Phytochemical profile of morphologically selected yerba-mate progenies
Perfil fitoquímico de progenies de erva-mate morfologicamente selecionadas

Alice Teresa Valduga1*, Itamar Luís Gonçalves1, Nessana Dartora2, Albain Aparecida Mielniczki-Pereira1, Lauro Mera de Souza2

1Universidade Regional Integrada do Alto Uruguai e das Missões/URI, Erechim, RS, Brasil
2Universidade Federal do Paraná/UFPR, Curitiba, PR, Brasil
*Corresponding author: valice@uricer.edu.br
Received in july 30, 2015 and approved in november 9, 2015

ABSTRACT
Yerba-mate (Ilex paraguariensis St. Hil) is a native South American species. Plant progenies are populations that differ in terms of their productivity, morphology and phytochemical profile. This study aimed to determine the concentration of primary and secondary metabolites, such as antioxidants, in leaves, of yerba-mate progenies selected based on morphological characteristics. We evaluated the centesimal composition of secondary metabolites in the leaves of five yerba-mate plants. Methylxanthines and phenolic compounds were determined by UPLC-PDA, and antioxidant activity by measuring DPPH scavenging. Significant differences were found in centesimal composition and the contents of caffeine, theobromine, rutin and chlorogenic acid, as well as antioxidant activities, in selected progenies. The IC50 values were correlated with the chlorogenic acid levels (r² = 0.5242) and soluble content (r² = 0.7686). The morphological characteristics observed in yerba-mate leaves can be used as a tool for plant selection, to obtain matrices with different phytochemical profiles as a genetic material source.

Index terms: Ilex paraguariensis St. Hil.; plant selection; secondary metabolites.

INTRODUCTION
Yerba-mate (Ilex paraguariensis St. Hil) is a native species of South America, which is cultivated in Brazil, Argentina and Paraguay (Burris et al., 2012). Saponins, phenolic compounds such as chlorogenic acid and flavonoids, methylxanthines, amino acids, sugars, and vitamins are found in yerba-mate (Jacques et al., 2007; Heck; De Mejia, 2007; Burris et al., 2012). High-performance liquid chromatography has been used to analyze phenolic compounds and methylxanthine in yerba-mate, using mainly a mobile phase gradient (Dutra; Ribani, 2010; Isolabella et al., 2010).

Several strategies have been developed to obtain yerba-mate extracts with high levels of bioactive compounds, including freeze-concentration technology (Boaventura et al., 2012) and nanofiltration (Murakami et al., 2011; Prudêncio et al., 2012). The processing steps that affect the levels of phenolic compounds and methylxanthine have been assessed (Isolabella et al., 2010; Valerga; Lanari, 2012; Zaions et al., 2014). The selection of plants that produce high concentrations of secondary metabolites can also be performed towards this aim. In this context, this study aimed to analyze how the morphological features of selected yerba-mate progenies are linked to the phytochemical composition, and to evaluate the in vitro antioxidant activity of extracts.
MATERIAL AND METHODS

Plant material

The plant material was collected in Barão de Cotegipe city, in January 2014. Leaves with petioles were collected from a full-sun homogeneous crop, following removal from extraneous material. The leaves were dried to constant weight (a moisture content less than 5%) at 35 °C in an oven with air circulation and were then crushed. Extracts were obtained from 3 g material using a Soxhlet extractor for 6 h, with 200 mL water as a solvent.

Centesimal composition determination

The moisture was determined by a gravimetric method according to IAL norms (Instituto Adolfo Lutz-IAL, 2008). The quantification of total lipids involved Soxhlet extraction with hexane for 4 h. The protein content was determined via the Kjeldahl method (Instituto Adolfo Lutz-IAL, 2008). The mineral residue was measured according to Brazilian Pharmacopeia (Brazil, 2010). Carbohydrates were determined indirectly (Instituto Adolfo Lutz-IAL, 2008).

