

Comparison of Infusions from Black and Green Teas (*Camellia sinensis* L. Kuntze) and *Erva-mate* (*Ilex paraguariensis* A. St.-Hil.) Based on the Content of Essential Elements, Secondary Metabolites, and Antioxidant Activity

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Abstract The aim of the research was to compare chemical composition and antioxidant activity of black and green teas with those of *erva-mate*. Therefore, essential elements (P, Mg, Fe, Zn, Mn, and Cu), phenolic acids (gallic, caffeic, *p*-coumaric, and ferulic), flavonoids (catechin, rutin, myricetin, apigenin, and quercetin), and alkaloids (caffeine, theobromine, and theophylline) were determined. Phenolics and alkaloids were quantified by HPLC, metallic elements by atomic absorption spectrometry, and antioxidant activity by a 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid method. Total P, inorganic P, and Mg expressed in milligrams per gram dry weight (d.w.) and other metals expressed in milligrams per kilogram d.w.) in the order Mn > Zn > Fe > Cu were assayed. Caffeine was found above 25 mg/g d.w. in all samples. Among the phenolic compounds, caffeic acid and quercetin were on the highest level, whilst apigenin and *p*-coumaric acid were on the lowest. Correlation analysis revealed frequently occurring relations between P and several metals and phenolics and also between Zn and other metabolites from flavonoids, phenolic acids, and alkaloids. Within organic compounds, correlations were often found among gallic and caffeic acids and other analytes, like *p*-coumaric, ferulic acids, and myricetin. Antioxidant activity of studied infusions from different types of teas was very similar. Cluster analysis grouped the studied materials into clear clusters, generally separating *erva-mate* samples from *Camellia sinensis*. Principal component analysis indicated total P, Mg, Cu,

caffeic acid, myricetin, and *p*-coumaric acid as factors influencing mostly the diversity among analyzed samples.

Keywords *Camellia sinensis* (L.) Kuntze · *Ilex paraguariensis* A. St.-Hil. · Essential elements · Secondary metabolites · Antioxidant activity

Introduction

Infusions prepared from green and black teas (*Camellia sinensis* (L.) Kuntze) have become very popular beverages among populations of many countries worldwide. Main exporters of tea are China, India, Turkey, Iran, and other Southern Asian countries (Salahinejad and Aflaki 2010). Beneficial effects of tea drinking for human health are numerous, including, for example, its stimulating influence on the brain; protective action against various types of cancers, like lung, prostate, and breast tumors; reduction of cholesterol levels in the blood; and also boosting the immune system. This positive impact on the human organism is caused by antioxidant properties of polyphenolic compounds that are present in tea infusions, among them phenolic acids (Ronowicz et al. 2014; Salahinejad and Aflaki 2010). Other chemical constituents in *C. sinensis* tea consist of volatile compounds, alkaloids, vitamins, carbohydrates, amino acids, proteins, chlorophyll, fluoride, minerals, and trace elements (Carbera et al. 2003). Polyphenols are compounds of great interest because they have potent antioxidant activity both in vitro and in vivo due to its reducing properties (Wu and Wei 2002). Among flavonoids, quercetin and myricetin are observed in considerable quantities in tea of *C. sinensis* (Saito and Miyata 2000).

However, especially in Southern America societies, common infusions of *erva-mate* in Brazil, also known as *yerba-*

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mate in Spanish language countries, are consumed. Erva-mate is a traditional beverage drunk by South American cowboys (*gauchos*) and is obtained from a shrub, *Ilex paraguariensis* A. St.-Hil. After Paraguay, Brazil is the second producer of erva-mate in the world (Neto et al. 2007). Methylxanthines, represented by caffeine, are among the compounds present in erva-mate (Bojic et al. 2013). Moreover, infusions prepared from erva-mate can influence human well-being; for instance, they can stimulate antirheumatic and anti-inflammatory and diuretic activities (Jacques et al. 2007).

Due to high consumption of these beverages worldwide, there are many studies, in which the chemical composition of tea and erva-mate infusions was examined. For example, the chemical composition of erva-mate samples was studied in order to detect differences in the level of essential elements in the samples harvested in seven locations in Southern Brazil (Reissmann et al. 1999). Toxic and nutrient elements were analyzed by ICP-OES in samples of erva-mate from different countries (Argentina, Brazil, Paraguay, and Uruguay) (Pozebon et al. 2015). Erva-mate infusions were also analyzed in order to detect differences in fluoride contents in samples originating from various countries (Lukomska et al. 2015).

Green tea leaves from *C. sinensis* among other herbal teas obtained from peppermint, *Echinacea*, red clover, Siberian ginseng, dandelion, red raspberry, and blueberry leaves were also investigated (Gallaher et al. 2006). Mineral contents of teas from several botanical species available in Brazil were also analyzed by XRF spectrometry (Wastowski et al. 2013).

