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A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of different Brazilian teas using multivariate statistical techniques



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ABSTRACT

A total of 51 Brazilian teas from the species *Camellia sinensis*, *Peumus boldus*, *Matricaria recutita*, *Baccharis trimera*, *Cymbopogon citratus*, *Pimpinella anisum*, *Mentha piperita*, and *Ilex paraguariensis* were analyzed in terms of phenolic compounds, color and *in vitro* antioxidant capacity using ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. Data were processed using principal component analysis (PCA), hierarchical cluster analysis (HCA) and linear discriminant analysis (LDA). Significant ($P < 0.01$) correlations between antioxidant activity measured by DPPH and FRAP assays with the total phenolic compounds ($r = 0.87$; $r = 0.90$, respectively) and flavonoids ($r = 0.79$; $r = 0.77$, respectively) were attained. The compounds that displayed significant ($P < 0.05$) correlations with the antioxidant activity were gallic acid, catechin, epicatechin, procyanidin B2, quercetrin, and caffeine. PCA was a suitable approach to check for similarities among tea samples, explaining up to 50% of data variability. Four groups were suggested using HCA, in which cluster 3 showed the highest content of total phenolic compounds, flavonoids, antioxidant activity, gallic acid, and caffeine. All samples included in this group were from *C. sinensis*. The overall classification capacity obtained by LDA was 82.00%, in which 100% of samples from *I. paraguariensis*, *C. citratus*, *M. recutita*, and *P. boldus* were adroitly classified, while 60% of teas from *P. anisum*, 80% of *M. piperita* teas, and 88.24% of *C. sinensis* teas were correctly classified.

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1. Introduction

Tea is the second most consumed beverage aside from water and has gained much attention due to its health-promoting benefits, including antimutagenic (Bunkova, Marova, & Nemeč, 2005), anticancer and antiapoptotic (Cai, Luo, Sun, & Corke, 2004; El-Beshbishy, Tork, El-Bab, & Autifi, 2011), neuroprotective (Mendel & Youdim, 2004), hypoglycemic and antihyperglycemic (Abeywickrama, Ratnasooriya, & Amarakoon, 2011), antioxidant (Morais, Cavalcanti, Costa, & Aguiar, 2009), antimicrobial (Oh, Jo, Cho, Kim, & Han, 2013), and inflammatory effects (Chaudhuri et al., 2005), among others listed by Pinto (2013). These biological activities are associated in part to the antioxidant activity of chemical compounds present in teas, especially flavonoids and phenolic acids. Thus, the evaluation of total and individual quantification of phenolic compounds is essential to correlate with the biological activity.

Characterization of the antioxidant capacity of teas is important to determine their health benefits. Vaquero, Serravalle, de Nadra, and de Saad (2010) assessed the association between antioxidant activity

measured using FRAP and DPPH assays and the content of total phenolic compounds of Argentinean herbs infusions and observed high correlations ($r > 0.81$, $P < 0.05$). Katalinic, Milos, Kulisic, and Jukic (2006) performed a screening of 70 medicinal plant extracts and verified a high correlation between antioxidant capacity measured by FRAP and total phenolic content ($r = 0.98$, $P < 0.001$). In this sense, the antioxidant effectiveness depends on the tea species and the content and type of phenolic compounds present in each species. Atoui, Mansouri, Boskou, and Kefalas (2005) and Bravo, Goyaa, and Lecumberria (2007) verified that phenolic acids and their derivatives were detected in all herbal infusions while the presence of flavonoids varied considerably: catechins were present mainly in *Camellia sinensis* and *Ilex paraguariensis*. Thus, it is important to monitor the phenolic composition and the biological activity of teas consumed by a large part of the population in order to correlate their benefits with human health.

The quality control of foods by using multivariate statistical techniques (chemometrics) has increased in many fields of food science and technology once these tools are able to extract the maximum amount of information from chemical data, including chemical composition and antioxidant activity of extracts (Granato, Katayama, & Castro, 2012; Hossain, Patras, Barry-Ryan, Martin-Diana, & Brunton, 2011; Patras et al., 2011). In relation to commercial teas, it is important to

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perform researches that assess their chemical composition and bioactivity, especially the antioxidant capacity, once consumers do not have access to these data. Herein, considering that teas are able to increase the antioxidant capacity in human's plasma and multiple organs (Benzie, Szeto, Strain, & Tomlinson, 1999; Frei & Higdon, 2003), the evaluation of such products is important to provide information about their possible health benefits. Therefore, this study aimed to assess the phenolic composition and *in vitro* antioxidant activity of a wide variety of teas widely marketed and consumed in Brazil using three different multivariate statistical techniques.

2. Material and methods

2.1. Chemicals

Folin–Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tri (2-pyridyl)-s-triazine), DPPH, and chemical HPLC-grade standards (purity \geq 95%) of gallic acid, chlorogenic acid, coumaric acid, (+)-catechin, (–)-epicatechin, procyanidin B1, procyanidin B2, quercetin, quercetrin, and caffeine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile and acetic acid were of HPLC grade, while the other reagents used in the experiments were of analytical grade. The aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore, São Paulo, SP, Brazil).