Ultra-Performance Liquid Chromatography (UPLC)

Ultra-Performance Liquid Chromatography was used for the quantification of xanthines and phenolic compounds. Calibration curves were prepared with standards of caffeine and theobromine (λ 270 nm), chlorogenic acid (λ 325 nm) and rutin (λ 255 nm), each at 50, 100, 200, and 250 μg/mL. Chromatography was carried out using an Acquity-UPLC™ system (Waters, MA, USA), composed of a binary pump, a sample manager and a column oven. Detection was performed at 210-400 nm using photodiode array detectors (PDA). The samples were maintained at room temperature (22 °C) and the column oven at 60 °C.

Xanthines and phenolic compounds were analyzed by reversed-phase (RP) chromatography using a BEH C18 column (Waters) with a column size of 50 × 2.1 mm, and a particle size of 1.7 μm. The mobile phase consisted of H₂O acidified with formic acid (solvent A) and methanol (solvent B). The linear gradient system was developed at a flow rate of 500 μL min⁻¹: increasing the solvent B concentration from 0% to 35% in 6 min, from 35% to 80% in 8 min, holding for 1 min, followed by a return to the initial conditions (100% A) for 10 min, then holding for 3 min to re-equilibrate. The samples (1 mg mL⁻¹) were prepared in MeOH-H₂O and were measured in triplicate with an injection volume of 1 μL.

In vitro antioxidant activity assessment

The in vitro antioxidant activity was determined via the DPPH method (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl). Yerba-mate extracts (400 μg/mL) solubilized in hydroethanolic solution (80% methanol) were used. For the assay, 500 μL 0.1 mM methanolic DPPH solution was incubated for 30 min with 500 μL sample solution with increasing extract concentrations. The percentage of DPPH scavenging, expressed as antioxidant activity (AA%), was calculated using Equation 1, where A_{control} is the initial absorbance of methanolic PPH solution, and A_{sample} is the reaction mixture at 515 nm (DPPH + sample).

\[
AA\% = 100 \cdot \left[ \left( A_{\text{sample}} - A_{\text{blank}} \right) / A_{\text{control}} \right]
\]

Statistical analysis

The contents of primary and secondary metabolites, and IC₅₀ values were compared using the Kruskal–Wallis test, with 5% as the significance level, using GraphPad Prism 6.0 software.

RESULTS AND DISCUSSION

Centesimal composition of yerba-mate progeny leaves

The centesimal composition of analyzed plants is represented in Figure 1. A difference in mineral residue, moisture and lipid levels between plants A and D was observed (p < 0.05). Significant differences were also found in the protein level between plants A and B.

A correlation was found between the level of soluble solids and the centesimal composition of yerba-mate leaves. The soluble fraction was positively correlated with the mineral residue and the carbohydrate content. The decreasing level of soluble compounds was correlated with the moisture and lipid contents (Figure 2). Few studies have been performed concerning the centesimal composition of yerba-mate leaves and only one reports the levels of primary metabolites in yerba-mate leaves (Esmelindro et al., 2002).
Figure 1: Centesimal composition of yerba-mate plant leaves. (a) Moisture content; (b) mineral residue; (c) lipids; (d) proteins; and (e) carbohydrates. (*) $p < 0.05$ in relation to plant A.

Figure 2: Correlation between the soluble solids and the centesimal composition in leaves of yerba-mate plants. Soluble solids were correlated with mineral residue in (a), moisture in (b), lipids in (c), and carbohydrates in (d).
Secondary metabolite profiles of yerba-mate progenies

The levels of secondary metabolites in yerba-mate progenies are presented in Figure 3. A difference in caffeine levels between plants B and D was observed. The amount of caffeine in Plant D was lower than that of other plants evaluated. Chlorogenic acid, rutin, and theobromine levels in plant E were higher than those in plant C (p < 0.05).

Differences in total methylxanthines, caffeine, theobromine, total phenols, chlorogenic acid, and caffeic acid contents were found in yerba-mate progenies according to location (Cardozo et al., 2007). A different phytochemical profile among plants was described in coffee (Garrett et al., 2013) and Camellia sinensis (Thomas et al., 2006) cultures. Some plant secondary metabolites can prevent the cellular damage produced by oxidative stress, including phenolic acids and flavonoids (Bubols et al., 2013; Evans, 2002).