Mineral composition, including assays of micro- and macro-elements in herbal teas obtained from mint and mixture of chamomile and lavender leaves, was studied, and it was found that metal bioavailability was significantly different depending on the element analyzed (Szymczycha-Madeja et al. 2013). Recently, the effect of the origin of medicinal herbs from distant regions of Europe on the contents of essential elements in infusions obtained from them was investigated (Konieczynski et al. 2016).

Taking all the above into account, the commercially available samples of tea (black, green) and erva-mate from different regions of the world were studied regarding their mineral and secondary plant metabolites (phenolics and alkaloids) and antioxidant activities. In the work, flame atomic absorption spectrometry (FAAS) was used to determine metallic essential elements (Fe, Mg, Zn, Mn, and Cu), HPLC-UV for phenolic compounds (gallic, caffeic, *p*-coumaric, and ferulic acids; rutin; myricetin; quercetin; apigenin; and catechin), and alkaloids (theobromine, theophylline, and caffeine), and UV/Vis spectrophotometry for determination of antioxidant activity (2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid, ABTS), total phosphorus (P), and P-PO₄. In order to detect similarities and differences among the studied tea and erva-mate samples, multivariate methods, such as cluster and principal component analyses, were applied.

Materials and Methods

Tea Material and Sample Preparation

The samples of tea and erva-mate under analysis are listed in Table 1. The infusions were prepared by pouring boiling redistilled water (100 mL) into 1.0 g of a tea material. After 20 min of brewing under cover, infusions were filtered through paper filters (Filtrak No. 389, Germany) and diluted to 100 mL using redistilled water, obtained in a Heraeus (Switzerland) distillation system.

Prior to HPLC analysis, the infusions were filtered through a 0.20- μ m nylon membrane filter (Witko, Łódź, Poland) into a HPLC vial. For each tea sample, the completed procedure was repeated in triplicate.

Reagents

Standards of four phenolic acids [gallic acid (GA), caffeic acid (CA), *p*-coumaric acid (*p*-CA), and ferulic acid (FA)], five flavonoids [catechin (CAT), rutin (RUT), quercetin (QUE), myricetin (MYR), and apigenin (API)], and three alkaloids [theobromine (TBR), theophylline (TPI), and caffeine (CAF)] were purchased from ChromaDex (CA, USA). HPLC grade methanol was obtained from POCh (Gliwice, Poland), and trifluoroacetic acid (TFA) was from Sigma-Aldrich (St. Louis, MO, USA).

Phosphorus Determinations

Total and extractable phosphorus were determined using the phosphate-molybdate complex ($\lambda = 650$ nm) (Konieczynski et al. 2016). Absorbance was measured using a Metertech SP-870 (South Korea) UV/Vis spectrometer.

ABTS Radical Scavenging

The antioxidant capacity on ABTS was assessed by adapting the method described by Re et al. (1999). A solution of 55 μ M ABTS was prepared in 10 mM phosphate buffer (pH 7.0), and infusions were diluted (1:1, 1:10, and 1:100) with water. The tests were performed by adding three different volumes of each diluted sample (10, 25, and 50 μ L), ABTS solution (500 μ L), and phosphate buffer to a final volume of 1 mL. The absorbance was taken after 30 min at 734 nm, and antioxidant activity was expressed by half maximal inhibitory concentration (IC₅₀) values.

Essential Element Determination

Essential metallic elements in infusions were assayed using standard analytical conditions (air/acetylene), by a flame atomic absorption spectrometer (250 Plus; Varian,

Table 1 Studied plant material

| No. | Studied material | Botanical plant species | Country of origin | Company (manufacturer) | Importer |
|------|---|---|-------------------|---|--|
| EM1 | <i>Erva-mate</i> (<i>yerba mate</i>) | <i>Ilex paraguariensis</i> St.-Hil. | Brazil | Chimarrão Dos Pampas, Fernandes Pinheiro, PR | Leão Junior S.A., Brazil |
| EM2 | | | Argentina | Taragui Sin Palo, Establecimiento Las Marias | Oranzada Robert Denatka, Otwock, Poland |
| EM3 | | | Argentina | Union Suave, Establecimiento Las Marias | |
| GT4 | Green tea | <i>Camellia sinensis</i> (L.) Kuntze | China | Chinese company | BiFIX W. Piasecki Sp. j. Górki Małe, Poland |
| GT5 | | | China | Chinese company | |
| GT6 | | | Japan | Japanese company | “Jahmajka” Sopot, Poland |
| BT7 | Black tea | <i>Camellia sinensis</i> (L.) Kuntze | Sri Lanka | MUF Holdings, Ltd. | Gourmet Foods, Sp. z o.o., Warszawa, Poland |
| BT8 | Black tea “Tippy Milima” | | Kenya | Kenyan company | “Jahmajka” Sopot, Poland |
| BT9 | Black tea Darjeeling | | India | Teesta Valley | |
| BT10 | Black tea | | China | Yunnan | |
| BT11 | Finest Oolong | | China, Taiwan | Chinese company | |

Australia), and the appropriate analytical wavelengths (nm) were 285.2 (Mg), 248.3 (Fe), 213.9 (Zn), 279.5 (Mn), and 324.8 (Cu). The tea and erva-mate samples were diluted 100 times before Mg determination, and to each diluted sample, 1% solution of La₂O₃ was added prior to measurement by FAAS. The analytical procedures used in the study have been validated recently, and the obtained data of linearity, accuracy, recovery, LOD, and LOQ were satisfactory (Konieczynski et al. 2015, 2016).

