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Characterization of the Constituents and Antioxidant Activity of Brazilian Green Tea (*Camellia sinensis* var. *assamica* IAC-259 Cultivar) Extracts

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Freeze-dried extracts from *Camellia sinensis* var. *assamica* IAC-259 cultivar named Brazilian green tea were prepared by hot water and ultrasound-assisted extractions using leaves harvested in spring and summer. Their caffeine and catechin contents were measured by high performance liquid chromatography–diode array detector. The antioxidant activity of the major green tea compounds and Brazilian green tea extracts was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The levels of caffeine were higher in the summer samples ($p < 0.05$); otherwise, there were no significant variations related to the catechin contents between spring and summer samples. The sonication method using water/acetone as solvent had a high efficiency to extract not only epigallocatechin gallate but also epicatechin gallate ($p < 0.05$). Antioxidant activities of the Brazilian green tea extracts were not significantly different among seasons and extraction systems. The antioxidant data (IC_{50}) of the Brazilian green tea extracts showed a significant correlation with their epigallocatechin gallate and epicatechin gallate contents ($p < 0.05$).

KEYWORDS: Brazilian green tea; antioxidant activity; epigallocatechin gallate; catechin; freeze-dried extract

INTRODUCTION

Green tea is an oriental millenary beverage; however, its consumption has increased in all countries because of its well proven nutraceutical benefits (*1*). Green tea contains many bioactive substances such as the catechins and methylxanthines, (–)-epigallocatechin gallate (EGCG) being the most abundant and biologically active green tea catechin (*2, 3*). This tea is derived from the leaves of *Camellia sinensis* that are exposed to steam or dry heat to inactivate the oxidative enzymes. The major producers of *C. sinensis* are China and India, whose teas are the subject of many studies (*4, 5*). Otherwise, the cultivation of tea on a large scale has spread subsequently to many other parts of Asia, Russia, Africa, and South America (*6*). Many factors, such as geographical location, cultivar species, season, age of the leaves, climate, and horticultural practices (soil, water, minerals, fertilizers) may influence the composition of tea (*5, 7*). Because of these factors, the chemical characterization and bioactivity evaluation of teas produced by new climate and edaphical site conditions, such as the Australian tea, Azorean tea, Brazilian tea, and Malaysian tea, are very important, considering that these studies are currently scarce (*8–12*).

The cultivation of *C. sinensis* var. *assamica* in Brazil is restricted to Vale do Ribeira in the State of São Paulo, where most of the harvest is used for black tea production. Recently, Brazilian farmers have started to produce green tea as well, to supply the new coming market, using the Brazilian cultivar *C. sinensis* var. *assamica* IAC-259 (*11*). Teas produced from var. *assamica* had higher polyphenol content (30%) than those from var. *sinensis* (20%) (*12, 13*).

The production of green tea extract was expanded in recent years to produce soft drinks, dietary supplements, and cosmetics to give these a healthier appeal to the consumers (*2, 3, 14, 15*). Many procedures for extract optimization of tea are found in the literature, such as ultrasound-assisted extraction (UAE), pressurized liquid extraction, microwave-assisted extraction, and supercritical fluid extraction (*15–17*). UAE has been widely used to isolate bioactive substances from different parts of plants (*17*) and is attributed to the disruption of cell walls, particle-size reduction, and enhanced mass transfer of the cell contents as a result of cavitation bubble collapse. Studies have shown that the sonication method using organic solvents is an alternative means of increasing the speed of sample extraction (*18, 19*).

Various biological effects have been attributed to green tea polyphenols as the source of antioxidant activity (AOA) (*1, 2*).

