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Chemical Composition and Antioxidant Activity of Yerba-Mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) Extract as Obtained by Spray Drying

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ABSTRACT: Yerba-mate or maté (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) leaves are typically used for their stimulant, antioxidant, antimicrobial, and diuretic activity, presenting as principal components polyphenolic compounds. In this study, the objective was to develop a yerba-mate dry extract by using spray drying technology and to evaluate the dry extract antioxidant activity and chemical composition. The results obtained by means of the DPPH assay show that the extract presents an IC_{50} of 2.52 mg/mL. The yerba-mate spray-dried extract presents high catalase-like activity, suggesting that it is a strong free-radical scavenger. The antioxidant activity as expressed as catalase-like activity was related to total polyphenol content. In addition, the results show that the spray-dried extract presents high polyphenol content, namely, high concentrations of caffeic acid (1.54 mg/g), 5-caffeoylquinic acid (91.40 mg/g), rutin (5.38 mg/g), and total phenolics (178.32 mg/g), which justifies its high antioxidant activity.

KEYWORDS: food technology, catalase-like, polyphenols, erva-mate, *Ilex paraguariensis*

INTRODUCTION

The yerba-mate or maté (*Ilex paraguariensis* A.St.-Hil.) is a tree of the Aquifoliaceae family. The Ilex genus comprises about 450 species which are naturally distributed in tropical regions (Asia and South America) and in temperate zones as well.[1] The yerba-mate tree occupies a 540,000 km^2 area located between Brazil, Argentina and Paraguay.[2] The "chimarrão", an infusion of yerba-mate, is widely consumed is this region, representing part of the local culture. Yerba-mate is listed by Council of Europe as a natural source of food flavoring. The category N2 for flavors indicates that yerba-mate can be added to foodstuffs in small quantities, with a possible limitation of an active principle in the final products. In the South America, yerba-mate tea-like is commonly consumed as a beverage. It is stated to be less astringent than tea (*Camelia sinensis*). In the USA, yerba-mate is listed as GRAS (generally recognized as safe).[3]

Previous studies dealing with yerba-mate phytochemistry point out the presence of different chemical groups, such as saponins, alkaloids, phenolics and essential oil. The leaves also present vitamins (A, C, B1 and B2), magnesium, calcium, iron, sodium, and potassium.[4,5] Phytochemical investigations on yerba-mate reported many classes of caffeoyl derivatives and flavonoids as chlorogenic acid, caffeic acid, 5-caffeoylquinic acid and rutin.[6,7] The substances contained in yerba-mate present functional and pharmaceutical properties, such as antioxidant, stimulant, antimicrobial, and diuretic. Beyond the traditional "chimarrão" preparation, yerba-mate has the potential to be used in the cosmetic, pharmaceutical and food industries.[3,4]

Phenolic compounds are natural antioxidants contained in vegetables in their free form and also bonded with sugars (glucosides) and proteins,[5,8,9] representing the most abundant antioxidants in the human diet. The quantities of total polyphenols that are consumed daily throughout the world is about 1 g/day, for a diet that includes fruits, vegetables, tea and red wine. Due to their abundant distribution in the human diet, the phenolics have received much attention since the past decade. Epidemiology studies have shown an inverse association between the daily consumption of fruits and vegetables and the risk to diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases.[10] The protective effects of fruits and vegetables have long been attributed to their antioxidant compounds, such as polyphenols, carotenoids, and vitamins C. Antioxidants act in various ways, which include the complexation of redox-catalytic metal ions, scavenging of free radicals and decomposition of peroxides.[8−10]

Yerba-mate is a potential source of polyphenols. Aqueous extract of *Ilex paraguariensis* is a typical antioxidant-containing beverage largely consumed in several South American countries. The mate is prepared as a hot infusion of the dried and minced leaves of yerba-mate and is very prized for its bitter taste and antioxidant properties. The yerba-mate phenolics present *in vitro* and *in vivo* antioxidant activity, being able to scavenge free radicals and reactive oxygen species (ROS).[6,11,12] Hydrogen peroxide (H_2O_2) is a ROS that is a substrate for the generation of more active biologically reactive oxygen species. In normal

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health conditions, antioxidant enzymes, enzyme cofactors and antioxidant substances naturally scavenge these free radicals. The most important antioxidant enzymes are catalase and superoxide dismutase. Low levels of these enzymes are implicated in degenerative and chronic diseases.[13,14]

In foods, antioxidants are added to minimize changes in nutritional value, color, flavor, and aroma. Antioxidants can protect the body against damage caused by free radicals and degenerative diseases. Synthetic antioxidants usually employed in industry are effective and stable, but their use is limited in many countries because they are not considered completely safe for human health.[12,15]

Yerba-mate is exported to the United States, Asia and Europe as an herbal drug (dry leaf) or extracts used in different preparations (food, pharmaceutical and cosmetic). There is an increasing number of yerba-mate products being developed, as well as a growing interest in this product by countries whose population does not traditionally consume yerba-mate tea-like. In this study, the objective was to develop a yerba-mate dry extract by using spray drying technology and to evaluate its chemical composition, antioxidant activity and catalase-like activity.

