DETERMINATION OF ANTIOXIDANT ACTIVITY IN Bunchosia glandulifera EXTRACTS BY CYCLIC VOLTAMMETRY AND CHEMICAL METHODS

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ABSTRACT – Extracts of the different parts of Bunchosia glandulifera (Jacq) Kunth (Malphiguiaceae) tree were obtained and studied for determination of the antioxidant activities. The total antioxidant activity, determined by ABTS and FRAP assays increased in the order seed < pulp < tree bark < leaf < root extract. The antioxidant activities were also determined by cyclic voltammetry. In this method, it was used dimethylsulfoxide as solvent, tetrabutylammonium hexafluorophosphate 0.1 mol dm⁻³ as supporting electrolyte, and ascorbic acid as a standard. The total antioxidant activity obtained by cyclic voltammetry showed, in generally, the same trend observed by the other methods.

PALAVRAS-CHAVE: extratos naturais; atividade antioxidante; voltametria cíclica.

KEYWORDS: natural extracts; antioxidant activity; cyclic voltammetry.

1. INTRODUCTION.

Antioxidants are present in different aliment and have important functions in our health, as retarding aging body, preventing cancer, atherosclerosis, diabetes, arthritis, heart disease and also show anti-viral, antibacterial and anti-inflammatory activities (Oroian and Escriche, 2015; Wang et al., 2011; Wootton-Beard and Ryan, 2011; Venkat Ratnam et al., 2006).

The determination of antioxidant is made by different chemical methods (Shahidi and Zhong, 2015; Oroian and Escriche, 2015; Magalhães et al., 2008; Karadag et al., 2009). In some of them the total antioxidant activity of a sample is determined, and in others a specific class of compounds is quantified. For total antioxidant capacity determination, methods as the ABTS or Trolox equivalent antioxidant capacity (TEAC) assay (Tian and Schaich, 2013), the FRAP method (Ferric Reducing Antioxidant Power) (Benzie and Strain, 1996) and the DPPH assay (Mishra et al., 2012) are used.

Electrochemical methods are used in analytical chemistry, electroanalysis, for a large amount of substances, including metal ions and organic compounds, as those present in agrotoxics, medicines,
food and many others. Normally, electrochemical methods are fast and inexpensive. The use of electrochemical methods for determination of antioxidant has been reported by some research groups (Oliveira-Neto et al., 2016; Przygodzka et al., 2014). Cyclic voltammetry and differential pulse voltammetry have been the most used electrochemical techniques. The main result obtained by these procedures is the total antioxidant activity. It is also possible to have an idea of the classes of antioxidant present in the sample, although not quantitatively. The individual classes of antioxidant compounds, as phenolic, carotenoids and ascorbic acid show different oxidation potential, and waves observed in the voltammograms at different regions indicate the different classes of antioxidant present. But in spite of this potentiality, electrochemical methods are not yet widely explored and used. There are a reduced number of works reported in the literature compared to the other methods, and the methodology is not well consolidated (Przygodzka et al., 2014).

In Brazil there are many native species of plants that are used by the population and popularly known to have beneficial effects to health (Giraldi and Hanazaki, 2010). The analysis of their chemical composition has been done by some research groups (Queiroz et al., 2015), but still there are many of these plants that have not yet been studied. Bunchosia glandulifera (Jacq) Kunth (Malphiguiaceae), popularly known as “Falso Guaraná”, is a native species found mainly in north of South America but also met in the South of the continent, and produces a small red fruit (Anderson, 2002). In the region of Santo Antônio da Patrulha-RS, the fruit pulp in nature or pulp juice or jam are consumed, and are considered to have stimulant and nutritious properties, and the frequent consumption is related to beneficial effects as physical vigor, mental lucidity force and longevity. Seeds are also consumed, ground in the form of powder added to hot or cold water, in a similar manner to the “guaraná” (Paullinia cupana) powder. The total antioxidant activity of the fruit pulp of Bunchosia glandulifera and the TP content were studied, and some phenolic compound were identified (Silva et al., 2016). Previous tests in our laboratory indicated that not only the fruit pulp and seeds, but that the bark tree, leaves and root also present high total antioxidant activity.

In this context, the objective of this work is the determination of the antioxidant activity of the extracts of fruits pulp, seeds, bark tree, leaves and roots of Bunchosia glandulifera (Jacq) Kunth (Malphiguiaceae). It is also objective the use of cyclic voltammetry for comparison of the results with the results obtained by other methods and the consolidation of the electrochemical assays.

2. MATERIALS AND METHODS

2.1. Preparation of the Extracts
B. glandulifera was identified and a voucher specimen is deposited at the Herbarium of Universidade Federal do Rio Grande do Sul (UFRGS), under number 167276 ICN. The fruits, seeds, bark, leaves and roots were collected from a tree in Santo Antônio da Patrulha-RS, Brazil, in September, 2012, and stored, in polyethylene bags of 100 g, in a freezer at -18 °C until needed for extraction.