In vitro antioxidant activity

The correlations of DPPH scavenging with extract concentration were linear between 5 µg mL$^{-1}$ and 60 µg mL$^{-1}$. In the right graphic of Figure 4, the concentration intervals that produced 50% of the response are shown. Lower concentrations of the plant D extract caused the scavenging of 50% of the initial DPPH amount. The extract concentrations of the different yerba-mate progenies required to produce 50% DPPH scavenging (IC$_{50}$), calculated from the equations generated by linear regression, are shown in Table 1. The extract from plant D showed a higher antioxidant activity (IC$_{50}$ = 30.87 ± 0.81 µg/ml) than that of plant A (p < 0.05).

The IC$_{50}$ values were negatively correlated with chlorogenic acid levels and the soluble solids. Plants with the highest levels of chlorogenic acid and soluble solids required a lower extract concentration to react with DPPH (Figure 5). The correlation between the rutin level and the IC$_{50}$ values was less strong ($r^2 = 0.2050; p = 0.0901$).

A recent literature review summarized 19 in vitro and 10 in vivo assays for measuring antioxidant activity, and among the methodologies analyzed, DPPH scavenging was the most frequent method utilized (Alam; Rafiquzzaman, 2013).

The presence of metabolites with antioxidant activity in yerba-mate has been explored through the use of extracts in functional food development. Recently, yerba-mate extracts have been used in yogurt formulations (Preci et al., 2011), in gelatin (Bérté et al., 2011) and cereal bars (Chiesa; Souza, 2012), and in relation to beverage development, efforts are being invested into producing black tea from yerba-mate leaves (Molin et al., 2014).

**Figure 3:** Methylxanthines and phenolic compounds in selected yerba-mate progenies. (a) Caffeine, (b) theobromine, (c) rutin, and (d) chlorogenic acid. (*) p < 0.05 compared with plant D; (**) p < 0.05 compared with plant C. The values are expressed as mean ± standard error.
Table 1: In vitro antioxidant activity of yerba-mate extracts from selected plants.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant A</td>
<td>36.22 ± 0.39</td>
</tr>
<tr>
<td>Plant B</td>
<td>35.31 ± 0.70</td>
</tr>
<tr>
<td>Plant C</td>
<td>35.41 ± 0.76</td>
</tr>
<tr>
<td>Plant D</td>
<td>30.87 ± 0.81*</td>
</tr>
<tr>
<td>Plant E</td>
<td>33.14 ± 0.22</td>
</tr>
</tbody>
</table>

* $p < 0.05$ compared to plant A. Values are expressed as mean ± standard deviation.

Figure 4: Linear correlation between the yerba-mate extract concentration and DPPH scavenging. The DPPH scavenging values are expressed as the mean ± standard deviation.

Figure 5: Factors that influence DPPH scavenging. (a) Correlation between the chlorogenic acid levels and the IC$_{50}$ values. (b) Correlation between the soluble solid percentages and the IC$_{50}$ values.
The progeny of Plant D possessed leaves that were more elongate and thin, and were darker green and less serrated than the leaves of other yerba-mate plants assessed in this study. Future research using clones from this progeny will verify whether the results reported here are reproducible.

CONCLUSIONS

Differences in primary/secondary metabolite levels and antioxidant activity were observed among the plants selected, that were based on their morphological characteristics. These results can be used as a tool in plant selection, to obtain plants with higher nutritional and economical value.

REFERENCES


JACQUES, R. A. et al. GC/MS characterization of mate tea leaves extracts obtained from high-pressure CO$_2$ extraction. Journal of Supercritical Fluids, 40(3):354-359, 2007.


MURAKAMI, A. N. N. et al. Concentration of phenolic compounds in aqueous mate (Ilex paraguariensis A. St. Hil) extract through nanofiltration. LWT - Food Science and Technology, 44(10):2211-2216, 2011.


