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Methylxanthines, phenolic composition, and antioxidant activity of bark from residues from mate tree harvesting (*Ilex paraguariensis* A. St. Hil.)

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ABSTRACT

Leaves and products made from them are the major focus of research on bioactive compounds of mate (*Ilex paraguariensis*). However, studies on other parts of the plant, are scarce. The aim of this study was to determine the methylxanthines, phenolic compounds, and antioxidant activity of mate bark (residual biomass) and compare with those of mate leaves. The high antioxidant activity of mate bark and its high concentration of total polyphenols were apparent in both the aqueous and the methanolic extracts, the values of which were greater than those detected in the leaves. Of the phenolic acids identified, the levels of chlorogenic acid and 4,5-dicaffeoylquinic acid in the samples were significantly higher in the methanolic bark extract. With regard to methylxanthines, considerable concentrations were detected in the samples. The results obtained contribute to the viability of exploiting this residue, broadening the use of the mate plant.

1. Introduction

Mate, or erva-mate, (*Ilex paraguariensis* A. St. Hil.) is an arboreal species that naturally grows in forests in the temperate and subtropical climatic regions of Brazil, Paraguay, and Argentina. It is widely known and used by the inhabitants of these countries, where it has considerable socioeconomic importance (Filip, Lopez, Giberti, Coussio, & Ferraro, 2001).

The commercial product made with it, named "mate", "erva-mate" or "yerba mate" is used in the preparation of several types of beverages, such as "*chimarrão*", "*tererê*", soft drinks, and teas. Branches of less than 10 mm diameter and mainly the leaves of mate are the basis for the preparation of hot or cold infusions, traditionally known for being tonic stimulants and digestive drinks.

The *per capita* consumption of mate in Brazil is estimated as 1.2 kg per year, while in Argentina and Uruguay people use around 5–7 kg of dried mate per year for the preparation of teas. More recently, mate-based products have no longer been limited to their producing countries. They have reached external markets such as the USA and Europe (Cardozo et al., 2007; Heck & Mejia, 2007). This diversity of markets creates different preferences for different degrees of mate bitterness. Pagliosa et al. (2009) associated the mate bitterness with age and cultivation of the plant, and noted that caffeine and sugar content have no influence on its bitterness.

The consumption of mate has increased due to its health benefits, attributed to the presence of secondary metabolites (methylxanthinic alkaloids, polyphenols, and saponins) in leaf tissue and in commercial products made with mate. Besides being associated with the flavour, odour, colour and oxidative stability of foods (Cansian et al., 2008), these compounds have important roles in the morphogenetic processes (flowering and fruiting, for instance) and in plant defence. Since the cellular components of the human body are not totally protected by endogenous antioxidants (Filip, Lotito, Ferraro, & Fraga, 2000; Schuldt et al., 2005), functional compounds, when present in a balanced food product, contribute to a lower incidence of chronic diseases, such as cancer, arteriosclerosis, and diabetes, mainly due to an antioxidant effect.

Many of the positive effects of mate on humans are related to the presence of methylxanthines (caffeine, theobromine, and theophylline) and phenolic acids (Bravo, Goya, & Lecumberri, 2007; Chandra & Mejia, 2004; Marques & Farah, 2009). In fact, several studies with plants have shown a relationship between the polyphenolic content and the free radical-scavenging ability, including studies with mate (Bixby, Spieler, Menini, & Gugliucci, 2005; Vieira et al., 2008).

However, there have been a number of investigations of whether mate is carcinogenic or not. Most of these studies have

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focused on oral, oesophageal, lung, laryngeal, and bladder cancers, and these have produced evidence of associations with mate consumption (Bates, Hopenhayn, Rey, & Moore, 2007). The synergic action between mate, alcohol, and tobacco gave a clear result in several studies; and in some studies nutritional deficiencies and poor oral hygiene played a role. No increased risk was associated with cold mate beverages. More research needs to be done before a definitive statement can be made regarding cancer risk associated with any of the various forms of mate consumption. Future research should include population-based studies, collection of data on consumption of tobacco, alcohol, hot drinks, fresh fruit, and vegetables, as well as the measurement of volume and temperature of mate intake (Loria, Barrios, & Zanetti, 2009). A recent study relating mate processing with carcinogenic compounds, such as PAHs (polycyclic aromatic hydrocarbons), showed that the concentrations of these compounds were lower than the maximum limit considered to be health risks (Vieira, 2009).

The leaves of *I. paraguariensis* and commercial products made with them are the major focus of research on methylxanthines and polyphenols. However, studies on the chemical constituents of other parts of the plant, such as the residues generated in the processing of the plants or at their cultivation sites, are scarce. Processing industries of the mate plant have demanded ever finer mate branches (<10 mm) and, in some cases, only the leaves. Consequently, ever greater quantities of plant parts which are not commercially exploited are being discarded at the cultivation sites. The mate branches that are thicker than 10 mm diameter are discarded due to their advanced state of lignification and because they are expected to have a low content of phenolic compounds (Tamasi, Filip, Ferraro, & Calviño, 2007), a trait that alters the taste of processed mate. However, the bark of these branches that are discarded may contain secondary metabolites, which are beneficial to health and may have added value for the formulation of new products in the food and pharmaceutical fields.

