.002	0.034 ± 0.001	0.059 ± 0.002
HT12	0.12 ± 0.02	1.13 ± 0.03	ND	ND	0.048 ± 0.001	0.015 ± 0.001	ND
HT13	0.11 ± 0.02	0.205 ± 0.001	ND	ND	0.173 ± 0.005	0.038 ± 0.002	ND
FT1	1.34 ± 0.22	ND	0.809 ± 0.005	ND	1.47 ± 0.04	0.23 ± 0.01	0.105 ± 0.003
FT2	0.21 ± 0.04	0.53 ± 0.01	0.605 ± 0.004	ND	0.127 ± 0.004	0.053 ± 0.002	1.96 ± 0.07
FT3	1.68 ± 0.28	ND	1.277 ± 0.008	ND	0.162 ± 0.005	0.186 ± 0.009	0.076 ± 0.003
FT4	0.16 ± 0.03	0.202 ± 0.004	0.076 ± 0.001	ND	0.056 ± 0.002	0.022 ± 0.001	ND
FT5	0.11 ± 0.02	0.131 ± 0.003	ND	ND	0.039 ± 0.001	0.019 ± 0.001	ND
FT6	0.17 ± 0.03	0.177 ± 0.004	0.080 ± 0.001	ND	0.066 ± 0.002	0.028 ± 0.001	ND
FT7	0.47 ± 0.08	0.258 ± 0.005	0.293 ± 0.002	ND	0.089 ± 0.003	0.036 ± 0.002	ND
FT8	0.37 ± 0.06	0.53 ± 0.01	0.228 ± 0.001	0.076 ± 0.001	0.101 ± 0.003	0.040 ± 0.002	ND
FT9	0.22 ± 0.04	0.259 ± 0.003	0.098 ± 0.001	ND	0.049 ± 0.001	0.016 ± 0.001	ND
FT10	0.73 ± 0.05	0.26 ± 0.01	ND	ND	0.028 ± 0.001	0.039 ± 0.002	ND
FT11	0.11 ± 0.02	0.166 ± 0.003	ND	ND	0.062 ± 0.002	0.025 ± 0.001	ND

Values are expressed as mean ± *SD* (n = 3). ND – not detected.

Tab. 2B. Contents of individual phenols in black, green, herbal and fruit tea infusions.

Tea	Quercetin [g·kg^{-1}]	Procyanidin B1 [g·kg^{-1}]	Procyanidin B2 [g·kg^{-1}]	Procyanidin B3 [g·kg^{-1}]	(–)-Epigallocatechin [g·kg^{-1}]	Protocatechuic acid [g·kg^{-1}]
BT	0.854 ± 0.003	0.117 ± 0.004	0.315 ± 0.001	0.023 ± 0.001	0.21 ± 0.01	ND
GT	0.015 ± 0.004	0.158 ± 0.004	0.35 ± 0.01	0.030 ± 0.001	0.36 ± 0.01	0.223 ± 0.006
HT1	ND	1.12 ± 0.03	0.94 ± 0.03	ND	1.07 ± 0.04	ND
HT2	ND	0.080 ± 0.002	0.236 ± 0.007	0.130 ± 0.003	0.105 ± 0.003	ND
HT3	0.078 ± 0.001	0.98 ± 0.03	0.100 ± 0.003	ND	ND	ND
HT4	ND	0.080 ± 0.002	0.027 ± 0.001	ND	0.035 ± 0.002	ND
HT5	1.061 ± 0.004	0.243 ± 0.008	0.194 ± 0.003	ND	0.155 ± 0.004	ND
HT6	ND	0.095 ± 0.002	0.031 ± 0.001	ND	ND	ND
HT7	ND	0.32 ± 0.01	0.027 ± 0.001	ND	0.195 ± 0.006	ND
HT8	0.254 ± 0.001	0.106 ± 0.003	0.019 ± 0.001	ND	0.056 ± 0.002	n.d.
HT9	ND	0.021 ± 0.001	0.201 ± 0.006	ND	0.017 ± 0.001	0.219 ± 0.008
HT10	1.726 ± 0.003	0.047 ± 0.001	0.025 ± 0.001	0.014 ± 0.001	ND	ND
HT11	0.199 ± 0.001	0.022 ± 0.001	0.023 ± 0.001	ND	0.024 ± 0.001	ND
HT12	ND	0.073 ± 0.002	0.309 ± 0.009	ND	ND	ND
HT13	ND	0.074 ± 0.002	0.037 ± 0.001	ND	ND	ND
FT1	ND	0.103 ± 0.003	ND	ND	1.27 ± 0.04	ND
FT2	0.413 ± 0.008	0.084 ± 0.003	0.022 ± 0.001	ND	0.095 ± 0.004	ND
FT3	0.368 ± 0.007	0.133 ± 0.004	ND	ND	1.60 ± 0.05	0.051 ± 0.002
FT4	ND	0.081 ± 0.003	0.072 ± 0.002	ND	0.063 ± 0.002	0.036 ± 0.002
FT5	ND	0.068 ± 0.001	0.056 ± 0.002	ND	0.098 ± 0.003	ND
FT6	ND	0.115 ± 0.003	0.085 ± 0.003	ND	0.109 ± 0.003	ND
FT7	0.152 ± 0.003	0.095 ± 0.003	0.028 ± 0.001	ND	0.174 ± 0.005	0.017 ± 0.001
FT8	0.026 ± 0.001	0.180 ± 0.005	0.023 ± 0.001	ND	0.100 ± 0.003	ND
FT9	ND	0.091 ± 0.003	0.065 ± 0.002	ND	ND	ND
FT10	0.018 ± 0.001	ND	ND	ND	ND	0.028 ± 0.001
FT11	ND	0.054 ± 0.001	ND	ND	ND	ND