Secondary Metabolites Determinations

Phenolic compounds were determined using a HPLC LaChrom (Merck, Darmstadt, Germany) system consisting of L-7100 pump, L-7360 column compartment, and L-7420 UV detector. The separation was performed on a Hypersil Gold C18 column (250 × 4.6 mm, 5-μm particles) (Thermo Scientific, Runcorn, UK), maintained at 30 °C using methanol-0.1% TFA solution (solvent A) and water-0.1% TFA solution (solvent B) as mobile phase. The HPLC mobile phase was prepared fresh daily, filtered through a 0.45-μm membrane filter, and then degassed before injection. A gradient program was chosen as follows: 0–40 min, linear 5–25% A; 40–50 min, linear 25–35% A; 50–55 min, isocratic 35% A; 55–60 min, linear 35–65% A; and 60–70 min, linear 65–5% A. The flow rate of mobile phase was 1.0 mL/min with an injection volume of 20 μL, and the runs were monitored at 280, 320, and 370 nm for benzoic and cinnamic acid derivatives, and flavonoids, respectively. The phenolic compounds and alkaloids were characterized according to their retention time and comparison with standards.

For quantitative analysis, a 6-point calibration curve was obtained by injection of known concentrations of different standards compounds: GA (10–130 μg/mL; $y = 44,647x - 221,211$; $R^2 = 0.9960$), CA (10–130 μg/mL; $y = 80,278x + 1673$; $R^2 = 0.9980$), *p*-CA (10–130 μg/mL; $y = 11,320x - 135,814$; $R^2 = 0.9950$), FA (10–130 μg/mL; $y = 119,637x + 818,017$; $R^2 = 0.9960$), CAT (10–130 μg/mL; $y = 4327x + 8602$; $R^2 = 0.9980$), RUT (10–130 μg/mL; $y = 72,071x + 554,160$; $R^2 = 0.9890$), MYR (10–130 μg/mL; $y = 12,740x - 2726$; $R^2 = 0.9970$), QUE (40–200 μg/mL; $y = 12,372x - 230,867$; $R^2 = 0.9985$), API (10–130 μg/mL; $y = 34,654x + 19,888$; $R^2 = 0.9970$), TBR (40.32–201.16 μg/mL; $y = 131,645x - 61,751$; $R^2 = 0.9951$), TPI (40.53–202.66 μg/mL; $y = 44,505x - 654,602$; $R^2 = 0.9983$), and CAF (40–200 μg/mL; $y = 72,600x - 1,777,442$; $R^2 = 0.9926$).

Statistical Calculations

All the assays were carried out in triplicate, and the results are expressed in milligrams per gram of dry weight (d.w.) as mean values ± standard deviation (SD). Analysis of variance (ANOVA) test, followed by Tukey’s and Duncan’s tests, was performed to check significant differences between samples. A Pearson correlation was used to assess the relationship between metals, phenolics, alkaloids, and antioxidant activity. Calculations were performed using Statistica 10 software (StatSoft, Inc., USA) on the basis of parametric tests with the level of significance of $p < 0.05$. Moreover, principal component analysis (PCA) and cluster analysis (CA) were used to interpret the obtained results.

Results and Discussion

Essential Elements

The concentration of total P was found in the range from 1.06 to 2.87 mg/g d.w. and that of its water-extractable inorganic form (P_i) from 0.38 to 1.17 mg/g d.w., as shown in Table 2. Total P was assayed in higher amounts in green and black tea leaves than that determined in erva-mate samples. On the other hand, P_i level was higher in black tea and erva-mate samples originating from Brazil (EM1).

Magnesium was determined in all studied tea and erva-mate samples in the similar range of concentrations, from 1.06 mg/g d.w. in case of green tea (GT4) to 2.14 mg/g d.w. in black tea (BT9). It can be noticed that Mg level in all erva-mate samples is practically the same, about 1.70 mg/g d.w., slightly higher than its concentration in tea samples. These results are consistent with the data found in the literature (Reissmann et al. 1999; Gallaher et al. 2006; Szymczycha-Madeja et al. 2013; Pohl et al. 2016), and the level of P and Mg determined in the studied samples of teas and erva-mate represents a quite similar level of concentrations.