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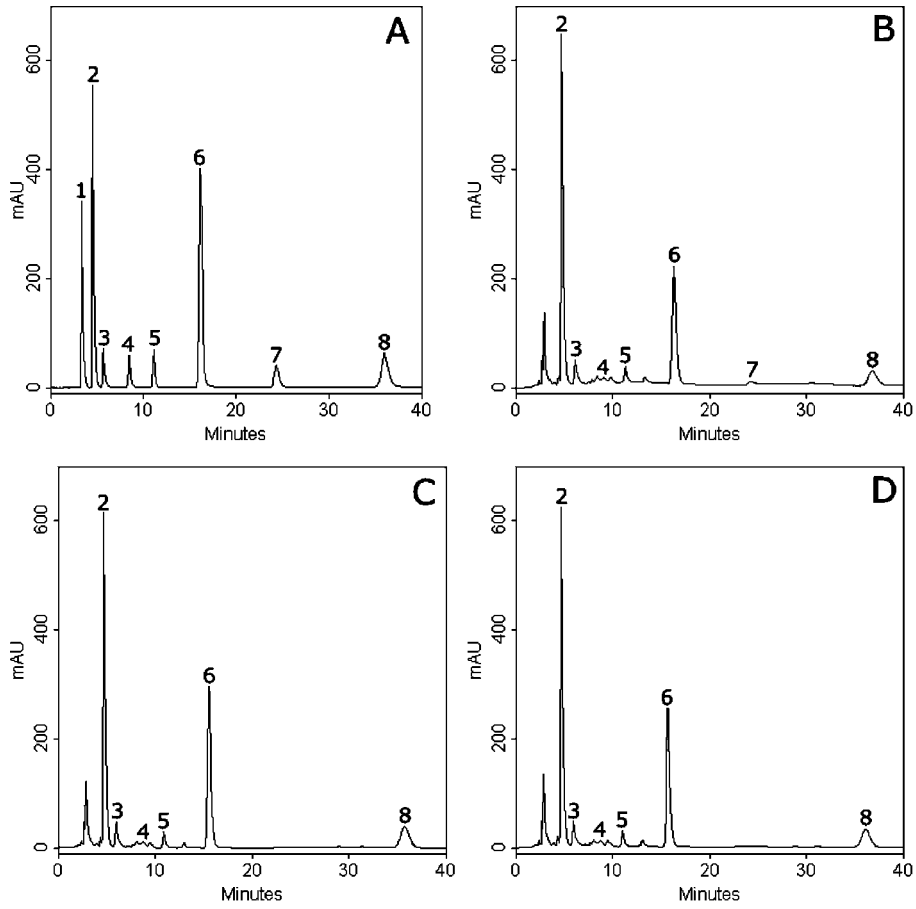

Figure 1. **A**. HPLC chromatogram of standard solutions: (1) gallic acid (76 $\mu g \cdot mL^{-1}$), (2) caffeine (136 $\mu g \cdot mL^{-1}$), (3) epigallocatechin (363 $\mu g \cdot mL^{-1}$), (4) catechin (75 $\mu g \cdot mL^{-1}$), (5) epicatechin (113 $\mu g \cdot mL^{-1}$), (6) epigallocatechin gallate (571 $\mu g \cdot mL^{-1}$); (7) gallocatechin gallate (76 $\mu g \cdot mL^{-1}$), and (8) epicatechin gallate (150 $\mu g \cdot mL^{-1}$). **B**. Chromatogram of the BGTE from Sum1 obtained by system 1. **C**. Chromatogram of the BGTE from Sum1 obtained by system 2. **D**. Chromatogram of the BGTE from Sum1 obtained by system 3. Detection was at 280 nm. See chromatographic conditions in the Materials and Methods section.

Herein, we used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test to compare the AOA of tea leaves from a Brazilian cultivar submitted to three extraction systems. This method is based on the bleaching of the stable radical of DPPH (*20*), and it was applied to determine the antioxidant power of wine, orange juices, fruits, and teas (*21*, *22*).

This paper presents the comparison of the chemical content of Brazilian green tea extracts (BGTE) prepared by three extraction systems using leaves harvested in spring and summer. The extraction systems tested were the hot water extraction and the UAE systems, using different solvents. The contents of caffeine and six catechins (catechin, epicatechin, epigallocatechin, epicatechin gallate, EGCG, gallocatechin gallate) together with the AOA in vitro of BGTE are also presented.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol and ethyl acetate (liquid chromatography (LC) grade), acetone p.a., and *ortho*-phosphoric acid p.a. were obtained from Vetec (Rio de Janeiro, RJ, Brazil). AA and gallic acid (GA) were obtained from Merck (Darmstadt, Germany). Caffeine (CAF) was obtained from Nuclear (São Paulo, SP, Brazil). (+)-Catechin (C), DPPH, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), EGCG, and (−)-gallocatechin gallate (GCG) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Ultrapure water was obtained with the Milli-Q plus system from Millipore (Billerica, MA, U.S.A.).