Values are expressed as mean ± *SD* (n = 3). ND – not detected.

(0.014 g·kg^{-1}). The contents of procyanidins B1 and B2 in tea infusions ranged from 0.021 g·kg^{-1} to 1.12 g·kg^{-1}, and from 0.019 g·kg^{-1} to 0.94 g·kg^{-1}, respectively. Bearberry, HT1 (2.06 g·kg^{-1}), hibiscus, HT3 (1.08 g·kg^{-1}), green tea, GT (0.508 g·kg^{-1}) and black tea, BT (0.432 g·kg^{-1}) had the highest B1 and B2 combined procyanidin contents. Among fruit tea infusions, raspberry, FT3 (0.231 g·kg^{-1}), apple, FT8 (0.203 g·kg^{-1}) and cherry, FT1 (0.185 g·kg^{-1}) had the highest B1 and B2 combined procyanidin contents. Differences in total phenolic content determined by Folin-Ciocalteu and HPLC methods confirmed the non-specificity of Folin-Ciocalteu method. HPLC analysis provided more information about chemical characteristics and antioxidant activity of the samples.

Generally, it is known that total content of polyphenols correlates with antioxidant activity [31]. As most natural antioxidants are multifunctional [32], the antioxidant capacities of samples cannot be fully described by one single method. In the present study, spectrophotometric techniques, such as DPPH radical-scavenging activity, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical cation-scavenging activity (ABTS), ferric reducing-antioxidant power (FRAP) and reduction power (RP) and electrochemical method (cyclic voltametry, CV) were used to determine

Tab. 3. The antioxidant capacities of black, green, herbal and fruit tea infusions obtained by different methods.