In Table 2, the results of determinations of microelements (Fe, Mn, Zn, and Cu) are presented, too. They followed the order $Mn > Zn > Fe > Cu$. The quantities of metallic elements assayed in the study are in agreement with the values found in other research on tea, erva-mate, or medicinal herb infusions (Pozebon et al. 2015; Polechonska et al. 2015; Salahinejad and Aflaki 2010; Konieczynski et al. 2015, 2016). However, the level of Zn and Cu is higher in all erva-mate samples than that determined in black and green teas, as confirmed by post hoc Tukey's test. For Mn and Fe results, differences between the samples of black and green teas, as well as between teas and erva-mate samples under investigation, appeared to be not significant.

Phenolic Compounds and Alkaloids

Table 3 shows the results of HPLC determination of selected phenolic compounds (namely GA, CA, *p*-CA, and FA), five flavonoids (CAT, RUT, QUE, MYR, and API), as well as three alkaloids (TBR, TPI, and CAF), along with antioxidant activity in the samples of green and black teas and erva-mate. In all samples, CAF is the compound present at a higher concentration (mean concentration is on the level of 34.30 mg/g d.w.), whilst API and *p*-CA were found at the lowest mean concentration, 1.00 and 0.23 mg/g d.w., respectively. Among analyzed teas, erva-mate was the poorest in the most compounds, not counting CA, which was found at the highest level (8.95 mg/g d.w.). Black teas were the richest in alkaloids, whilst green teas in phenolic compounds, as shown in Fig. 1.

The range of concentrations (mg/g d.w.) of phenolic compounds determined in this investigation is consistent with that

Table 2 Results of determinations of elements in the studied samples (mg/g d.w. for P, P_i , and Mg; for other elements, mg/kg d.w.)

| | EM1 | EM2 | EM3 | GT4 | GT5 | GT6 | BT7 | BT8 | BT9 | BT10 | BT11 |
|-------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| P | 1.44 ± 0.14 ^a | 1.12 ± 0.09 ^a | 1.06 ± 0.04 ^a | 1.66 ± 0.15 ^b | 2.14 ± 0.01 ^b | 1.96 ± 0.01 ^b | 2.20 ± 0.09 ^b | 2.05 ± 0.01 ^b | 2.87 ± 0.01 ^b | 1.77 ± 0.01 ^b | 1.94 ± 0.01 ^b |
| P_i | 0.76 ± 0.03 ^a | 0.56 ± 0.06 ^a | 0.53 ± 0.01 ^a | 0.52 ± 0.04 ^a | 0.50 ± 0.01 ^a | 0.38 ± 0.01 ^a | 1.04 ± 0.07 ^b | 0.63 ± 0.01 ^b | 1.17 ± 0.02 ^b | 0.96 ± 0.02 ^b | 0.97 ± 0.01 ^b |
| Mg | 1.73 ± 0.01 ^a | 1.71 ± 0.04 ^a | 1.75 ± 0.01 ^a | 1.06 ± 0.01 ^a | 1.56 ± 0.04 ^a | 1.61 ± 0.01 ^a | 1.37 ± 0.02 ^a | 1.83 ± 0.01 ^a | 2.14 ± 0.01 ^a | 1.83 ± 0.01 ^a | 1.81 ± 0.01 ^a |
| Fe | 16.70 ± 2.86 ^a | 6.40 ± 1.79 ^a | 14.87 ± 2.43 ^a | 10.81 ± 1.10 ^a | 12.03 ± 0.13 ^a | 12.46 ± 0.04 ^a | 4.07 ± 0.58 ^a | 15.42 ± 0.31 ^a | 11.16 ± 0.26 ^a | 9.75 ± 0.09 ^a | 14.50 ± 0.25 ^a |
| Mn | 641.0 ± 1.0 ^a | 819.5 ± 1.0 ^a | 1016.5 ± 0.5 ^a | 424.5 ± 1.0 ^a | 696.0 ± 2.5 ^a | 684.2 ± 2.0 ^a | 724.8 ± 0.6 ^a | 722.0 ± 0.5 ^a | 428.5 ± 0.5 ^a | 720.0 ± 0.5 ^a | 651.5 ± 0.5 ^a |
| Zn | 91.85 ± 5.68 ^a | 78.10 ± 4.74 ^a | 75.01 ± 4.24 ^a | 20.94 ± 1.45 ^b | 21.13 ± 0.05 ^b | 19.37 ± 0.18 ^b | 25.63 ± 0.66 ^b | 24.41 ± 0.32 ^b | 30.55 ± 0.11 ^b | 24.73 ± 0.15 ^b | 25.56 ± 0.10 ^b |
| Cu | 1.86 ± 0.05 ^a | 1.82 ± 0.07 ^a | 1.93 ± 0.05 ^a | 1.63 ± 0.02 ^b | 1.50 ± 0.01 ^b | 1.36 ± 0.06 ^b | 1.64 ± 0.05 ^b | 2.03 ± 0.01 ^b | 1.86 ± 0.01 ^b | 1.74 ± 0.01 ^b | 1.80 ± 0.01 ^b |

The arithmetic mean ± SD is presented ($n = 6$). Mean values by the same letter within a row do not differ significantly according to Tukey's post hoc test at $\alpha < 0.05$