2.2. Tea samples

A total of 51 tea bag samples were acquired in the commerce of Ponta Grossa, Brazil: *C. sinensis* (n = 18), *Peumus boldus* (n = 5), *Matricaria recutita* (n = 5), *Baccharis trimera* (n = 4), *Cymbopogon citratus* (n = 5), *Pimpinella anisum* (n = 5), *Mentha piperita* (n = 5), and *I. paraguariensis* (n = 4). It is noteworthy that two brands share more than 60% of the total sales in Brazil, and the samples evaluated in this study represent well the nationwide marketed teas.

2.3. Extraction procedure

Initially, 30 g of each dried leaves was mixed and homogenized by constant agitation (150 rpm). Then, a total of 2.0 g of each dried leaves sample was extracted with 100 mL distilled water at 80 °C in a glass flask covered with a lid in order to avoid evaporation. The extraction procedure was carried out under magnetic stirring for a period of 7.5 min. Then, the mixture was filtered and the tea was transferred to Falcon tubes and immediately frozen at –20 °C until further analysis.

2.4. Total phenolic content (TPC)

The TPC of tea samples was determined by colorimetric analysis using Folin–Ciocalteu reagent as described by Singleton and Rossi (1965). In a test tube, 8.4 mL of distilled water, 100 μ L of diluted tea (1:7) and 500 μ L of Folin–Ciocalteu reactive were added. After 3 min, 1.0 mL of saturated sodium carbonate was added into each tube and the tube was agitated for 10 s in a vortex. After 1 h, the absorbance was measured using a spectrophotometer (model Mini UV 1240, Shimadzu, Tokyo, Japan) at a wavelength of 725 nm. The measurement was compared to a calibration curve of gallic acid (GA) [TPC = $497.52 \times$ absorbance; $R^2 = 0.998$; $P < 0.001$] and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter of tea [mg GAE/L].

2.5. Determination of total flavonoid content (TFC)

The TFC was quantified in triplicate by an aluminum chloride colorimetry assay (Jia, Tang, & Wu, 1999). Briefly, 250 μ L of diluted tea sample (1:3) was mixed with 2720 μ L of a 30% ethanolic solution and

120 μ L of sodium nitrite (0.5 mol/L). This solution was mixed well and allowed to react for 5 min and then 120 μ L of a 10% aluminum chloride solution was added to the test tubes and the solution was allowed to react for 5 min more. Then, 800 μ L of a 1 mol/L NaOH solution was added to the tubes and the absorbance was read against a reagent blank (ethanol) at a wavelength of 510 nm using a spectrophotometer (model Mini UV 1240, Shimadzu, Tokyo, Japan). The measurement was compared to a calibration curve of catechin (CT) [TFC = $434.78 \times$ absorbance; $R^2 = 0.999$; $P < 0.001$] and the results were expressed as milligrams of catechin equivalents (CTE) per liter of tea [mg CTE/L].

2.6. Measurement of *in vitro* antioxidant activity

The total antioxidant potential of tea samples was determined in triplicate using the ferric reducing antioxidant power (FRAP) assay according to the method proposed by Benzie and Strain (1996), with minor changes. The assay is based on the reducing power of antioxidant present in teas, in which a potential antioxidant reduces the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+} /TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mmol/L, pH 3.6), a solution of 10 mmol/L TPTZ in 40 μ M HCl, and 20 mmol/L $FeCl_3$ at 10:1:1 (v/v/v). Freshly prepared FRAP reagent (300 μ L) and diluted tea samples (10 μ L) were added to each well and mixed thoroughly for 10 s. Measurements were performed using a microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA), and the absorbance was recorded at a wavelength of 593 nm after 30 min. A standard curve [FRAP = $462.96 \times$ absorbance; $R^2 = 0.999$; $P < 0.001$] was plotted using different concentrations of Trolox (100–1000 μ mol/L). The results were expressed in μ mol Trolox equivalents per liter of tea (μ mol TE/L). All determinations were performed in triplicate.

Free-radical scavenging activity of teas was determined in triplicate by the DPPH assay according to the method of Brand-Williams, Cuvelier, and Berset (1995), with minor changes. This method determines the hydrogen donating capacity of a molecule and does not produce oxidative chain reactions or react with free radical intermediates. Briefly, every diluted tea sample (1:10) was added (100 μ L) to 3.9 mL of a 125 μ mol/L methanolic DPPH solution. The absorbance at a wavelength of 517 nm was measured using a spectrophotometer (model Mini UV 1240, Shimadzu, Tokyo, Japan) after the solution had been allowed to stand in the dark for 30 min. Methanol was used as a negative control (blank). The free-radical scavenging activity of each tea sample towards DPPH radical was calculated using Eq. (1):

$$\text{Antioxidant activity (\% inhibition)} = \left[\frac{1 - (\text{Abs}_{517} \text{ sample} / \text{Abs}_{517} \text{ blank})}{\times 100} \right] \quad (1)$$

2.7. Color attributes

Instrumental color attributes (L^* , a^* , b^*) of tea samples were determined by reflectance using an UltraScan PRO Hunter Lab. (Reston, VA, USA) spectrophotometer. Samples were filled in a 1-cm cell and L^* , a^* and b^* values were determined using Illuminant D_{65} and 10° observer angle. The parameters' chroma (C^*) was estimated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and hue angle (h^*) was calculated by: $h^* = \tan^{-1}(b^* / a^*) + 180^\circ$ when $a^* < 0$ and $h^* = \tan^{-1}(b^* / a^*)$ when $a^* > 0$ (Granato, Ribeiro, Castro, & Masson, 2010).