Tea	Antioxidant activity [mol·kg^{-1}]			
	DPPH	ABTS	FRAP	RP
BT	0.181 ± 0.001^{m}	3.21 ± 0.00^{f}	0.788 ± 0.001^{o}	2.57 ± 0.04^{i}
GT	0.180 ± 0.004^{m}	3.21 ± 0.00^{g}	1.825 ± 0.027^{u}	7.45 ± 0.14^{n}
HT1	0.177 ± 0.001^{l}	3.19 ± 0.01^{f}	1.725 ± 0.009^{t}	7.27 ± 0.11^{n}
HT2	0.173 ±0 .003^{l}	3.17 ± 0.00^{f}	0.438 ± 0.037^{h}	1.57 ±0 .08^{d}
HT3	0.124 ± 0.00^{h}	3.20 ± 0.01^{g}	0.454 ± 0.034^{h}	1.34 ± 0.03^{cb}
HT4	0.173 ± 0.002^{l}	3.22 ± 0.02^{g}	0.482 ± 0.029^{j}	2.06 ± 0.05^{g}
HT5	0.175 ± 0.001^{l}	3.24 ± 0.02^{h}	0.695 ± 0.011^{m}	2.20 ± 0.08^{h}
HT6	0.084 ± 0.005^{c}	2.98 ± 0.04^{c}	0.127 ± 0.005^{a}	2.40 ± 0.09^{h}
HT7	0.163 ± 0.003^{k}	3.21 ± 0.00^{g}	1.148 ± 0.039^{s}	3.52 ± 0.03^{k}
HT8	0.118 ± 0.00^{f}	3.03 ± 0.04^{c}	0.402 ± 0.011^{f}	1.30 ± 0.02^{b}
HT9	0.173 ± 0.002^{l}	3.18 ± 0.01^{f}	0.420 ± 0.018^{g}	1.94 ± 0.01^{f}
HT10	1.180 ± 0.001^{n}	3.27 ± 0.00^{h}	0.755 ± 0.035^{n}	2.65 ± 0.05^{i}
HT11	0.172 ± 0.001^{l}	3.18 ± 0.01^{f}	0.923 ± 0.066^{p}	3.76 ± 0.02^{l}
HT12	0.148 ± 0.002^{j}	3.24 ± 0.00^{h}	0.493 ± 0.027^{j}	2.35 ± 0.09^{h}
HT13	0.062 ± 0.003^{b}	3.19 ± 0.03^{f}	0.194 ± 0.005^{b}	1.38 ± 0.01^{c}
FT1	0.181 ± 0.007^{m}	3.18 ± 0.01^{f}	0.915 ± 0.062^{p}	3.77 ± 0.01^{l}
FT2	0.142 ± 0.003^{i}	3.20 ± 0.00^{g}	0.417 ± 0.008^{g}	2.01 ± 0.05^{g}
FT3	0.180 ± 0.001^{m}	3.17 ± 0.01^{f}	1.121 ± 0.002^{r}	3.93 ± 0.04^{m}
FT4	0.126 ± 0.003^{h}	2.72 ± 0.01^{b}	0.372 ± 0.021^{e}	1.85 ± 0.02^{f}
FT5	0.114 ± 0.005^{e}	3.23 ± 0.00^{h}	0.344 ± 0.009^{d}	1.73 ± 0.18^{e}
FT6	0.126 ± 0.002^{h}	2.66 ± 0.01^{ba}	0.491 ± 0.023^{j}	2.12 ± 0.04^{g}
FT7	0.140 ± 0.002^{i}	3.05 ± 0.00^{d}	0.636 ± 0.019^{l}	2.68 ± 0.06^{i}
FT8	0.122 ± 0.001^{g}	3.12 ± 0.01^{e}	0.349 ± 0.006^{d}	2.37 ± 0.08^{h}
FT9	0.138 ± 0.002^{i}	3.04 ± 0.01^{d}	0.537 ± 0.024^{k}	2.95 ± 0.13^{j}
FT10	0.107 ± 0.001^{d}	3.18 ± 0.01^{f}	0.477 ± 0.007^{i}	2.02 ± 0.07^{g}
FT11	0.034 ± 0.00^{a}	2.59 ± 0.03^{a}	0.301 ± 0.017^{c}	0.42 ± 0.02^{a}

Values are mean ± *SD* (n = 3). Values with different letters within columns are statistically different at $p < 0.05$ by paired Student t-test.