Table 3 Results of determinations of phenolic compounds and alkaloids (mg/g d.w.) and antioxidant activity in the studied samples

| | EM1 | EM2 | EM3 | GT4 | GT5 | GT6 | BT7 | BT8 | BT9 | BT10 | BT11 |
|------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| GA | 0.49 ± 0.09 ^a | 0.48 ± 0.01 ^a | 0.45 ± 0.01 ^a | 0.53 ± 0.02 ^c | 1.20 ± 0.43 ^f | 1.11 ± 0.54 ^e | 0.78 ± 0.03 ^d | 3.19 ± 0.16 ^g | 3.73 ± 0.91 ^h | 3.46 ± 0.32 ^b | 3.40 ± 0.12 ^b |
| CAT | 3.69 ± 0.07 ^c | 4.09 ± 0.45 ^f | 4.24 ± 0.22 ^c | 6.71 ± 0.13 ^g | 0.93 ± 0.23 ^b | 2.61 ± 0.08 ^e | 0.50 ± 0.23 ^a | 0.48 ± 0.32 ^d | 1.74 ± 0.32 ^d | 0.61 ± 0.54 ^{ab} | 0.93 ± 0.75 ^b |
| CA | 6.92 ± 2.22 ^c | 9.26 ± 2.79 ^d | 9.96 ± 0.22 ^c | 7.71 ± 0.03 ^c | 3.45 ± 0.14 ^{ab} | 3.39 ± 0.23 ^{ab} | 5.66 ± 0.55 ^b | 1.03 ± 0.45 ^a | 2.19 ± 0.08 ^{ab} | 2.80 ± 0.04 ^{ab} | 3.02 ± 0.12 ^{ab} |
| p-CA | 0.06 ± 0.01 ^a | 0.07 ± 0.01 ^a | 0.09 ± 0.00 ^d | 0.12 ± 0.01 ^e | 0.31 ± 0.09 ^b | 0.37 ± 0.05 ^b | 0.09 ± 0.01 ^c | 0.39 ± 0.65 ^f | 0.33 ± 0.74 ^f | 0.34 ± 1.09 ^g | 0.31 ± 0.97 ^b |
| FA | 1.12 ± 0.00 ^d | 1.32 ± 0.01 ^f | 1.48 ± 0.02 ^e | 1.09 ± 0.07 ^c | 2.29 ± 0.06 ^c | 2.34 ± 0.54 ^f | ND ^b | 2.18 ± 0.69 ^g | 2.25 ± 0.99 ^g | 2.20 ± 1.07 ^h | 2.28 ± 1.06 ^a |
| RUT | 1.41 ± 0.01 ^f | 1.92 ± 0.04 ^b | 2.26 ± 0.02 ^f | 1.41 ± 0.02 ^f | 0.70 ± 0.05 ^a | 2.46 ± 1.00 ^b | ND ^c | 1.86 ± 0.89 ^h | 1.17 ± 0.87 ^e | 1.56 ± 0.12 ^g | 0.54 ± 0.05 ^d |
| MYR | 4.40 ± 0.01 ^c | 5.96 ± 0.46 ^d | 8.11 ± 1.16 ^c | 4.39 ± 0.03 ^a | 0.32 ± 0.11 ^a | 3.21 ± 1.35 ^b | 0.26 ± 0.03 ^a | 0.78 ± 0.08 ^a | 0.44 ± 0.34 ^a | 0.18 ± 0.45 ^a | 0.83 ± 0.75 ^a |
| QUE | 8.16 ± 0.64 ^f | 1.93 ± 0.23 ^a | 7.74 ± 0.82 ^f | 2.19 ± 0.32 ^c | 4.11 ± 0.64 ^f | 3.28 ± 0.97 ^g | 2.27 ± 0.50 ^d | 2.50 ± 0.56 ^e | 1.92 ± 0.95 ^f | 2.65 ± 1.08 ^f | 1.84 ± 0.76 ^b |
| API | 1.17 ± 0.05 ^{ab} | 1.28 ± 0.13 ^{ef} | 1.32 ± 0.01 ^{bd} | 1.21 ± 0.06 ^{ab} | 0.83 ± 0.54 ^c | 1.45 ± 0.76 ^f | 1.14 ± 0.04 ^a | 0.18 ± 0.92 ^g | 0.90 ± 0.21 ^c | 0.29 ± 0.54 ^h | 1.32 ± 0.87 ^{de} |
| TBR | 2.52 ± 0.98 ^h | 6.96 ± 0.21 ^j | 3.66 ± 0.09 ⁱ | 0.13 ± 0.01 ^a | 0.14 ± 0.06 ^c | 0.21 ± 0.04 ^b | 0.48 ± 0.03 ^f | 0.23 ± 0.65 ^c | 0.45 ± 0.02 ^e | 0.49 ± 0.10 ^g | 0.28 ± 0.04 ^d |
| TPI | 1.54 ± 0.65 ^{bc} | 1.65 ± 0.05 ^g | 1.55 ± 0.22 ^c | 1.52 ± 0.35 ^d | 1.57 ± 0.38 ^d | 1.53 ± 0.14 ^{ab} | 1.59 ± 0.44 ^e | 1.57 ± 0.12 ^d | 1.59 ± 0.13 ^e | 1.72 ± 0.16 ^h | 1.62 ± 0.23 ^f |
| CAF | 25.83 ± 2.45 ^b | 44.53 ± 3.76 ^f | 26.32 ± 2.08 ^c | 34.26 ± 1.59 ^a | 34.26 ± 3.38 ^a | 36.24 ± 4.50 ^g | 33.31 ± 2.55 ^e | 30.53 ± 2.12 ^d | 39.47 ± 1.22 ⁱ | 36.52 ± 3.29 ^h | 35.86 ± 2.43 ^f |
| IC50 | 6.59 ± 0.02 ^a | 6.41 ± 0.01 ^b | 6.54 ± 0.02 ^a | 6.80 ± 0.03 ^c | 6.42 ± 0.01 ^b | 6.60 ± 0.02 ^a | 6.72 ± 0.01 ^d | 6.85 ± 0.03 ^e | 6.73 ± 0.01 ^d | 6.78 ± 0.01 ^d | 6.70 ± 0.01 ^d |