2.8. HPLC analysis of phenolic compounds and caffeine

The phenolic profile analysis was based on the methodology described by Zuo, Chen, and Deng (2002), with some minor modifications. The teas were filtered through a syringe filter 0.22 μ m of nylon

(Millipore, São Paulo, Brazil) prior to analysis and 10 μL of sample was injected. The HPLC apparatus (model 2695 Alliance Waters, Milford, MA, USA) was coupled with a photodiode array detector (model PDA 2998 Waters, Milford, MA, USA), a quaternary pump and an auto sampler. Separation was performed on a Symmetry® C18 column with dimensions of 4.6 mm \times 150 mm, 3.5 μm (Waters, Milford, MA, USA) at 20 °C. The mobile phase was composed of solvent A (2.5% acetic acid, v/v) and solvent B (acetonitrile). The following gradient was applied: 3–9% B (0–5 min), 9–16% B (5–15 min), and 16–36.4% B (15–33 min), followed by washing and reconditioning of the column. The flow rate was 1.0 mL·min⁻¹, and the runs were monitored at 280 nm (gallic acid, catechin, epicatechin, procyanidin B1, procyanidin B2 and caffeine), 320 nm (chlorogenic acid and coumaric acid) and 350 nm (quercetin and quercetrin). Quantification was performed using calibration curves of standards. The regression equations of pure chemical standards, limits of detection, limits of quantification, retention time, and wavelength used to quantify the phenolic compounds in the tea samples are presented in Table 1, while some examples of chromatograms obtained are presented in Fig. 1.

2.9. Statistical analysis

Data were presented as mean \pm standard deviation. A chemometric approach composed of principal component analysis (PCA), hierarchical cluster analysis (HCA) and linear discriminant analysis (LDA), implemented in the STATISTICA 7.0 software (Stat-Soft Inc., Tulsa, OK, USA) was used to analyze the retail price, caffeine content, phenolic compounds, antioxidant activity and color parameters. Prior to chemometrics application, all variables were autoscaled (transformation into z-scores) to standardize the statistical importance of all responses. Then, a matrix of samples ($n = 51$) and response variables ($n = 20$) was built, in which samples were adopted as lines and variables as rows, totaling 1020 data points. The Pearson correlation coefficient is usually used to express the strength between two continuous variables. However, it is essential to analyze the statistical significance of each correlation coefficient and, in a practical way, this can be achieved by calculating the P -value. In the current study, linear correlation analysis was performed in order to check the significance (if present) of each correlation coefficient between variables. Furthermore, the determination coefficient, R^2 , was determined to demonstrate how

Table 1
Chromatographic parameters of caffeine and phenolic compounds analyzed by HPLC.

Chemical compounds	Retention time (min)	UV bands (nm)	Regression equation	R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Gallic acid	3.22	271.5	$y = 1.27 \text{ E} + 07$ $x + 24,693$	0.999	0.15	0.50
Chlorogenic acid	9.29	326.9	$y = 1.86 \text{ E} + 07$ $x + 877$	0.997	0.19	0.62
Coumaric acid	15.28	309.6	$y = 5.29 \text{ E} + 07$ $x + 88,036$	0.999	0.03	0.09
Catechin	8.80	278.7	$y = 6.36 \text{ E} + 06$ $x + 2309$	0.997	0.08	0.28
Epicatechin	12.45	278.4	$y = 5.53 \text{ E} + 06$ $x + 161$	0.997	0.07	0.23
Procyanidin B1	7.08	278.7	$y = 4.31 \text{ E} + 06$ $x - 3176$	0.997	0.54	1.81
Procyanidin B2	11.24	279.8	$y = 4.80 \text{ E} + 06$ $x - 2352$	0.997	0.17	0.56
Quercetin	23.50	376.2	$y = 1.28 \text{ E} + 06$ $x + 9269$	0.999	0.98	3.26
Quercetrin	22.24	349.0	$y = 1.56 \text{ E} + 07$ $x + 4352$	0.998	0.27	0.89
Caffeine	10.00	273.9	$y = 2.03 \text{ E} + 06$ $x + 45,945$	0.999	1.74	5.81

Note: LOD: limit of detection; LOQ: limit of quantification.