Plant Material. Brazilian green tea leaves (*C. sinensis* var. *assamica* IAC-259 cultivar), comprising a bud and three leaves, were harvested between spring 2005 (right after the first new shoots) and summer 2006 in the same plantation of Vale do Ribeira in the State of São Paulo, Brazil. Plants were cultivated in an open field. The samples (1 kg) were named Sum1 (harvested on February 1, 2005), Sum2 (January 16, 2006), Sum3 (March 1, 2006), Spr1 (October 5, 2005), Spr2 (October 17, 2005), and Spr3 (November 5, 2005). The vegetable material was dried as follows: plucked leaves were steamed for 40–60 s and then curled and dried in hot air at 90–110 °C for 40–50 min. This primary drying and rolling process reduced the leaf moisture from 76% to about 50%. The leaves were rolled for another 15 min without heat and then pressed and dried for 30–40 min in hot air at 50–60 °C. This secondary drying reduced the moisture content of the tea leaves to about 30%. Further curling was followed by the third drying stage in which the tea leaves were dried directly on a hot pan at 80–90 °C and twisted for 40 min under pressing and rolling by a curling hand mounted on a pan. Finally, tea leaves were dried at 80 °C until a moisture content of 6% was achieved.

Hot Water Extraction—System 1. Conventional extraction by hot water (*15*) using a heating magnetic stirrer (VELP Scientifica model ARE, Italy) was used as a reference method compared to the UAE method. Ground tea leaves (2.5 g) were soaked in 100 mL of water (80 ± 2 °C) for 20 min, the rotational speed of magnetic stirring being set to about 250 rpm, and then centrifuged at 3500 rpm (2000*g*) for 5 min. The supernatant was filtered and kept at −20 °C for 24 h until freeze-drying (BOC Edwards model Modulyo 4K, Brazil) to obtain the BGTE.

UAE—Systems 2 and 3. Ground tea leaves (5.0 g) were transferred to a 200 mL Erlenmeyer flask, and 100 mL of extraction solution (system 2, water/acetone, 1:1 (v/v); system 3, water/ethanol, 75:25 (v/v)) was added. The flask was then immersed in an ultrasonic bath (Thornton model T50, Brazil) set to 30 min, and the temperature was controlled so as not to exceed 30 °C. Afterwards, the extract was

Table 1. Composition (% w/w, dry weight) of Major Components[a] in the BGTE[b] Obtained by Three Extraction Systems and Measured by HPLC-DAD