MATERIALS AND METHODS

Reagents. The reagents used were of analytical grade. Folin−Ciocalteau, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl, catechin, caffeic acid, 5-caffeoylquinic acid, rutin, theobromine and caffeine were obtained from Sigma Chemical Co. (St. Louis, MO). HPLC-grade methanol and acetic acid were from Tedia (Fairfield, OH, USA). The membrane filter 0.45 μm was from Millipore (Bedford, USA). All other chemicals were purchased from E. Merck (Darmstadt, Germany).

Plant Material. The cropping system of yerba-mate is dense, combining native forest with replanting of plants. Our sample consisted of 100 kg of 15-year old, mature yerba-mate (*Ilex paraguariensis* A. St.-Hil.) leaves, harvested in São Mateus do Sul city, Paraná State, Brazil, in May 2010. The samples were donated by the company Baldo S/A. The fresh leaves underwent several stages of processing, namely, blanching (a rapid drying at 700 °C/30 s), partial drying (at 300 °C/2 min), drying (at 95 °C/6 h), and milling (passing through a 60 mesh sieve). The exsiccate was identified by Professor Dra. Elide Pereira dos Santos of the Botany Dept of Federal University of Paraná (UFPR). Voucher Lenchinski, L.F., n. 1; collected in 16/11/2010, herbarium (UPCB).

Extraction and Spray Drying. For obtaining the liquid extract, 10 kg of dehydrated yerba-mate leaves (milling 60 mesh) was added with 30 L of potable water and heated for 30 min at 85 °C. The obtained liquid extract was fed into a spray dryer that presented the following characteristics: model K 22/27, KOHLS Co., equipped with a 3 mm atomizer. The assay conditions were as follows: inlet air temperature of 185 °C, outlet air temperature of 83 °C, air pressure of 4.5 bar, drying air flow rate of 5.5 m^3/h and feeding solution inlet rate of 200 g/min.

Chemical Composition. Protein, lipid, moisture, total ash and dietary fiber were determined through AOAC methods.[16] The total carbohydrate content was calculated by the difference between 100 and the relative percent sum (in w/w) of the protein, lipid, moisture, ash, and dietary fiber. Energy values (kilocalories) were obtained by applying factor 4, 9, and 4 for each gram of protein, lipid and carbohydrate.[17]

Determination of Total Phenolics. The content of total phenolic compounds was determined by using the Folin−Ciocalteau colorimetric method with slight modifications.[18] A sample of 5 g was diluted in 100 mL of ethanol solution at 50%. An aliquot of 0.2 mL was transferred into a test tube containing 1.0 mL of Folin−Ciocalteau reagent. This mixture was hand-shaken, and, after 5 min of rest, 0.8 mL of 7.5% (v/v) sodium carbonate was added. After incubation for 30 min in the absence of light, the absorbance at 765 nm was measured in a spectrophotometer (model UV-1700, Shimadzu, Kyoto, Japan). The content of total phenolics was derived by comparison with a catechin standard curve (0.1; 0.3; 0.6; 1.25; 2.5; and 7.5 mg/mL $r^2 = 0.9948$) and expressed as mg of catechin equiv/g of sample.

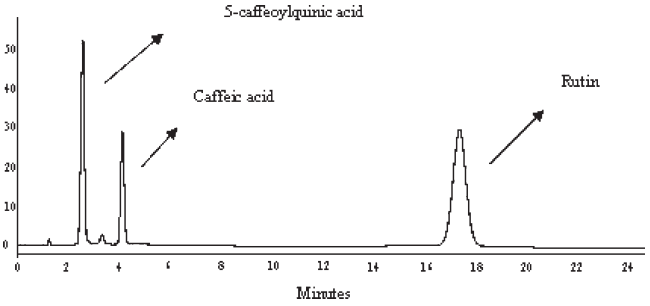

Figure 1. Chromatogram of the polyphenols.