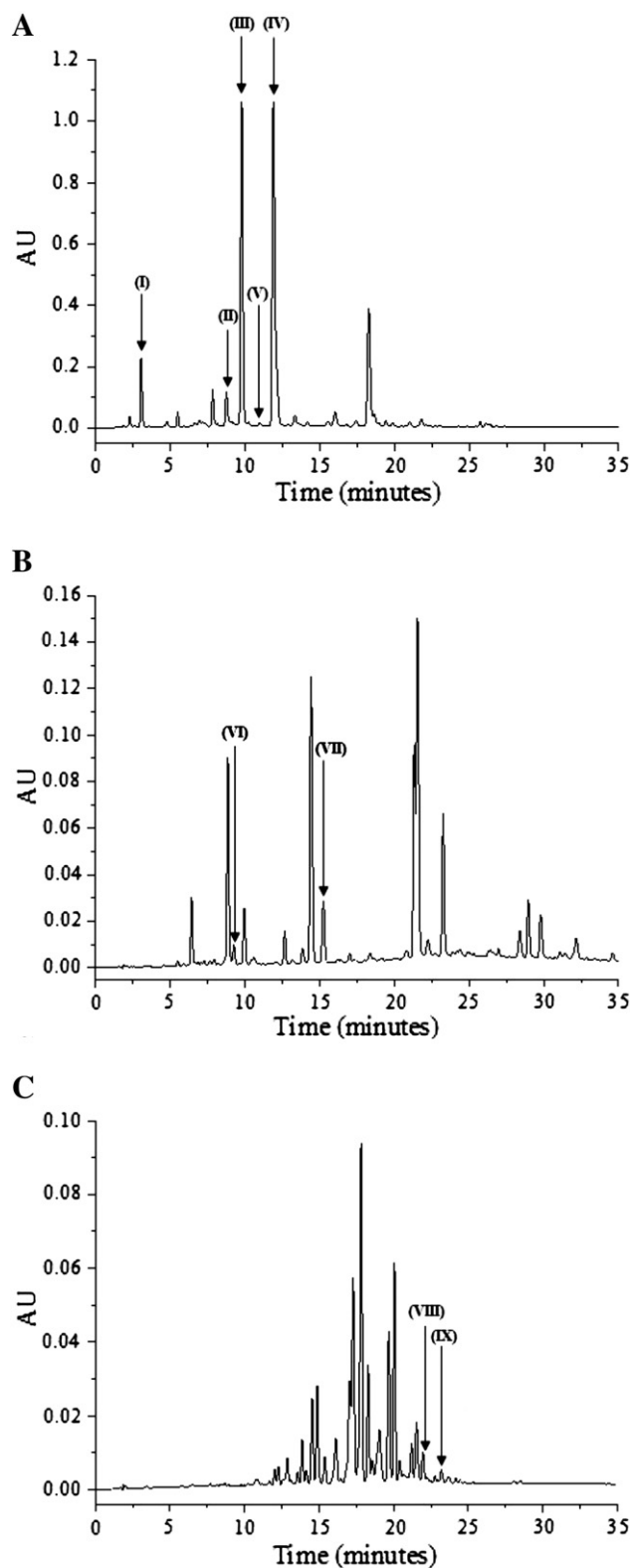


Fig. 1. Chromatograms obtained for different teas from phenolic compounds determined at (A) 280 nm from *Camellia sinensis*, (B) 320 nm from *Baccharis trimera* and (C) 350 nm from *Peumus boldus*. (I) Gallic acid, (II) catechin, (III) caffeine, (IV) epicatechin, (V) procyanidin B2, (VI) chlorogenic acid, (VII) coumaric acid, (VIII) quercetrin, (IX) quercetin.

the response variables were related mathematically and to understand the proportion of the variance (fluctuation) of one variable that is predictable from the other variable.

Table 2
Color attributes, total phenolics, flavonoids and *in vitro* antioxidant capacity of Brazilian teas.

Herbal species	Price and color attributes				Phenolic compounds			Antioxidant activity		
	Retail price (US\$/10 g)	L*	a*	b*	C*	h*	Total phenolics (mg GAE/L)	Flavonoids (mg CTE/L)	DPPH (% reduction)	FRAP (µM TE/L)
<i>Camellia sinensis</i> (n = 18)	1.62 ± 0.96	24.61 ± 0.55	0.20 ± 0.31	0.88 ± 0.49	0.99 ± 0.41	74.91 ± 28.38	1034.48 ± 416.24	179.88 ± 32.41	68.60 ± 22.40	10,331.19 ± 4802.91
<i>Peumus boldus</i> (n = 5)	0.96 ± 0.44	25.07 ± 0.21	0.12 ± 0.06	1.06 ± 0.18	1.07 ± 0.19	83.81 ± 2.62	716.40 ± 124.19	126.87 ± 50.04	46.91 ± 8.31	4463.75 ± 607.19
<i>Matricaria recutita</i> (n = 5)	0.88 ± 0.11	24.84 ± 0.16	-0.04 ± 0.14	1.13 ± 0.09	1.14 ± 0.09	86.18 ± 18.67	285.96 ± 203.06	45.22 ± 8.04	12.32 ± 7.25	897.37 ± 239.56
<i>Baccharis trimera</i> (n = 4)	0.76 ± 0.17	24.93 ± 0.23	-0.28 ± 0.18	0.74 ± 0.24	0.81 ± 0.21	111.63 ± 15.43	176.60 ± 50.86	58.65 ± 4.61	18.32 ± 9.36	1080.01 ± 196.34
<i>Cymbopogon citratus</i> (n = 5)	0.93 ± 0.39	24.90 ± 0.49	-0.08 ± 0.15	0.82 ± 0.11	0.83 ± 0.12	94.79 ± 9.72	147.12 ± 30.74	46.35 ± 9.40	17.10 ± 12.68	757.22 ± 133.25
<i>Pimpinella anisum</i> (n = 5)	1.00 ± 0.11	25.23 ± 0.25	-0.41 ± 0.16	0.47 ± 0.37	0.72 ± 0.11	100.88 ± 50.60	100.45 ± 16.24	34.09 ± 3.28	14.30 ± 9.63	654.98 ± 49.33
<i>Mentha piperita</i> (n = 5)	0.78 ± 0.7	24.23 ± 0.30	0.23 ± 0.11	0.43 ± 0.08	0.50 ± 0.09	62.99 ± 11.10	470.07 ± 208.13	125.57 ± 45.67	35.24 ± 16.42	3464.59 ± 901.86
<i>Ilex paraguariensis</i> (n = 4)	1.22 ± 0.17	23.95 ± 0.14	0.30 ± 0.02	0.19 ± 0.08	0.37 ± 0.04	31.17 ± 11.28	672.87 ± 126.25	176.04 ± 40.50	49.66 ± 9.59	5065.75 ± 298.61

Note: Results expressed as mean ± SD (n = 3).