The arithmetic mean ± SD is presented (*n* = 6). IC50 values for ABTS radical scavenging of tea infusions are shown. Mean values by the same letter within a row do not differ significantly according to Duncan's multiple test at $\alpha < 0.05$

ND not detected

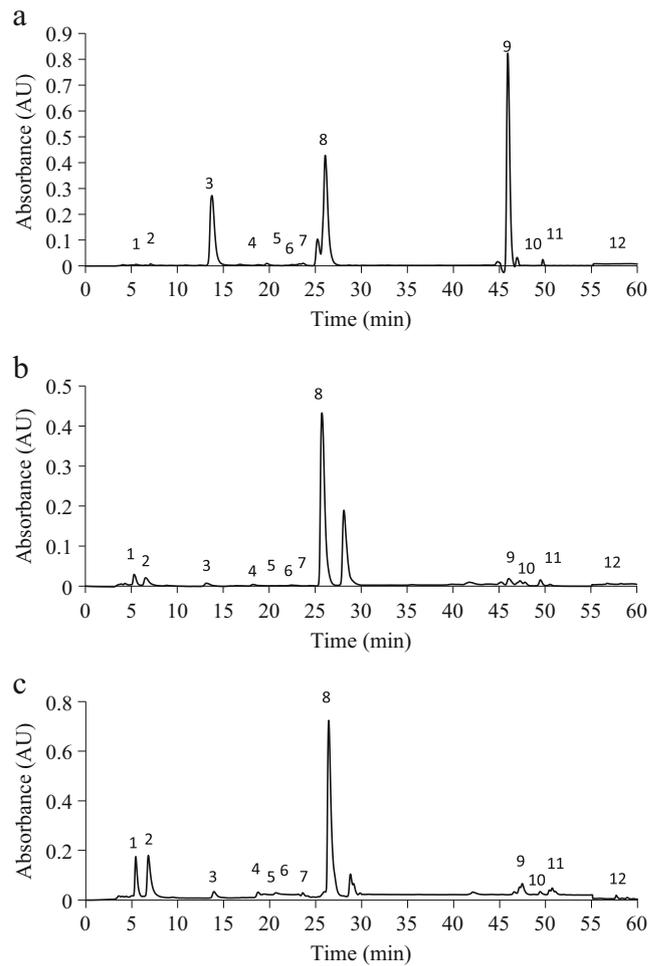


Fig. 1 The HPLC chromatograms of infusions. **a** *Erva-mate*. **b** Green tea. **c** Black tea. Peak identification: 1 gallic acid, 2 catechin, 3 theobromine, 4 theophylline, 5 *p*-coumaric acid, 6 caffeic acid, 7 ferulic acid, 8 caffeine, 9 myricetin, 10 quercetin, 11 rutin, 12 apigenin

reported in the literature concerning studies of phenolics in *erva-mate* (Bojic et al. 2013) and herbal teas (da Silva Port's et al. 2013). Veljković et al. (2013) determined individual phenols in black and green tea infusions and found that green teas are richer in phenolic compounds than black teas. In the green teas, the mean content of GA, CA, RUT, and CAT was on the level of 1.98, 0.261, 1.091, and 0.40 mg/g, respectively, whilst in the black teas, their mean content was on the level of 1.34, 0.195, 1.485, and 0.263 mg/g, respectively. The content of RUT is in agreement with that in this study but higher in the case of other analytes obtained in this study. Manchón et al. (2013) determined phenolic compounds and alkaloids in fermented green teas and found GA, CAF, TBR, TPI, and CAT on the same level as in this study, whilst CA and *p*-CA were on the level below the detection. In unfermented green teas, the concentration of CAT (in the range of 0.29 to 6.28 mg/g) was in agreement with that obtained in this study. The level of CAF (from 15.4 to 26.8 mg/g) was slightly lower, and the levels of TBR (from 0.40 to