Determination of Selected Phenolics by HPLC. A sample of 2 g was mixed with 100 mL of ethanol solution at 50% (v/v) and was let to rest for 12 h at room temperature. Each sample was extracted three times with 25 mL each, for 30 min in reflux. The three parts extracted by reflux were mixed and completed the volume to 250 mL in a volumetric flask. The obtained samples were filtered through a membrane filter (Millipore 0.45 μm). These samples were submitted to high performance liquid chromatography (HPLC) analysis through dilution in the mobile phase. A Shimadzu chromatograph equipped with a 20 μL Rheodyne injector, a LC-10AD pump and a SPD-10A UV−vis detector operating at 325 and 370 nm were used, together with the Class-VP software. A Phenomenex Bondclone C-18 (3.9 × 300 mm), 10 μm was used as column, where methanol−water (35:65 v/v with 0.5% acetic acid) was used as mobile phase at a 1 mL/min flux.[19] For the quantitative evaluation, a standard calibration curve was obtained by plotting the area of generated peaks against different concentrations (5.0−15.0 μg/mL; $r^2 = 0.9986$) of rutin, (0.5−10.0 μg/mL; $r^2 = 0.9981$) of caffeic acid, and (10.0−100.0 μg/mL; $r^2 = 0.9992$) of 5-caffeoylquinic acid (Figure 1). All analyses were made in triplicate.

Determination of Methylxanthines by HPLC. A sample of 2 g was mixed with 4.0 mL of H_2SO_4 and was let for 15 min in a hot water bath. To each sample, 50 mL of boiled water was added, and the mixture was let to rest in hot water bath for another 15 min. Samples were filtered with filtering paper (Whatman No. 1), neutralized to pH 7.0 with NaOH 40% (w/v), transferred to a volumetric flask and the volume was then completed to 250 mL with water. The obtained samples were filtered through a membrane filter (Millipore 0.45 μm). These samples were submitted to high performance liquid chromatography (HPLC) analysis through dilution in the mobile phase. A Shimadzu chromatograph equipped with a 20 μL Rheodyne injector, a LC-10AD pump and a SPD-10A UV−vis detector operating at 272 nm was used, together with the Class-VP software. A Phenomenex Sinergi Hidro-RP 80A (3.9 × 150 mm), 4 μm was used as column, where methanol−water (25:75 v/v) was used as mobile phase at a 1 mL/min flux.[20] For the quantitative evaluation, a standard calibration curve was obtained by plotting the area of the generated peaks against different concentrations of caffeine (10.0−100.0 μg/mL; $r^2 = 0.9978$) and theobromine (0.5−4.0 μg/mL; $r^2 = 0.9939$) (Figure 2). All analyses were made in triplicate.

Antioxidant Activity. The antioxidant activity of the spray-dried yerba-mate extract was measured by *in vitro* assays, namely, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^•$) scavenging activity and enzyme-like activities (catalase-like and superoxide dismutase-like). DPPH$^•$ radical scavenging activity was measured using a method modified from that Yamaguchi et al.[21] in which 200 μL of spray-dried extract of yerba-mate

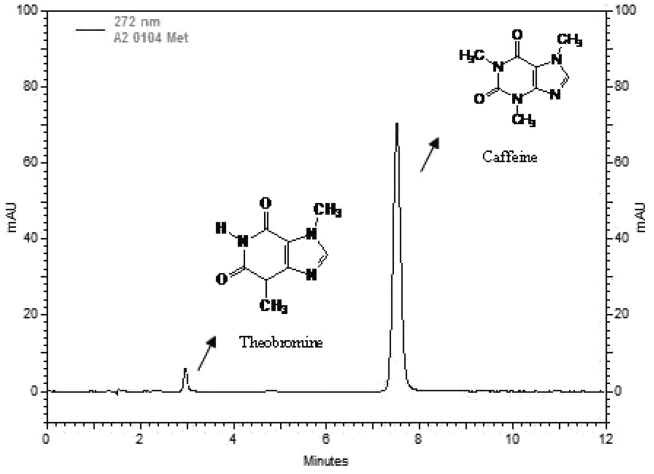

Figure 2. Chromatogram of the methylxanthines.

solutions, in different concentrations (10, 15, 20, 25, 30, 35, 40, 45, and 75 mg/mL), were added to 800 μL of a buffer solution (Tris HCl 100 mM, pH 7.4). To such a mixture was added 1000 μL of a 500 μM DPPH ethanolic solution. Tubes were stored in the dark for 20 min, after which absorbance was measured at 517 nm (model UV-1700 spectrophotometer, Shimadzu, Kyoto, Japan). The blank solution was composed of distilled water in substitution to the extract of yerba-mate. Ascorbic acid (10.0−75.0 mg/mL; $r^2 = 0.9962$) was used as positive control. Results were expressed as IC_{50}, i.e., amount of yerba-mate extract necessary to scavenge 50% of the DPPH radical ($y = -169.78x + 96.06$; $r^2 = 0.9815$).