PCA involves a mathematical procedure that identifies patterns in data, and expresses them in such a way as to highlight similarities and differences. PCA was applied to separate the samples according to the phenolic composition, color, and antioxidant activity. For this purpose, the results obtained for each parameter were adopted as columns and the tea samples as rows. Eigenvalues higher than 1.0 were adopted to explain the projection of the samples on the two-dimensional graph and analysis was based on linear correlations (Granato, Branco, Cruz, & Faria, 2011).

HCA is a preliminary method to study datasets in the search for natural groupings among samples characterized by the values of a set of measured features. Owing to its unsupervised character, HCA is a pattern recognition technique that can be used to reveal the structure residing in a dataset. Sample similarities were calculated on the basis of the Euclidean distance, and the Ward hierarchical agglomerative method was used to group the samples (Granato, Calado, Oliveira, & Ares, 2013). The dendrogram imposes a hierarchy on this similarity so that it is possible to have a two-dimensional vision of the similarity or dissimilarity of the entire set of samples used in the study (Granato, Katayama, & Castro, 2011). In order to compare the results among the formed clusters, Levene's test was carried out to check for homogeneity of variances, while one-way analysis of variance (ANOVA) and Fisher's LSD *post hoc* tests were then applied to identify remarked differences among clusters. For the variables that presented non-homogeneous variances ($P < 0.05$), the non-parametric multiple comparison Kruskal–Wallis test was used. *P*-values below 0.05 were considered to reject the null hypothesis.

LDA is a supervised statistical technique used to find a linear combination of features which characterizes or separates two or more classes of objects. LDA is closely related to analysis of variance and linear regression analysis, which also attempts to express one dependent variable as a linear combination of other features or measurements (Statsoft Electronic Statistics Book, 2013). With the aim of classifying the tea samples according to the species, a univariate analysis using Fisher's test was carried out in the first place. In the current work, the contents of quercetin, quercetrin, flavonoids, catechin, and chromaticity (C^*) were used as independent variables, and types of teas were used as responses. Classification scores, given in percentage, for each case for each group were calculated by applying Eq. (2):

$$S_i(\%) = C_i + W_{i1} * X_1 + W_{i2} * X_2 + \dots + W_{im} * X_m \quad (2)$$

where the subscript *i* denotes the respective group; the subscripts 1, 2, ..., *m* denote the *m* variables; c_i is a constant for the *i*'th group, w_{ij} is the weight for the *j*'th variable in the computation of the classification score for the *i*'th group; x_j is the observed value for the respective case for the *j*'th variable and S_i is the resultant classification score, given in percentage.

3. Results and discussion

The content of the total phenolic compounds of the Brazilian teas ranged from 100.45 to 1034.48 mg GAE/L and the flavonoid content ranged from 34.09 to 179.88 mg CTE/L. The results showed that the inhibition of DPPH ranged from 12.62 to 68.60% of reduction, and the FRAP results varied from 654.98 to 10,331.19 µmol TE/L (Table 2). Color properties of extract tea samples showed differences ($P < 0.05$) among species. With regard to lightness (L^*), all samples present low values ($L^* < 26$). The color intensity (C^*) also showed low values, ranging from 0.50 to 1.14 and hue values (h^*) varied from 31.17 to 111.63. Atoui et al. (2005) found results for total phenolic content of *M. piperita*, *M. recutita*, and *C. sinensis* ranging from 442 to 5067 mg GAE/L. The results found by Gião et al. (2007) for *M. recutita* and *B. trimera* varied from 196 to 308 mg GAE/L. The antioxidant activity (DPPH) of different herbs ranged from 6.15 to 94.50% of reduction (Tsai, Tsai, Chien, Lee, & Tsai, 2008). The data found by Vaquero et al. (2010) for 13 different

Table 3
Chemical composition (mg/L) of selected teas marketed in Brazil.

Herbal species	Gallic acid	Chlorogenic acid	Coumaric acid	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2	Quercetin	Quercitrin	Caffeine
<i>Camellia sinensis</i> (n = 18)	198.73 ± 78.02	5.22 ± 6.33	n.d.	31.76 ± 58.49	68.55 ± 105.39	n.d.	3.67 ± 8.77	n.d.	21.13 ± 15.76	5485.23 ± 1637.22
<i>Peumus boldus</i> (n = 5)	n.d.	n.d.	n.d.	116.65 ± 40.09	29.94 ± 35.30	169.07 ± 46.31	n.d.	7.49 ± 1.34	41.19 ± 9.22	n.d.
<i>Marricaria recutita</i> (n = 5)	n.d.	41.51 ± 21.48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>Baccharis trimera</i> (n = 4)	n.d.	63.43 ± 34.19	11.41 ± 21.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	69.63 ± 113.35
<i>Cymbopogon citratus</i> (n = 5)	n.d.	37.97 ± 26.17	0.06 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>Pimpinella anisum</i> (n = 5)	n.d.	26.24 ± 11.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19.33 ± 18.38	n.d.
<i>Mentha piperita</i> (n = 5)	n.d.	3.25 ± 5.91	4.31 ± 2.46	n.d.	0.27 ± 0.60	n.d.	n.d.	59.77 ± 34.36	n.d.	n.d.
<i>Ilex paraguariensis</i> (n = 4)	n.d.	199.42 ± 149.38	n.d.	6.67 ± 13.33	n.d.	n.d.	n.d.	n.d.	n.d.	1244.63 ± 711.13

Note: Results expressed as mean ± SD (n = 3), n.d. = not detected or values below LOD.

herbs showed that the total phenolic content ranged from 50.30 to 925.00 mg GAE/L and the antioxidant activity measured by the DPPH assay varied from 21.50 to 86.50%.