	CAF	EGC	C	EC	EGCG	GCG	ECG	TC
				BGTE—System 1				
Sum1	5.41 ± 0.02[c]	11.74 ± 0.52	1.50 ± 0.04	3.30 ± 0.06	13.83 ± 0.14	0.85 ± 0.05[c]	3.67 ± 0.14	34.92
Sum2	7.02 ± 0.02[c]	7.02 ± 0.02	1.60 ± 0.06	2.95 ± 0.01	10.62 ± 0.15	0.56 ± 0.00[c]	3.57 ± 0.01	26.46
Sum3	6.50 ± 0.00[c]	7.98 ± 0.02	0.70 ± 0.00	2.29 ± 0.02	13.59 ± 0.01	0.70 ± 0.00[c]	3.17 ± 0.06	28.43
Spr1	4.56 ± 0.01	19.04 ± 0.12	0.53 ± 0.04	3.53 ± 0.01	12.29 ± 0.32	0.57 ± 0.00	2.51 ± 0.01	38.47
Spr2	4.88 ± 0.04	15.08 ± 0.10	1.58 ± 0.05	5.13 ± 0.00	9.86 ± 0.15	0.39 ± 0.01	3.07 ± 0.06	35.11
Spr3	5.68 ± 0.01	9.42 ± 0.03	1.80 ± 0.00	2.84 ± 0.02	14.28 ± 0.10	0.50 ± 0.01	3.43 ± 0.01	32.27
				BGTE—System 2				
Sum1	5.31 ± 0.01	13.21 ± 0.23	1.62 ± 0.04	3.21 ± 0.03	18.58 ± 0.00	nd	5.11 ± 0.05	41.73
Sum2	6.84 ± 0.06	7.27 ± 0.01	1.20 ± 0.06	2.81 ± 0.02	14.02 ± 0.05	nd	4.99 ± 0.01	30.29
Sum3	6.68 ± 0.02	8.91 ± 0.20	0.91 ± 0.00	2.78 ± 0.05	20.13 ± 0.35	nd	4.75 ± 0.02	37.48
Spr1	4.70 ± 0.01	17.57 ± 0.09	1.11 ± 0.03	4.08 ± 0.07	18.69 ± 0.19	nd	4.41 ± 0.22	45.86
Spr2	4.84 ± 0.01	16.75 ± 0.03	1.58 ± 0.02	4.76 ± 0.01	16.21 ± 0.18	nd	4.66 ± 0.01	43.95
Spr3	6.01 ± 0.05	9.37 ± 0.09	1.61 ± 0.02	2.86 ± 0.03	19.91 ± 0.06	nd	5.02 ± 0.07	39.26
				BGTE—System 3				
Sum1	5.40 ± 0.03[c]	13.00 ± 0.28	1.38 ± 0.01	3.31 ± 0.01	16.72 ± 0.05	nd	4.40 ± 0.03	38.81
Sum2	6.85 ± 0.08[c]	7.31 ± 0.01	1.53 ± 0.03	2.97 ± 0.00	12.03 ± 0.04	nd	4.22 ± 0.06	28.06
Sum3	6.43 ± 0.03[c]	8.77 ± 0.01	0.32 ± 0.01	2.44 ± 0.04	16.18 ± 0.08	nd	3.85 ± 0.01	31.57
Spr1	4.61 ± 0.02	23.04 ± 0.22	0.14 ± 0.01	3.64 ± 0.04	16.38 ± 0.36	nd	3.63 ± 0.04	47.06
Spr2	4.76 ± 0.00	15.64 ± 0.20	1.47 ± 0.01	4.99 ± 0.18	12.26 ± 0.13	nd	3.69 ± 0.01	38.06
Spr3	5.69 ± 0.16	9.16 ± 0.04	1.24 ± 0.01	2.76 ± 0.05	16.45 ± 0.06	nd	4.07 ± 0.00	34.09

[a] CAF, caffeine; EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate; ECG, epicatechin gallate; TC, total catechins. [b] Each value represents the mean ± SD of three individual determinations. [c] Significant difference, comparison between seasons by Student *t* test, $p < 0.05$; nd, not detectable.

Table 2. Comparison of Efficiency Extraction of Major Components[a] (% w/w, dry weight) among Extraction Systems and EGCG/CAF and ECG/CAF Ratios in the BGTE[b]

extraction system	CAF	EGC	C	EC	EGCG	GCG	ECG	TC	EGCG/CAF ratio[b]	ECG/CAF ratio[b]
1	5.68 ± 0.38[a]	11.74 ± 1.87[a]	1.29 ± 0.22[a]	3.34 ± 0.40[a]	12.42 ± 0.75[b]	0.60 ± 0.06	3.24 ± 0.17[c]	32.62 ± 1.84[a]	2.23 ± 0.44[b]	0.58 ± 0.07[c]
2	5.73 ± 0.38[a]	12.18 ± 1.77[a]	1.34 ± 0.12[a]	3.42 ± 0.34[a]	17.92 ± 0.97[a]	nd	4.82 ± 0.11[a]	39.76 ± 2.26[a]	3.20 ± 0.64[a]	0.86 ± 0.11[a]
3	5.62 ± 0.36[a]	12.82 ± 2.40[a]	1.01 ± 0.25[a]	3.35 ± 0.37[a]	15.00 ± 0.91[b]	nd	3.98 ± 0.12[b]	36.28 ± 2.71[a]	2.73 ± 0.61[ab]	0.72 ± 0.08[b]

[a] CAF, caffeine; EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate; ECG, epicatechin gallate; TC, total catechins. [b] Each value represents the mean ± SD of six individual determinations. Values in each column with the same letter were not significantly different by ANOVA (SNK), $p < 0.05$. nd, not detectable.

centrifuged for 5 min at 3500 rpm (2000*g*), and the supernatant was filtered. Then, the organic solvent was eliminated by using a rotary evaporator (Büchi model R-114, Switzerland) at a temperature below 30 °C. The resulting aqueous solution was kept at −20 °C for 24 h until freeze-drying to obtain the BGTE.