The CAT-like activity determination was performed through the method adapted by Aebi,[22] where the decomposition of hydrogen peroxide (H_2O_2) is quantified spectrophotometrically at 240 nm. Ascorbic acid (100 μg/mL) was used as positive control. A total of 1 unit of CAT-like activity decomposed 1 mmol of H_2O_2 /minute at pH 7.4.[22] Results are expressed as nmol of hydrogen peroxide/min.

The SOD-like activity of the yerba-mate extract was spectrophotometrically determined by measuring the inhibition of self-catalytic adrenochrome formation rate at 480 nm (model UV-1700 spectrophotometer, Shimadzu, Kyoto, Japan), in a reaction medium containing 1 mmol/L of adrenaline (pH 2.0) and 50 mmol/L of glycine (pH 10.2). This reaction was performed at 30 °C for 3 min. Ascorbic acid (100 μg/mL) was used as positive control. One unit of SOD-like activity is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50%.[23]

Statistical Analyses. The generated data were statistically evaluated by using the MSTAT-C software version 2.10 for DOS (Michigan State University, East Lansing, MI, USA).[24] Mean and standard deviation calculations were performed. Data were subjected to analysis of variance, and means were compared using Tukey's test. Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition. The chemical composition of spray-dried extract and dehydrated yerba-mate leaves (milling 60 mesh) is shown in Table 1. An analysis of Table 1 indicates that the chemical composition of the investigated compounds was strongly affected by the extraction and by the spray-drying method. The results of the chemical analyses revealed that spray-dried extract of yerba-mate increased the carbohydrate, moisture, total ash, and energy contents. The dietary fiber, protein and lipid contents of dehydrated yerba-mate leaves were higher than those observed for the spray-dried extract. The yerba-mate extract powder is also a source of total ash, which yields a high mineral content.

Table 1. Chemical Composition in the Spray-Dried Extract and in Dehydrated Yerba-Mate Leaves[a]

component	spray-dried extract	dehydrated leaves
carbohydrate (g)	80.71 ± 0.39 a	26.07 ± 0.62 b
protein (g)	4.09 ± 0.02 b	7.97 ± 0.17 a
lipid (g)	0.90 ± 0.02 b	4.25 ± 0.29 a
dietary fiber (g)	0.00 ± 0.00 b	52.97 ± 0.59 a
moisture (g)	4.76 ± 0.12 a	3.60 ± 0.42 a
total ash (g)	9.54 ± 0.44 a	5.14 ± 0.15 b
energy (kcal/100 g)	347.27 ± 1.66 a	174.41 ± 0.88 b

[a] The values are mean ± SD ($n = 3$). Means with different letters in the same line were significantly different ($p < 0.05$).

Table 2. Polyphenols and Methyxanthines Contents in the Spray-Dried Extract and in Dehydrated Yerba-Mate Leaves[a]

composition	spray-dried extract (mg/g)	dehydrated leaves (mg/g)
total polyphenols	178.32 ± 7.35 a	96.16 ± 4.12 b
rutin	5.38 ± 0.09 a	3.09 ± 0.49 b
caffeic acid	1.54 ± 0.11 a	0.72 ± 0.13 b
5-caffeoylquinic acid	91.40 ± 1.75 a	24.78 ± 2.05 b
caffeine	18.55 ± 0.80 a	5.59 ± 0.17 b
theobromine	7.14 ± 0.33 a	1.88 ± 0.12 b

[a] The values are mean ± SD ($n = 3$). Means with different letters in the same line were significantly different ($p < 0.05$).