Using a bivariate correlation between color attributes and the other response variables, it was possible to observe a significant ($P < 0.01$) correlation between the flavonoid content and L^* ($r = -0.42$; $R^2 = 17.64\%$), a^* coordinate ($r = 0.56$; $R^2 = 31.36\%$), and h^* ($r = -0.37$; $R^2 = 13.69\%$). Besides being statistically significant, the correlation coefficient only shows a trend on how the response variables are associated. In order to fully understand the relationship between variables, the coefficient of determination (R^2) was also calculated. For flavonoid vs. L^* , for example, only 17.64% of the variance in the flavonoid content was explained when the L^* values changed.

The contents of individual phenolic compounds varied within tea species, as observed in Table 3. Gallic acid and procyanidin B2 were found only in *C. sinensis* teas, and the contents of chlorogenic acid ranged from 3.25 to 199.42 mg/L, coumaric acid from 0.06 to 11.41 mg/L, catechin from 6.67 to 116.65 mg/L, epicatechin from 0.27 to 68.55 mg/L, quercetin from 7.49 to 59.77 mg/L, quercitrin from 19.33 to 41.19 mg/L, and caffeine from 69.63 to 5485.23 mg/L. Procyanidin B1 was found only in *P. boldus* (169.07 ± 46.31 mg/L). A high variation on the phenolic composition inter- and intraspecies is due to the manufacturing conditions employed by each company and also because of differences in composition of teas, that is, each company uses different leaf-to-stem ratios. Moreover, the phenolic composition and therefore the antioxidant capacity of teas from the same species may be discrepant because of differences in the environmental conditions, physiopathological conditions of the plant, water stress, and soil composition, among others.

The results of this research showed significant ($P < 0.01$) correlations between antioxidant activity measured by DPPH and FRAP assays with the retail price ($r = 0.35$ and $R^2 = 12.25\%$; $r = 0.34$ and $R^2 = 11.56\%$, respectively), and total phenolic compounds ($r = 0.87$ and $R^2 = 75.69\%$; $r = 0.90$ and $R^2 = 81.00\%$, respectively), and total flavonoids ($r = 0.79$ and $R^2 = 62.41\%$; $r = 0.77$ and $R^2 = 59.29\%$, respectively). Likewise, Souza, Oldoni, Cabral, and Alencar (2011) analyzed the total phenolic content of 15 herbal teas marketed in Brazil, including the species of *C. sinensis*, *I. paraguariensis*, and *M. recutita* and found $r = 0.92$. Oh et al. (2013) analyzed 11 water-soluble extracts from numerous herbal species, including *I. paraguariensis* and *C. sinensis*, and verified a correlation coefficient of 0.6258 ($R^2 = 39.16\%$) between total phenolic compounds and antioxidant capacity (DPPH). As well outlined by Lin, Liu, and Mau (2008) and Granato et al. (2011), the antioxidant capacity of flavonoids might be due to the presence of double bonds in the C ring, which enhances the nucleophilic power. In addition, catechins represent the major phenolic compounds from tea water extracts (infusions), and they contain a saturated single bond at 2 and 3 positions (Fig. 2). Furthermore, it is speculated that the total content, chemical structure and number of hydroxyl groups present in flavonoids may have been responsible for a high scavenging ability observed in the current study.

The phenolic compounds that displayed significant ($P < 0.05$) correlations with either DPPH or FRAP assays were gallic acid ($r = 0.75$ and $R^2 = 56.25\%$; $r = 0.79$ and $R^2 = 62.41\%$, respectively), catechin ($r = 0.36$ and $R^2 = 12.96\%$; $r = 0.34$ and $R^2 = 11.56\%$, respectively), epicatechin ($r = 0.34$ and $R^2 = 11.56\%$; $r = 0.53$ and $R^2 = 28.09\%$, respectively), procyanidin B2 ($r = 0.38$ and $R^2 = 14.44\%$ only for FRAP), quercetin ($r = 0.31$ and $R^2 = 9.61\%$; $r = 0.36$ and $R^2 = 12.96\%$, respectively), and caffeine ($r = 0.71$ and $R^2 = 50.41\%$; $r = 0.78$ and $R^2 = 60.84\%$, respectively). Although only some phenolic compounds correlated to the antioxidant capacity, it is noteworthy that the chemical interactions (synergism, antagonism, and additional effects) among various phenolic compounds may take place in teas, as in any other complex food matrix. These chemical reactions are very usual in complex food matrices such as teas and may explain the results observed in the current study. A positive and significant ($P < 0.001$)