Mass Yield among Extraction Systems. Mass yields (% w/w, dry weight) were determined for the three extraction systems, according to the methodology above, using Sum1 as a sample. Mass yields among the extraction systems were compared by analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) method and presented as the mean values ± SD of 13 individual determinations.

High Performance Liquid Chromatography–Diode Array Detector (HPLC-DAD) Analysis. The analysis was performed on a Shimadzu (Kyoto, Japan) LC-10ADvp equipped with an SPD-M10Avp diode array detector, a DGU-14A degasser, an SCL-10Avp system controller, and a Class-VP v.6.14 chromatography data system. A Gemini RP-18 column (150 mm × 4.6 mm i.d., 5 μm, 110 Å) protected by a precolumn RP-18, both from Phenomenex (Torrance, CA, U.S.A.), were used throughout this study. The mobile phase was composed of a water/methanol/ethyl acetate (850:130:22.5 v/v/v) mixture, and the aqueous phase was adjusted to pH 2.25 with 20% *ortho*-phosphoric acid. It was employed at a flow rate of 1.7 mL min^{-1} as well as an injection volume of 20 μL, and peaks were detected at 280 nm. This HPLC method was previously validated by us (*11*). CAF and catechin standards (catechin, EC, EGC, ECG, EGCG, and GCG) and the BGTE were dissolved in the mobile phase and sieved through a 0.45 μm Millipore filter before the injection in the HPLC system. Each calibration curve used five concentrations of CAF and catechins. The results represent the average of three injections of each concentration. Total catechin (TC) content was the sum of the dry weight (%) of the catechins determined herein by HPLC in the BGTE.

DPPH Assay. The free radical scavenging capacity of the standards and the BGTE were determined using the DPPH discoloration method (*20*). Five concentrations of the BGTE, AA, GA, and the catechin standards (catechin, EC, EGC, ECG, EGCG, and GCG) in methanol were used to obtain the curves and calibration curves, respectively (**Table 3**). Dilutions of BGTE and standards (500 μL) in methanol were added to 1000 μL of DPPH (10 mg/100 mL MeOH) and allowed to stand for 30 min before absorbance was measured at 515 nm using a Shimadzu spectrophotometer model UV-1602PC (Kyoto, Japan). The DPPH solution (1000 μL) and methanol (500 μL) were used as a negative control. This experiment was conducted in triplicate. AOA was expressed as IC_{50} (inhibitory concentration in μg/mL of samples or standards necessary to reduce the absorbance of DPPH by 50% compared to the negative control). The lower the IC_{50}, the higher the AOA. Results were also expressed as AEAC (AA equivalent antioxidant capacity) in grams and calculated as follows:

$$\text{AEAC } (\mu\text{g AA/g}) = IC_{50(AA)}/IC_{50(sample)} \times 1\text{ g}$$

Statistical Analysis. The tests were in triplicate, and their mean values were presented. Student *t* test, ANOVA, and SNK tests were carried out with the SPSS 8.0 for Windows (SPSS Inc., 1997) software. Differences in *p* value ≤0.05 were considered significant.

RESULTS AND DISCUSSION

The methods used herein to extract green tea leaves were chosen according to those found in the literature. Pan et al. (*15*) described that extractions using an aqueous mixture of acetone or ethanol and a liquid/solid ratio above 20:1 (mL g^{-1}, solvent/plant) were sufficient to reach the high extraction of polyphenols

Table 3. Calibration Curves Used To Calculate IC_{50} for DPPH Assay of Phenolic Compounds[a] and the BGTE and the AEAC