DPPH and ABTS scavenging activities are expressed in moles of Trolox equivalents per kilogram of tea sample. FRAP is expressed in moles of Fe^{2+} equivalents per kilogram of tea sample. RP is expressed in moles of ascorbic acid equivalents per kilogram of tea sample.

antioxidant activity of tea infusions. The DPPH, ABTS, FRAP and RP values of tea infusions are shown in Tab. 3. DPPH and ABTS assays are based on the ability of antioxidants to scavenge free radicals, while the FRAP and RP assays are based on the capacity of antioxidants to reduce ferric(III) ions to ferrous(II) ions. All tea infusion samples exhibited strong antioxidant activities. Similar to the polyphenols content, the antioxidant activity of tea infusions showed significant differences depending on tea type. DPPH radical-scavenging activity ranged from 0.034 mol·kg^{-1} for fruit to 1.180 mol·kg^{-1} for herbal tea infusions. Infusion of exotic tea, FT11, showed the lowest, and sage tea, HT10, infusion the highest DPPH radical-scavenging activity. ABTS activity varied from 2.66 mol·kg^{-1} for fruit to 3.27 mol·kg^{-1} for herbal tea infusions. The lowest and the highest ABTS radical cation-scavenging activities were found in exotic tea, FT11 and sage tea, HT10, respectively, which was consistent with the results of the DPPH radical-scavenging activity assay. It is known that polyphenols have a higher antioxidant and antiradical activity than monophenols. Among polyphenols, gallic acid and caffeic acid are the most efficient antiradical compounds [21]. The values obtained by the ABTS assay were consistently higher than those obtained by the DPPH assay. The same phenomenon was found in recent studies on antioxidant activity of guava fruit reported by Thaipong et al. [33], and in that on malting barley and beers by Zhao et al. [34, 35]. Actually, the ABTS radical cation-scavenging activity may also reflect hydrogen-donating ability. Tea infusions with a higher ABTS radical cation-scavenging activity may stabilize active oxygen radicals and have better flavour stability. Also, different reaction kinetics between phenol and the ABTS radical cation and DPPH radical over a similar range of contents might lead to the different results from two methods [36]. The antioxidant activity of tea infusions measured by the FRAP and RP assays ranged from 0.301 mol·kg^{-1} for fruit to 1.825 mol·kg^{-1} for herbal tea infusions, and from 0.42 mol·kg^{-1} for fruit to 7.45 mol·kg^{-1} for herbal tea infusions, respectively. Obtained results confirmed that the antioxidant power of green tea, GT and bearberry tea, HT1, is considerably higher than black tea, BT. Raspberry, FT3 and cherry, FT1 among fruit tea infusions showed the highest antioxidant power. Furthermore, the antioxidant power of all teas correlated strongly with the total phenolics content (FRAP, $R^2 = 0.92246$, $p < 0.0001$; RP, $R^2 = 0.88084$, $p < 0.0001$), indicating that the number of phenolic hydroxyl groups is a major determinant of the antioxidant power of tea. Previous studies also showed that the tea antioxidants with a greater number of phenolic hydroxyl groups had a greater antioxidant power, namely, epigallocatechin gallate (8 groups) > epicatechin gallate (7 groups) > epigallocatechin (6 groups) > epicatechin (5 groups) [37]. When the potential of all types of tea infusions is compared with vitamin C, one cup of the tested teas (150 ml of a 1.5% tea infusion) would supply as much antioxidant power as 150 mg of vitamin C.

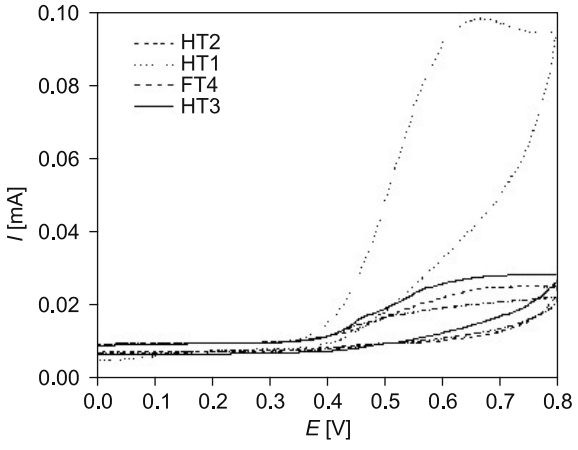

Fig. 2. Cyclic voltammograms of herbal (HT1, HT2, HT3) and fruit (FT2) tea infusions, taken to 800 mV at a scan rate of 100 mV·s^{-1}.