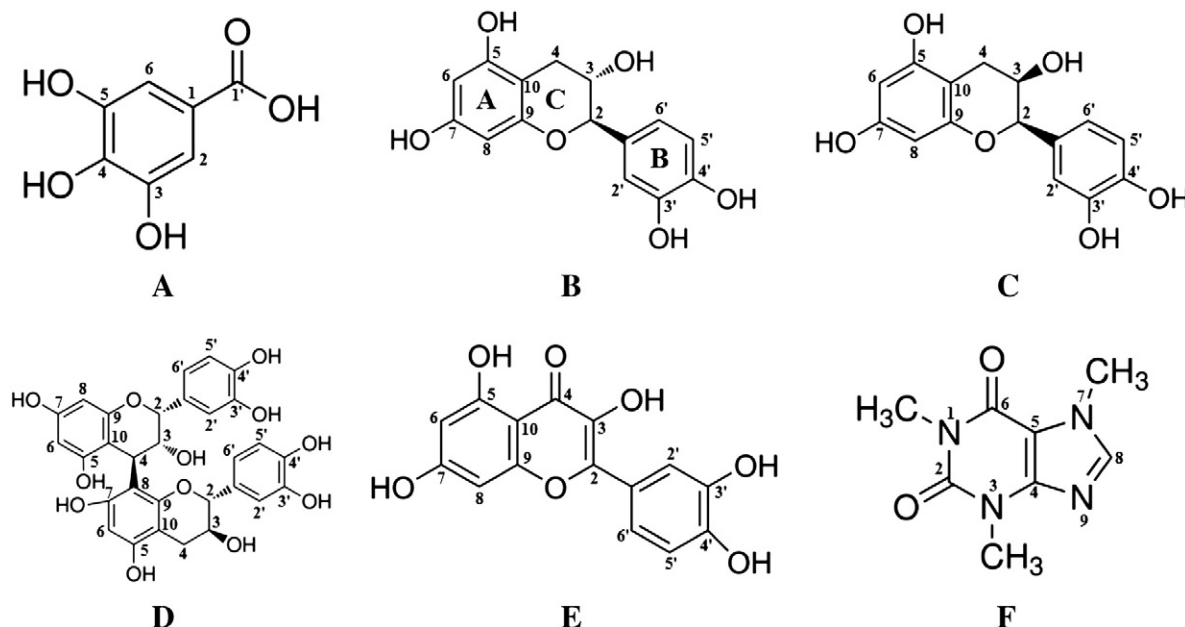


Fig. 2. Chemical structures of some compounds measured in Brazilian herbal teas: (A) gallic acid, (B) catechin, (C) epicatechin, (D) procyanidin B2, (E) quercetin, and (F) caffeine.

correlation between caffeine content and the antioxidant activity measured by DPPH and FRAP assays ($r = 0.71$, $r = 0.78$, respectively) was attained. This finding is in-line with those reported by Shi, Dalal, and Jain (1991) who concluded that caffeine presents good hydroxyl radical scavenging ability and attributed the anticarcinogenic properties of this compound to its antioxidant capacity.

In our study, gallic acid showed significant difference ($P < 0.001$) among clusters, and a correlation with antioxidant activity measured by DPPH and FRAP assays ($r = 0.75$ and $R^2 = 52.50\%$, $r = 0.79$ and $R^2 = 62.41\%$, respectively). According to Granato et al. (2011), trihydroxy benzoic acids, such as gallic acid, have a strong antioxidant activity because of the nucleophilic power (high reducing capacity) of their three available hydroxyl groups (Fig. 2).

In the current study, catechin and epicatechin showed a positive and significant ($P < 0.05$) correlation with DPPH ($r = 0.36$ and $R^2 = 12.96\%$; $r = 0.34$ and $R^2 = 10.20\%$, respectively) and FRAP ($r = 0.34$ and $R^2 = 10.20\%$; $r = 0.53$ and $R^2 = 28.09\%$, respectively), but only catechin content was significantly different among clusters ($P < 0.001$). The chemical structure of flavan-3-ol determines the relative ease of oxidation and free-radical scavenging activity. Although the presence of galloyl groups, number and position of hydroxyl groups (based on redox potential) are recognized to enhance the antioxidant activity, the methoxylation and glycosylation of position 3 apparently inhibit the reducing ability (Aron & Kennedy, 2008). In the same sense, procyanidin B2 ($r = 0.38$ and $R^2 = 14.44\%$, $P < 0.01$) correlated only with FRAP. Tsao, Yang, Xie, Sockovie, and Khanizadeh (2005) stated that procyanidins present higher antioxidant capacity measured by FRAP as compared to DPPH. The antioxidant capacity of procyanidins is due to the presence of the catechol unit on the aromatic B-ring (Rice-Evans, Miller, & Paganga, 1996), which stabilizes free radicals and has the ability to chelate metals and proteins due to several *o*-dihydroxy phenolic groups (Santos-Buelga & Scalbert, 2000).

Quercetrin contents correlated ($P < 0.05$) with DPPH and FRAP assays ($r = 0.31$ and $R^2 = 9.61\%$, $r = 0.36$ and $R^2 = 12.96\%$, respectively) and significant differences ($P < 0.001$) in concentrations of this compound were also observed among clusters. For flavonols (quercetin glycosides), the combination of the catechol moiety with double bonds at C2–C3 and 3–OH (Fig. 2) provides an extremely active free-radical scavenger (Van Acker et al., 1996).