	linear range ($\mu g \cdot mL^{-1}$)	slope	intercept	correlation coefficient (r)	IC_{50} ($\mu g \cdot mL^{-1}$)[b]	AEAC ($\mu g \cdot g^{-1}$)
AA	2.0–12.0	−8.2065	103.670	0.9983	6.54 af	1.0000
GA	1.0–4.0	−26.964	127.420	0.9887	2.87 b	2.2787
C	2.0–10.0	−6.7130	104.770	0.9891	8.16 acf	0.8015
EC	1.0–10.0	−6.4603	88.389	0.9973	5.94 deh	1.1010
EGC	2.0–11.0	−8.3386	97.275	0.9985	7.26 def	0.9008
ECG	2.0–10.0	−6.8906	100.000	0.9967	5.67 egh	1.1535
EGCG	1.0–6.0	−13.1640	105.150	0.9989	4.19 bg	1.5608
GCG	2.0–9.0	−10.6320	105.270	0.9981	5.20 gh	1.2577
			BGTE—System 1			
Sum1	7.0–10.0	−4.2236	107.630	0.9918	13.64	0.4795
Sum2	6.0–19.0	−3.6397	105.690	0.9987	15.30	0.4275
Sum3	7.0–20.0	−3.8758	109.010	0.9990	15.23	0.4294
Spr1	6.0–19.0	−2.5027	87.820	0.9921	15.11	0.4328
Spr2	5.0–15.0	−3.6576	107.900	0.9972	15.83	0.4131
Spr3	7.0–20.0	−4.2820	108.910	0.9898	13.76	0.4753
			BGTE—System 2			
Sum1	2.0–15.0	−4.7567	89.634	0.9991	8.33	0.7851
Sum2	4.0–13.0	−4.0630	92.038	0.9761	10.35	0.6319
Sum3	2.0–15.0	−5.0852	92.777	0.9910	8.41	0.7776
Spr1	4.0–13.0	−5.4775	100.260	0.9964	9.18	0.7124
Spr2	5.0–14.0	−4.1514	103.520	0.9906	12.89	0.5074
Spr3	5.0–14.0	−4.1662	96.513	0.9873	11.16	0.5860
			BGTE—System 3			
Sum1	8.0–16.0	−5.7558	135.390	0.9984	14.84	0.4407
Sum2	5.0–16.0	−4.3446	114.270	0.9964	14.79	0.4422
Sum3	8.0–16.0	−4.8808	128.570	0.9910	16.10	0.4062
Spr1	8.0–16.0	−4.3650	115.530	0.9900	15.01	0.4357
Spr2	8.0–16.0	−5.4819	135.100	0.9906	15.62	0.4187
Spr3	5.0–16.0	−4.4534	114.290	0.9964	14.44	0.4529

[a] AA, ascorbic acid; GA, gallic acid; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate. [b] Values in columns with the same letters were not significantly different by ANOVA (SNK), $p < 0.05$. Antioxidant activities of the Brazilian green tea samples were not significantly different.

Table 4. Correlation Coefficient among the IC_{50} for DPPH Assay of the BGTE ($n = 18$) and Their Corresponding Content of Each Analyzed Phenolic Standard

	correlation coefficient of IC_{50} (DPPH)
IC_{50} (DPPH)	1
catechin (C)	−0.461031403
total catechins (TC)	−0.628436337[a]
epicatechin (EC)	−0.268363823
epigallocatechin (EGC)	−0.155065167
epicatechin gallate (ECG)	−0.824081491[a]
epigallocatechin gallate (EGCG)	−0.818776633[a]

[a] Significant correlation by Student t test, $p < 0.05$.

and CAF from green tea leaves. Perva-Uzunalić et al. (*16*), studying extraction efficiency of major catechins and CAF from green tea leaves, described that the highest extraction efficiency using water was obtained at 80 °C after 20–30 min of extraction and a ratio of 40:1 (mL g^{-1}, solvent/plant). We decided to use these last extraction conditions as the reference method in comparison to the UAE.

UAE conditions were initially evaluated by using Sum1 as the sample. Some solvents (water; water/acetone, 1:1 v/v; water/ethanol, 75:25 v/v) and times of extraction (30, 60, and 90 min) were investigated. The extraction using water and sonication had the lowest mass yield; besides, there were no significant differences when using more than 30 min of extraction among all the solvents tested (data not shown). Consequently, the above binary solvents used with a 30 min extraction time were chosen as sonication extraction conditions.

The selected extraction conditions were further evaluated by examining the extraction efficiency of the major components (CAF and catechin contents) from green tea leaves.

Comparison of Mass Yield among Extraction Systems. On the basis of mass yield, UAE using water/acetone (1:1 v/v) showed the highest extraction efficiency with a yield of 36.29 ± 1.03% (w/w, $p \leq 0.05$). The hot water extraction and the UAE using water/ethanol (75:25 v/v) presented yields of 33.66 ± 0.73% and 32.98 ± 0.81%, respectively, that were not significantly different ($p \leq 0.05$). The sonication method of extraction proved to be a useful and rapid procedure for green tea analysis using a low temperature of extraction.