Cyclic voltammograms of tea infusions were obtained in the potential range 0–800 mV in order to cover all groups of antioxidant compounds (Fig. 2). The area under the anodic peak corresponds to the charge used up in the experiment up to the potential of 600 mV (Q_{600}), and is used as a measure of the content of the antioxidants present in the analysed sample, i.e. antioxidant capacity. All the peak potentials (E_p) and peak currents (I_p) determined from the cyclic voltammograms as well as Q_{600} and *TEAC* values of analysed tea infusions are presented in Tab. 4. From this table it is evident that green tea, GT had the highest *ACI*, followed by bearberry, HT1, black tea, BT and hibiscus, HT3, which is in accordance with its high total flavonoid contents. Among fruit tea infusions, raspberry, FT3 and cherry, FT1 had the highest *ACI*, followed by blueberry, FT7.

The *ACI* indices, along with the results from all five antioxidant capacity analyses (scaled to relative percentages) are shown in Tab. 5. The highest *ACI* has green tea, GT, followed by the bearberry, HT1 and black tea, BT, which is in accordance with their high total phenol contents and antioxidant capacity obtained by FRAP, RP and

Tab. 4. Peak potential (E_p) and currents (I_p) from cyclic voltammograms of black, green, herbal and fruit tea infusions.

Tea	E_p [V]			I_p [μA]			Q_{600} [μC]	*TEAC*
	1	2	3	1	2	3		
BT	0.46	0.55	0.69	8.73	16.5	21.23	33.6	72
GT	0.41	0.53	0.69	3.72	10.83	17.75	46.8	100
HT1	–	–	0.67	–	–	51.14	45.7	98
HT2	–	0.53	0.69	–	9.85	13.52	21.4	46
HT3	–	0.55	–	–	6.87	–	0.4	0.8
HT4	–	–	0.66	–	–	24.31	10.8	23
HT5	–	0.53	0.68	–	8.74	14.52	6.87	15
HT6	–	–	–	–	–	–	0	0
HT7	–	–	0.66	–	–	98.16	32.7	70
HT8	–	0.56	0.66	–	11.12	18.35	1.1	2
HT9	0.43	0.57	0.68	5.15	10.83	15	5.88	13
HT10	–	0.53	0.68	–	6.95	11.83	0.5	1
HT11	–	0.52	0.69	–	8.14	11.64	1.21	3
HT12	–	–	0.68	–	–	21.14	5.14	11
HT13	–	0.53	0.67	–	6.69	8.92	1	2
FT1	–	–	0.66	–	–	20.71	3.68	84
FT2	0.44e	0.57	–	5.68	11.9	–	2.79	63
FT3	0.45	0.55	0.68	7.95	13.12	16.97	4.38	100
FT4	0.44	0.54	0.67	5.7	10.01	12.71	2.94	67
FT5	0.44	0.54	0.69	6.47	10.07	12.51	2.13	49
FT6	0.44	0.54	0.69	5.71	9.88	13.16	2.63	60
FT7	0.45	0.55	0.69	7.45	13.1	16.14	3.55	81
FT8	0.45	0.55	0.68	6.02	10.2	13.16	2.36	54
FT9	0.43	–	0.66	5.32	–	17.8	2.94	67
FT10	0.44	0.54	0.65	4.96	–	14.7	2.89	61
FT11	–	0.54	0.69	–	9.64	14.24	2.13	49

CV assays. Fruit teas, raspberry, FT3 and cherry, FT1, ranked the highest on the *ACI* scale, which is also in accordance with total phenol contents and values from FRAP, RP and CV antioxidant assays.