The main goal of this study was to determine the methylxanthinic alkaloids, the phenolic compounds, and the antioxidant activity of bark aqueous extract and bark methanolic extract of mate branches ($\varnothing$ > 10 mm), as well as in fresh mate leaves.

2. Materials and methods

2.1. Reagents

For the chemical analysis, the following standard compounds were used: caffeine, theobromine, theophylline, *p*-coumaric acid, gallic acid, ferulic acid, and caffeic acid (Sigma Chemical Co., St. Louis, MO); syringic acid (Acros Organics, Geel, Belgium); chlorogenic acid (Sigma–Aldrich, Bangalore, India) and 4,5-dicaffeoylquinic acid (Chengdu Biopurify Phytochemicals Ltd., Chengdu, China).

The 2,2-diphenyl-1-picrylhydrazyl (stable free radical, DPPH$^{\cdot}$), Folin–Ciocalteau reagent, sodium carbonate, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma Chemical Co. All reagents were either of analytical or of chromatographic grade.

2.2. Samples

Samples of branches with a diameter greater than 10 mm (residue) and leaves of *I. paraguariensis* A. St. Hil. were obtained by collecting and mixing samples from thirty mate trees in Catanduvas, Santa Catarina state, Brazil, in August 2007. The samples were randomly chosen from 20-year-old mate trees, cultivated in an agroforestry system along with *Araucaria angustifolia* pines.

2.3. Sample preparation

The bark tissue was manually separated from the branches. The bark and the leaves of mate were oven-dried in a forced-air oven (50 ± 2 °C, 24 h) and ground in a hammer mill (Model 15A, Inbras-Eriez, Diadema, Brazil). The samples were frozen (−40 ± 2 °C), packed firstly in airtight plastic bags and then in aluminium packs, and stored (−20 ± 2 °C) until analysis.

2.4. Preparation of extracts

The bark and leaf samples (2 g each) of *I. paraguariensis* A. St. Hil., were separately mixed in methanol/water solutions (80:20; v/v) and distilled water at 85 ± 1 °C and both extracts were submitted to ultrasonication for 10 min. The extracts were filtered and transferred to amber bottles and purged with a stream of nitrogen. These extracts were used for the estimation of the total polyphenol content, through chromatographic analysis of those secondary metabolites by RP-HPLC-UV, and their antioxidant activity by measuring their free radical-scavenging capacity.

2.5. Total polyphenol content and antioxidant activity

The total polyphenol content (TPC) was determined following a modified Folin–Ciocalteau procedure (Singleton, Joseph, & Rossi, 1965). The appropriate dilutions of extracts were oxidised with Folin–Ciocalteau reagent and its reaction was neutralised with sodium carbonate. The absorbance of the resulting blue colour was measured at 725 nm, after 60 min, with a UV–Vis spectrophotometer (Model U-1800; Hitachi, Tokyo, Japan). The TPC was expressed as gallic acid equivalents in grams per 100 g of dry material.

The free radical-scavenging capacity of the extracts (methanolic and aqueous) was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) in accordance with the method of Kim, Guo, and Packer (2002). One hundred microlitres of each sample were added to 2.9 ml of DPPH methanol solution and then transferred to a quartz capillary. The colour changed from purple to yellow and its absorbance decrease was determined at 515 nm with a UV–Vis spectrophotometer (Hitachi model U-1800) every 0.5 min, for 15 min, and then every 15 min until the reaction reached a plateau. Antioxidant Trolox was used for the calibration and the results were expressed in micromoles of Trolox per gram of dry matter.

2.6. Identification and quantification of phenolic compounds

2.6.1. Sample preparation

Fifty millilitres of each extract were mixed with 150 ml of ethyl acetate (1:3; v/v) for 12 h, at 10 ± 1 °C in the dark. The organic phase (2.5 ml) was collected and the ethyl acetate was removed by evaporation using an N_2 stream. The final residue was re-dissolved in 500 μl of methanol and centrifuged at 5000 rpm/min. The resultant extract was stored (−20 ± 2 °C) until HPLC analysis (Schuldt et al., 2005).

Liquid chromatography The chromatographic analysis was performed on a Shimadzu LC-10 high-performance liquid chromatograph (HPLC) system, equipped with a reverse-phase column (Shim-pack C_{18}, 4.6 mm $\varnothing \times$ 250 mm) thermostatted at 40 °C, and a UV–Vis detector (Shimadzu SPD 10A, λ = 280 nm). The analytes were eluted isocratically at a flow rate of 0.8 ml/min, using a water:AcOH:*n*-butanol (350:1:10, v/v/v) mixture as the mobile phase. The injection volume was 10 μl/sample.

The identification of phenolic acids was carried out by comparing the retention times and absorption spectra of the peaks of the mate samples with those of standard compounds. The quantification of the phenolic acids was based on calibration curves built