Contents of CAF and Catechins in Extracts. The BGTE were quantified by HPLC-DAD as stated by a previous paper of ours (*11*). This method was validated as having intraday relative standard deviation (RSD) in the range 0.14–3.49%, interday relative standard deviation in the range 1.42–6.65% according to the component, and recovery in the range of 96–110% also according to the component. The HPLC chromatogram of the analyzed standards obtained under the chromatographic conditions described above is presented in **Figure 1A**. The chromatograms of BGTE from Sum1 obtained using extraction systems 1, 2, and 3 are shown, respectively, in **Figures 1B**,**C**,**D**; differences about GCG content could be observed.

The contents of major constituents of green tea samples in relation to seasons and extraction methods are presented in **Table 1**. It also presents the TC content. The catechins and CAF contents were determined in the BGTE obtained by using the hot water extraction (system 1), the sonication and water/acetone (1:1 v/v) extraction (system 2), or the sonication and water/ethanol (75:25 v/v) extraction (system 3). BGTE presented a heterogeneous composition of catechins and CAF between the seasons. Summer samples obtained by systems 1 and 3 had a significantly higher content of CAF when compared to spring

samples (Student t test, $p \leq 0.05$). Otherwise, there was no significant variation relating the catechins between the seasons. Epigallocatechin gallate was the major component in the BGTE, followed in the order of contents (% w/w, dry weight) by epigallocatechin > caffeine > epicatechin gallate > epicatechin > catechin > gallocatechin gallate.

Contents of CAF and Catechins Related to the Extraction Systems. The statistical analyses for extraction efficiency considering the three extraction systems are shown in **Table 2**. Only EGCG and ECG had higher mean yields by extraction system 2 than by systems 1 and 3 ($p \leq 0.05$). This demonstrated that the extraction method using sonication and water/acetone (1:1 v/v, system 2) could be more efficient to extract the major components than the usual method of decoction. Wang and Helliwell (*23*) and Ikeda et al. (*24*) observed heat epimerization at the 2-position in the ring C of catechins when either decoction or pasteurization was used. This epimerization is undesirable because a large amount of the most important catechin, EGCG, is transformed into GCG (*7*).

Moreover, considering the ratios of EGCG/CAF (EGCG/CAF) and ECG/CAF (ECG/CAF), the BGTE obtained by system 2 contain more than three times the amount of EGCG than CAF (% w/w, dry weight, $p < 0.05$, **Table 2**). These results could be useful when searching to do an extract with a smaller ratio of CAF considering that several studies in the literature attempt to reduce its content in tea (*25–27*).

AOA of Phenolic Compounds and BGTE. Prior to calculating the IC_{50}, calibration curves of each standard and curves of BGTE (collected in summer and spring and by using the three extraction methods) were determined by a DPPH assay using five data points. The curves proved to be linear in the concentration range shown in **Table 3** using linear regression and resulted in a correlation coefficient (r) > 0.98 to standards and $r > 0.97$ to samples.

AOA and AEAC of the standards and the BGTE are shown on **Table 3**. Antioxidant activities of BGTE were not significantly different among seasons and extraction systems. We also present the significant differences among standards in relation to the AOA ($p < 0.05$). The greatest antioxidant activities among standards were observed for GA and EGCG followed by GCG, ECG, and EC. In addition, AA, EGC, and (+)-catechin showed lesser AOA than the other phenolic compounds assayed. These results suggested the importance of the galloyl residue to the AOA in these series of compounds. Additionally, in relation to nongalloylated catechins, it is interesting that EC is more active than its 3-epimer catechin, suggesting that the configuration may play a role in the AOA of these latter compounds.

Correlations among AOA of the BGTE and Their Contents in Phenolic Compounds. Because system 2 proved to be more efficient to extract EGCG and ECG (**Table 2**) and these compounds demonstrated the best AOA, we wondered whether the observed AOA could be more correlated to the content of both compounds. Having this in mind, we determined the correlation coefficient (r) among the IC_{50} of the BGTE ($n = 18$) and their corresponding content of each phenolic standard (**Table 4**). Only ECG, EGCG, and the TC contents were significantly correlated with the AOA of the samples (Student t test, $p < 0.05$).

Some studies have suggested that EGCG could be used as a quality indicator of black tea (*7*, *28*). In this study, we show that EGCG and ECG contents could also be used as better fingerprints for Brazilian green tea when a raw material with maximum AOA is wished.

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