In order to quantify the contribution of individual phenolic compounds to tea antioxidant activity, stepwise linear regression was used. Thirteen independent variables (contents of individual phenolic compounds identified in tea) were used to explain variability for the dependent variable (antioxidant activity determined by different assays). Data on the contents of protocatechuic acid, rutin, morin, quercetin, (–)-epigallocatechin gallate, procyanidin B3 did not help to explain the variation in tea antioxidant activity in the current study. On the other hand, gallic acid, caffeic acid, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, procyanidin B1, procyanidin B2 were found to make significant ($p < 0.05$) contributions to the antioxidant activity of tea. Stepwise linear regression (partial R^2 and cumulative R^2) showed that procyanidin B2 alone was able to explain 52.9% of the variation in DPPH radical-scavenging activity observed in this study. Sequential addition of procyanidin B1 and (+)-catechin increased this to 78.3% and 86.9%, respectively. For ABTS radical cation-scavenging activity, caffeic acid was the most important factor that alone explained 43.6% of the observed variation. Sequential addition of (+)-catechin increased the predictive value of the model to 53.8%. Furthermore, (–)-epicatechin and (+)-catechin were able to explain 83.6% and 88.3% of the total variation in ferric reducing antioxidant power (FRAP) and reduction power (RP).

Tab. 5. Antioxidant potency composite index (*ACI*) of herbal and fruit tea infusions calculated from five antioxidant capacity measures scaled to relative percentages.

Tea	DPPH index	ABTS index	FRAP index	RP index	Q_{600} index	*ACI*
BT	15.3	98	43.2	34.5	71.8	52.6
GT	15.3	98.2	100.0	100.0	100.0	82.7
HT1	15.0	97.6	94.5	97.6	97.6	80.4
HT2	14.7	96.9	24.0	21.1	45.7	40.5
HT3	10.5	97.8	24.9	18.0	0.8	30.4
HT4	14.7	98.5	26.4	27.7	23.1	38.1
HT5	14.8	99.1	38.1	29.5	14.7	39.2
HT6	7.1	91.1	6.9	32.2	0.0	27.5
HT7	13.8	98.1	62.9	47.2	69.9	58.4
HT8	10.0	92.7	22.1	17.4	2.3	28.9
HT9	14.7	97.2	23.0	26.0	12.6	34.7
HT10	100.0	100.0	41.4	35.6	1.1	55.6
HT11	14.6	97.2	50.6	50.5	2.6	43.1
HT12	12.5	99.1	27.0	31.5	11.0	36.2
HT13	5.2	97.6	10.6	18.5	2.1	26.8
FT1	100.0	98.5	81.6	95.9	84.0	92.0
FT2	78.4	99.1	37.2	51.1	63.7	65.9
FT3	99.4	98.1	100.0	100.0	100.0	99.5
FT4	69.7	84.2	33.2	47.1	67.1	60.3
FT5	63.0	100.0	30.7	44.0	48.6	57.3
FT6	69.6	82.4	43.8	53.9	60.0	61.9
FT7	77.3	94.4	56.7	68.2	81.1	75.5
FT8	67.4	96.6	31.1	60.3	53.9	61.9
FT9	76.2	94.1	47.9	75.1	67.1	72.1
FT10	59.1	98.5	42.6	51.4	66.0	63.5
FT11	18.8	80.2	26.9	10.7	48.6	37.0

For antioxidant activity obtained from CV, (–)-epicatechin was the most important factor, since it alone explained 66.8% of the observed variation. Sequential addition of (+)-catechin, (–)-epigallocatechin procyanidin B2, gallic acid and procyanidin B1 increased this to 84.5%, 96.8%, 98.7%, 99.7% and 99.9%, respectively.

CONCLUSIONS

The total polyphenol content as well as determination of individual phenolic compounds and antioxidant activity are parameters of quality of tea regarding its biological properties. This research demonstrates clear evidence of in vitro antioxidant activity of the tested tea infusions. Green tea had the highest antioxidant capacity followed by bearberry and black tea. Among fruit tea infusions, raspberry and cherry showed the highest antioxidant capacities. Furthermore, results from stepwise regression analysis showed that antioxidant activity of tea can mainly be attributed to their phenolic constituents. The contribution of phenolic compounds indentified in this study to tea antioxidant activity was between 43.6% and 99.9%, which depended on the methods of antioxidant activity evaluation. Regarding the important place that tea infusions have as a popular beverages, the obtained results could be applied for the quality control of the manufacturer. It would also be useful for consumers to make good choices and to protect their interests, because today they are challenged by an expanding choice of products.

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