Table 4 Results of correlation analysis

| | P | Fe | Zn | GA | CA | <i>p</i> -CA | FA | MYR | QUE | IC50 |
|----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|--------------|--------------|
| P _i | | | | <i>0.61</i> | | | | | | |
| Mg | | | | <i>0.66</i> | | | | | | |
| Mn | <i>-0.63</i> | | | | | | | | | |
| Zn | <i>-0.70</i> | | | | | | | | <i>0.70</i> | |
| GA | <i>0.61</i> | | | | | | | | | |
| CA | <i>-0.79</i> | | <i>0.71</i> | <i>-0.82</i> | | | | | | |
| <i>p</i> -CA | <i>0.62</i> | | <i>-0.71</i> | <i>0.78</i> | <i>-0.91</i> | | | | | |
| FA | <i>0.71</i> | | <i>-0.70</i> | <i>0.73</i> | <i>-0.86</i> | <i>0.95</i> | | | | |
| MYR | <i>-0.83</i> | | <i>0.74</i> | <i>-0.69</i> | <i>0.88</i> | <i>-0.66</i> | <i>-0.77</i> | | | |
| API | | | | <i>-0.61</i> | <i>0.61</i> | | | | | |
| CAT | | | | <i>-0.64</i> | <i>0.77</i> | <i>-0.62</i> | <i>-0.87</i> | <i>0.81</i> | | |
| CAF | | <i>-0.62</i> | | | | | | | <i>-0.78</i> | |
| TBR | <i>-0.72</i> | | <i>0.83</i> | | <i>0.74</i> | <i>-0.63</i> | | <i>0.73</i> | | <i>-0.63</i> |

The values of statistically significant correlation coefficients are shown in italic font

2.37 mg/g) and GA (from 0.62 to 5.65 mg/g) were slightly higher than the results obtained in this work. Lee et al. (2014) determined TBR, CAF, and CAT in green teas and found TBR in the range from 2.83 to 8.81 mg/g, CAF from 26.74 to 29.54 mg/g, and CAT from 0.99 to 2.48 mg/g. These concentrations are slightly lower than those obtained in this study. The differences in phenolic composition could be dependent on biological and environmental factors (Ksouri et al. 2008). Besides, there are different reports of major compound changes in green tea depending on the leaf age (Lee et al. 2014).

Antioxidant Activity

Antioxidant activity of the studied teas and erva-mate samples was assessed by an ABTS radical scavenging method (Re et al. 1999), and the results expressed as IC50 values are also presented in Table 3. Samples that have lower IC50 values

correspond to those that have stronger antioxidant activity. IC50 values showed that antioxidant activities of analyzed infusions from different types of teas are very similar, because the values ranged from 6.41 µg/mL for EM2 to 6.85 µg/mL in the case of BT8. In general, IC50 values represent a slightly higher level than the ones found for tea prepared from *I. paraguariensis* (Fernandes et al. 2016). These differences can be due to the fact that in this study, aqueous extracts were used to determine antioxidant activity, whereas the other researchers applied ethanolic/aqueous and ethanolic solvents. Comparing the obtained results with those present in the literature, similar IC50 values were obtained by Pereira et al. (2014) for green tea samples, but for black tea samples, these values were slightly higher. In comparison with the IC50 value (1.10 µg/mL) obtained for the standard substance—quercetin—the values of IC50 calculated for the studied samples of teas and erva-mate are about six times higher, which means that they have weaker antioxidant properties than quercetin.

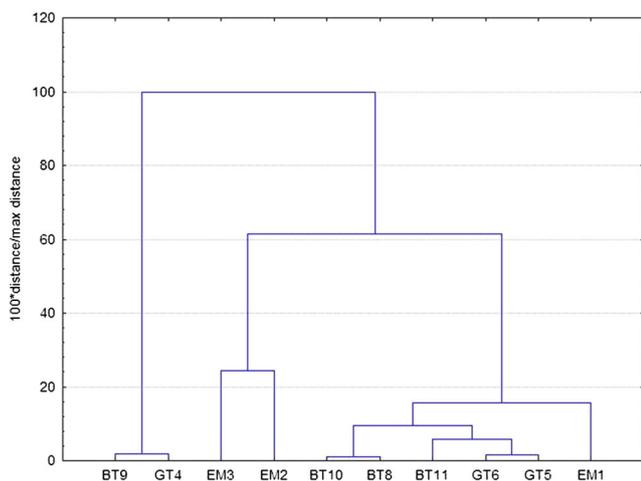


Fig. 2 Results of cluster analysis using the Wards method and Euclidean distance for the studied samples of black and green teas and erva-mate