Analyses of Polyphenol Compounds. Quantitative data for polyphenols, shown in Table 2, confirm that the extraction followed by spray-drying significantly increased the total phenolics, rutin, caffeic acid, and 5-caffeoylquinic acid concentrations when compared to dehydrated yerba-mate leaves. The development of the spray-dried extract yielded a final product which is homogeneous and rich in phenolic compounds, being a promising raw material for the food and pharmaceutical industries. Beyond these characteristics, the spray-dried extract might present better technological characteristics. The homogeneity in the chemical compound distribution facilitates the extract standardization for the development of different pharmaceutical and food products.[25]

The concentration of polyphenols in the yerba-mate leaves were above those reported previously. The difference in the yerba-mate chemical composition might be due to climate, soil characteristics, plant variety, seasonality, leaves age, processing type and the part of the plant which was used.[26,27] For Argentinean yerba-mate, Filip et al.[28] detected 0.23 mg/g of caffeic acid and 0.60 mg/g of rutin. For yerba-mate harvested in different Brazilian regions, the 5-caffeoylquinic acid concentration varied from 13.1 to 24.7 mg/g and the rutin concentration varied from 2.37 to 8.03 mg/g.[7] Bastos et al.[6] found 94.17 mg/g of total phenolics in dehydrated leaves and 59.16 mg/g for the roasted yerba-mate.

Methylxanthines. Results for methylxanthines are shown in Table 2. Caffeine and theobromine were detected in the samples, which is in accordance with results on yerba-mate reported in the literature.[23,29] The spray-dried extract methylxanthine contents

Table 3. Antioxidant Activity of the Spray-Dried Extract of Yerba-Mate[a]

analyses	spray-dried extract	acid ascorbic (positive control)
DPPH IC_{50} (mg/mL)	2.52 ± 0.51	260.9 ± 0.12
CAT-like[b] (nmol of H_2O_2/min)	22.80 × 10^2 ± 31.82	30.00 ± 0.01
SOD-like[c] (μL)	nd[d]	41.98 ± 1.16

[a] The values are mean ± SD (n = 3). [b] CAT-like: in nmol of decomposed H_2O_2/min. [c] SOD-like: IC_{50} value (μL of amount of sample needed to reduce by 50% the adrenochrome formation). [d] Not detectable.

were within the range reported for extracts obtained from high pressure CO_2 extraction of mate tea leaves.[26]

Antioxidant Activity. The antioxidant activity as measured by the DPPH method and the catalase enzyme activity (Table 3) showed that the yerba-mate extract presented great antioxidant capacity, as confirmed by a low IC_{50} value (2.52 mg/mL). The same test was performed with ascorbic acid, a well-known antioxidant compound, yielding a 260.9 mg/mL IC_{50} value, i.e., 99.04% less effective than the yerba-mate extract. The greater the free-radical scavenging capacity, the lower the IC_{50} value. Therefore, it can be affirmed that a low amount of yerba-mate extract is able to inhibit the DPPH radical oxidation in 50%. A compound's antioxidant capacity can also be reflected by its ability to decompose the hydrogen peroxide, as demonstrated by the catalase enzyme analysis. The catalase and superoxide dismutase enzymes act as ROS scavengers, protecting the cell membranes and thus keeping ROS at low intracellular concentration.[30]

Results show (Table 3) that the yerba-mate extract does not present superoxide dismutase-like (SOD-like) activity, but the high catalase-like (CAT-like) activity makes the extract a strong free-radical scavenger. Recent studies relate a high activity of superoxide dismutase and catalase enzymes in humans to the consumption of spices, fruits and other vegetables presenting high antioxidant activity.[31,32] Rover Júnior et al.[33] states that the antioxidant enzymes activity depends on the factors that lead to their activation or inactivation, their activation being a protective factor against free radicals. Antioxidant activity of *Ilex paraguariensis* was previously demonstrated in other systems. It has been proved that polyphenols exert antioxidant activity.[6,11,12] The higher enzymatic activity found in yerba-mate extract could be related to the higher polyphenol content.

The physiological actions promoted by phenolic compounds are related to a reduction is disease risk due to their great antioxidant capacity.[34] Phenolic compounds present various physiological properties, such as antiallergenic, antiatheriogenic, anti-inflammatory, and antimicrobial, but the greatest attention has been given to their antioxidant capacity.[35] The more the antioxidants sequestrate the free radicals, the lower is the chance of an individual developing a chronic-degenerative disease.[10,34] Previous studies show that phenolic compounds contained in different matrices present antioxidant activity, being considered efficient free-radical scavengers, such as demonstrated in studies dealing with coffee,[36] cocoa, green tea, black tea, red wine,[37] herbal infusions[9] spices[5] and yerba-mate.[6]

This trial demonstrated that the obtaining of spray-dried yerba-mate is promising in the generation of a new raw material for the industry. The results show that the spray-dried extract presents high polyphenol (caffeic acid, 5-caffeoylquinic acid and rutin) content, which justifies its high antioxidant activity, as confirmed by the DPPH free-radical scavenging assay and catalase-like activity.

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