Principal component analysis was applied in order to evaluate the data of phenolic compounds determined by HPLC, total phenolic compounds, total flavonoids, antioxidant capacity and color properties of Brazilian teas. Principal component 1 (PC1) explained up to 31.33% of total variance and PC2 explained 18.67%. Thus the two-dimensional graph presented was able to explain 50.00% of the variability in the experimental data (Fig. 3). Samples were separated along the first principal component by differences observed in total phenolic compounds, total flavonoids, DPPH, FRAP, gallic acid, epicatechin, and caffeine. The second PC separated the samples on the basis of L^* , a^* , b^* , C^* and h^* . It is noteworthy that all samples from *C. sinensis* were located in the 2nd and 3rd quadrants (left side), and *P. boldus* teas were located near the origin. In this sense, it is possible to assume that PCA was a suitable approach to check for similarities among tea samples. Indeed, the scatter plot provided by PCA is very important once it projects all samples in

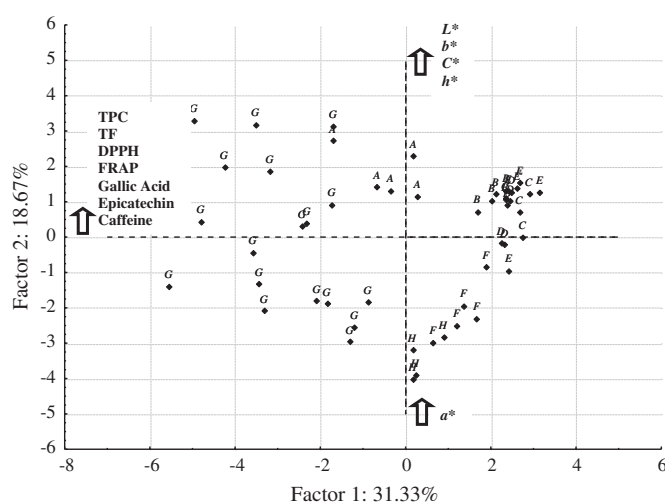


Fig. 3. A scatter plot (PC1 vs. PC2) on the main sources of variability between the Brazilian tea samples. Abbreviations: A (*Peumus boldus*); B (*Matricaria recutita*); C (*Baccharis trimera*); D (*Cymbopogon citratus*); E (*Pimpinella anisum*); F (*Mentha piperita*); G (*Camellia sinensis*); H (*Ilex paraguariensis*).

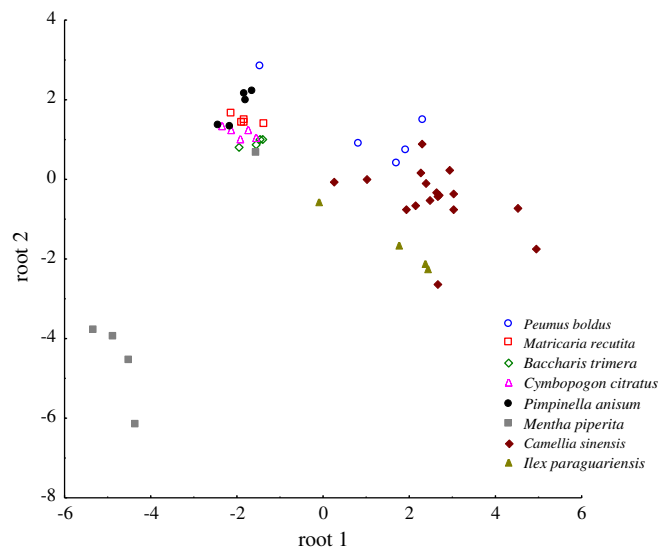


Fig. 5. 2D scatter plot (root 1 vs. root 2) using linear discriminant analysis to classify Brazilian teas based on the contents of quercetin, quercetrin, chromaticity, flavonoids and catechin.

$\Lambda = 0.0100$, $F = 3.01$, $P = 0.0128$). The classification capacity of the model proposed by LDA was 82.00%, in which 100% of samples from *I. paraguariensis*, *C. citratus*, *M. recutita*, and *P. boldus* were adroitly classified. Samples from *B. trimera* were not correctly classified using the selected independent variables employed in the current study. A total 60% of teas from *P. anisum*, 80% of *M. piperita* teas, and 88.24% of *C. sinensis* teas were correctly classified using LDA. Although a limited number of samples were used, LDA seemed to be a suitable approach to discriminate Brazilian commercial tea samples according to the phenolic composition and instrumental color. Thus, more samples should be used to validate the findings of our work and other statistical approaches should be tested to explain the experimental results.

4. Conclusions

Brazilian teas from eight different species were analyzed for their phenolic composition, color and antioxidant capacity. Using PCA, only 50% of data variability was explained but a better visualization of all results was attained. HCA separated the samples into four different clusters and pinpointed that the species with higher contents of phenolic compounds and free-radical scavenging activity was *C. sinensis*. On the contrary, samples from *M. recutita*, *B. trimera* and *C. citratus* presented the lowest antioxidant activity and phenolic compounds. LDA was effective in classifying samples from *I. paraguariensis*, *C. citratus*, *M. recutita*, *P. boldus*, *M. piperita*, *C. sinensis*, and *P. anisum* based on the contents of quercetin, quercetrin, total flavonoids, catechin and chromaticity. Therefore, multivariate statistical techniques used in the current study seemed to be suitable to monitor and assess the phenolic composition and *in vitro* antioxidant capacity of commercial teas.

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