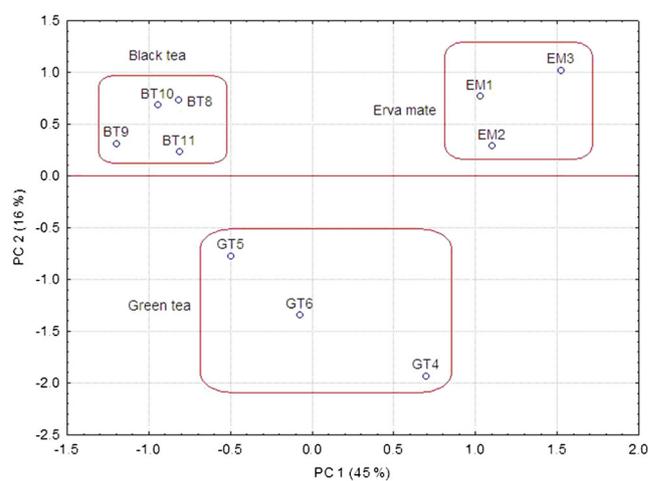


Fig. 3 Scatter plot of black and green tea and erva-mate samples in two-dimensional area PC1 vs. PC2

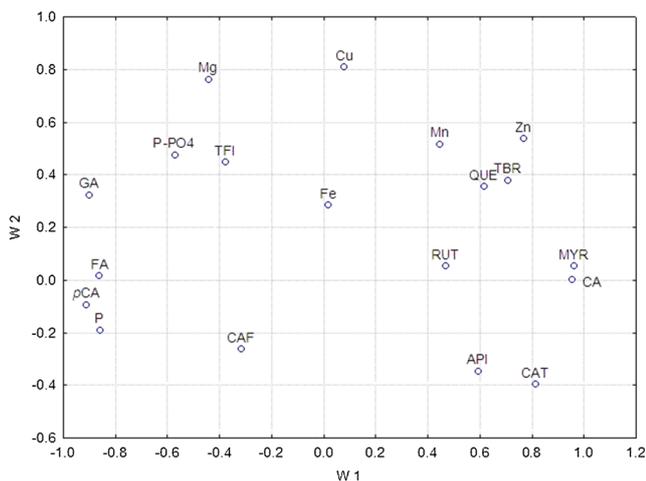


Fig. 4 Loadings of principal components obtained for the studied samples of black and green tea and *erva-mate* samples

Statistical Evaluation

Several statistically significant correlations among studied elements, phenolic compounds, alkaloids, and antioxidant activity were revealed (Table 4). P correlated with phenolic compounds (GA, CA, *p*-CA, FA, and MYR) and TBR. Zn was related to CA, *p*-CA, FA, MYR, and CAF. Regarding secondary plant metabolites, significant correlations occurred between GA and elements (Pi, Mg) and other metabolites, like CA, *p*-CA, FA, MYR, API, and CAT. Also, CA was related to *p*-CA, FA, MYR, API, CAT, and TBR. In other cases, only single relations were found, as can be noticed in Table 4.

In order to detect differences and similarities between the investigated samples as well as to classify them based on the content of elements, phenolic compounds, alkaloids, and antioxidant activity, highly advanced multivariate statistical methods were applied (Otto 2007; Konieczynski et al. 2016). Therefore, dendrogram obtained after calculations of CA using the Wards method and Euclidean distance is shown in Fig. 2. There are clear clusters grouping two samples of *erva-mate* (EM3 and EM2) in one aggregation in the left part of the plot, and another cluster with the samples of green and black teas can be found in the central and right parts of the plot. However, there are single exceptions from this pattern, like the samples of BT9 and GT4 clustered together in the left side of the dendrogram and one sample of EM1 located in the far right part of the plot. The location of these particular examples can be explained by their different chemical compositions in comparison with the other studied samples of black and green teas or *erva-mate*.

PCA confirmed these results, and the distribution of the studied samples shown in Fig. 3 clearly indicates all the samples of *erva-mate* as one group in the right upper corner of the plot, whereas the remaining green and black tea samples are well separated. Two first principal components accounted for

61% of the variability among the studied infusions. Moreover, PCA indicated total P, Mg, Cu, CA, MYR, and *p*-CA as factors mostly influencing the diversity among analyzed samples (Fig. 4).

Conclusions

Based upon the content of essential elements, selected phenolic compounds, alkaloids, and antioxidant activity of black and green tea infusions in comparison with the data obtained for *erva-mate*, it was possible to distinguish clearly the studied samples. Principal factors, which caused this differentiation between the black and green teas with those of *erva-mate*, were total P, Mg, Cu, CA, MYR, and *p*-CA. Therefore, it can be stated that the proposed scheme of analysis, including essential elements, phenolics, alkaloids, and antioxidant activity determinations, can be applied for comparison studies of commercially available samples of *C. sinensis* and *erva-mate*.

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Compliance with Ethical Standards

Conflict of Interest Pawel Konieczynski declares that he has no conflict of interest. Agnieszka Viapiana declares that she has no conflict of interest. Marek Wesolowski declares that he has no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants for whom identifying information is included in this article.

Ethical Approval This article does not contain any studies with human participants performed by any of the authors.

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