The fruit pulp, seeds, bark, leaves and roots were extracted with ethanol to exhaustion. The extract was filtered and subsequently the solvent was evaporated under rotavaporator (40 °C), thus obtaining the crude extract of the pulp (PE), seed (SE), bark (BE), leaves (LE) and root (RE).

2.2. Chemical Analysis
The total antioxidant activity was determined by two chemical methods: the ABTS (Rufino et al., 2007b) and the FRAP (Rufino et al., 2006) assays.

2.3. Cyclic voltammetry
Solutions of the extracts dissolved in dimethylsulfoxide (DMSO), containing tetrabutylammonium hexafluorophosphate (TBAPF6) 0.1 mol dm$^{-3}$ as supporting electrolyte, were prepared for the analysis. Calibration curves were constructed using ascorbic acid solutions also in DMSO containing TBAPF6 0.1 mol dm$^{-3}$, and the antioxidant activity obtained by cyclic voltammetry were expressed in terms of equivalent of ascorbic acid. The total charges under the anodic waves of the background signal (solvent + supporting electrode) were subtracted from the total charges under the anodic waves and peak currents obtained for each sample measured within the range of 0.0 to 1.5V.

3. RESULTS AND DISCUSSION

In Table 1 are shown the results of the analysis of the total antioxidant activity determination by ABTS and FRAP assays. The trends in variations of the antioxidant capacity obtained by these methods are similar. In generally, it can be observed low values for seed and pulp and increasingly higher values for bark, leaf and root extracts, in this order. The exception in this trend is the higher value of antioxidant activity of the leaf extract (1,746.1 µmol FeSO$_4$ g$^{-1}$) compared to the root extract (1,592.86 µmol FeSO$_4$ g$^{-1}$) when using the FRAP assay.

In Figure 1 are the cyclic voltammograms of all the extracts solutions. In the voltammograms of seed, leaf and root extracts two oxidation peaks were observed, at the regions of 0.9 and 1.3 V. In the tree bark extract voltammogram only the 1.3 V peak is present, and for the pulp extract, no oxidation peak was detected.

In Table 1 are shown the antioxidant activities determined for each extract using the values of the charges under the voltammograms. In the seed extract a low value was observed, 76.9 mg ascorbic acid g$^{-1}$ extract. The pulp extract showed no antioxidant activity. The bark tree extract showed a higher value of antioxidant capacity, 119.3 mg g$^{-1}$, and leaf and root even higher and comparable values, 341.1 and 348.2 mg g$^{-1}$, respectively.

The results of the different methods for total antioxidant activity determination showed results following the same trend in a general way, but some more or less intense variations, were observed. A comparison between the results of different methods may not be done using absolute values, but trends of the variations in antioxidant capacities must be compared. Normally the results of different methods are expressed in different units, but even if the same units are used, discrepancies between the results of antioxidant activities are intrinsic to the process, as observed in a previous work (Rebelo et al., 2013) where the results of measurements by cyclic voltammetry, differential pulse voltammetry, ABTS, and Folin-Ciicltou assays were expressed in mg Gallic Acid mL$^{-1}$. In this work, in generally, the antioxidant activities increase in the order seed < pulp < tree bark < leaf < root extract.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Seed</th>
<th>Pulp</th>
<th>Bark</th>
<th>Leaf</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS$^1$</td>
<td>0</td>
<td>0</td>
<td>186.4±20.6</td>
<td>309.4±25.2</td>
<td>1,149.0±42.1</td>
</tr>
<tr>
<td>FRAP$^2$</td>
<td>25.4±1.9</td>
<td>96.84±5.2</td>
<td>429.8±32.3</td>
<td>1,746.1±56.1</td>
<td>1,592.9±62.1</td>
</tr>
<tr>
<td>CV$^3$</td>
<td>76.9±1.9</td>
<td>-2.6±0.7</td>
<td>119.3±6.1</td>
<td>341.1±19.2</td>
<td>348.2±24.0</td>
</tr>
</tbody>
</table>

Units: $^1$µmol trolox g$^{-1}$, $^2$µmol FeSO$_4$ g$^{-1}$ and $^3$mg ascorbic acid g$^{-1}$.
Figure 1 - Cyclic voltammograms of 4.0 mg cm$^{-3}$ solutions of the a) fruits pulp, b) seeds, c) tree bark, d) leaves and e) roots extracts, in DMSO, 0.1 mol dm$^{-3}$ tetrabutylammonium hexafluorophosphate supporting electrolyte, scan rate = 0.100 V s$^{-1}$.

4. CONCLUSIONS
Root and leaf extracts of B. glandulifera tree showed very high antioxidant activity, followed by bark tree, fruit pulp and seeds, that showed decreasing values.
Cyclic voltammetry assay demonstrated to be a practical and easy method for total antioxidant capacity determination. DMSO solvent and tetrabutylammonium hexafluorophosphate supporting electrolyte is a convenient medium for electrochemical analysis of these kind of vegetable extract.

5. ACKNOWLEDGMENT
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6. REFERENCES


