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## **ASSESSMENT OF ANTIOXIDANT QUALITY IN COMMERCIAL CARQUEJA (*BACCARIS TRIMERA*) SAMPLES BY ELECTROCHEMICAL TOOLS**

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**ABSTRACT:** Carqueja (*Baccharis trimera*) (Ba) is an Asteraceae member widely known in Brazil due to its therapeutic applications in folk medicine. Such applications rely on antioxidant activity, which can be assessed through different methods. Although the most used tools for antioxidant activity evaluation rely on colorimetry as analytical principle, they are not reliable for complex vegetal samples assessment. Therefore, non-colorimetric methods for antioxidant analysis such as voltammetry are noteworthy due to their enhanced selectivity when vegetal samples are concerned. Thus, the aim of this work is to provide a wide electrochemical study concerning Ba antioxidant quality. Different voltammetric assays were used, namely: Cyclic Voltammetry, Square Wave Voltammetry and Differential Pulse Voltammetry. Findings indicated remarkable antioxidant activity quality, as well as one reversible redox system. The electrochemical processes implicate in electropolymerization which is further corroborated by sample adsorption dynamics. The small potentials to which anodic peak amplitude was recorded imply high antioxidant quality, which justify Ba use in therapeutics.

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**KEYWORDS:** Carqueja, antioxidant, voltammetry.

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## 1. INTRODUCTION

Carqueja (*Baccharis trimera*) (Ba) is an Asteraceae member widely known in Brazil due to its therapeutic applications in folk medicine. Ba is traditionally employed as an anti-inflammatory agent and overall antioxidant due to the presence of polyphenolic secondary metabolites, whose antioxidant activity promotes a myriad of health benefits. Amongst the main phytochemical markers present in this plant, shikimate pathway derivatives such as flavonoids are the most abundant. These compounds are known to reduce high energy oxidative species such as hydroxyl and superoxide radicals, therefore contributing to Ba antioxidant capability [1-2]. Antioxidant activity can be assessed by a plethora of methods, whose analytic principles may differ considerably. Although the most used rely on colorimetry as analytical principle, they are not reliable for vegetal sample assessment, hence plant secondary metabolite chromophores may interfere mid analysis. Therefore, non-colorimetric methods for antioxidant analysis such as voltammetry are noteworthy due to their enhanced selectivity when vegetal samples are concerned. Moreover, such electrochemical assessment provides data about redox processes which are intimately linked to antioxidant activity and quality [3-4-5]. The voltammetric assessment of antioxidant activity is mostly done by differential pulse voltammetry (DPV), however the information obtained solely by this method is insufficient to properly study redox processes. Therefore, methods such as cyclic voltammetry (CV) and square wave voltammetry (SWV) are widely employed to provide clear data towards sample reducing power. Through CV, redox processes dynamics can be assessed in an overall manner, gathering data regarding diffusion/adsorption dynamics, whereas SWV allows redox reversibility and electrocatalysis to be studied. The combined use of these methods provides deep information concerning sample electroactivity [6-7]. Electrochemical methods are furthermore highly versatile, and their applicabilities extend beyond antioxidant analysis, as voltammetric assays can be employed to assess phytochemical markers and even authenticity using minute solvent volumes and little to none reagents. Moreover, these methods are easy, highly reproducible, require no specialized personal and are cheaper than standard assessment tools, such as chromatography [8-9]. Due to Ba relevance in traditional medicine practices and the importance of a better understanding of its antioxidant profile, pharmacognostic investigations focused heavily on the quantification of Ba antioxidant activities. However, since these reports are heavily concerned on colorimetric assays, their results may contain bias whose are not present through electrochemical assessment. Nevertheless, quantification through spectrophotometric tests such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonate) may not provide enough data on antioxidant quality [10-11]. This work is therefore intended to shed light on Ba antioxidant quality through an all-encompassing voltammetric assessment of commercially available Carqueja vegetal mater. The study protocol was adapted from previous works published by our group [18-21], henceforth, samples were assessed and studied concerning their antioxidant

capabilities as well their redox processes through CV, SWV and DPV.

## 2. MATERIALS AND METHODS

### 2.1. Samples and Reagents

Two Ba samples comprising crude vegetal matter from different distributors, namely Ba1 and Ba2 were collected from a local market in Goiânia, Goiás State, Brazil. The collected samples were assessed on their quality and found out to be in accordance to legislation. All electrolyte salts, solvents and reagents were of analytical grade. Gallic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standard solution of gallic acid was prepared at the concentration of 1mM in Milli-Q water. Electrolyte solutions were prepared with double distilled Milli-Q water (conductivity  $\leq 0.1 \mu\text{S cm}^{-1}$ ) (Millipore S. A., Molsheim, France). Alumina solution from Arotec S/A Ind. e Comércio was used to polish the glassy carbon electrode's surface between assays in order to further allow reproducibility.

### 2.2. BT Sample preparation

The protocol herein employed was adapted from previous works from our group [18]. Therefore, 1.8 g of Ba plant material was macerated in 10 mL analytical grade ethanol, the macerated was extracted for 15 minutes by sonication at room temperature (25°C) and then filtered. 100 $\mu\text{L}$  of this solution was mixed with 2 mL of each buffer solution (pH 4.0, 5.5, 7.0, 8.5, 10.0) for voltammetric assays.

### 2.3. Voltammetric assay

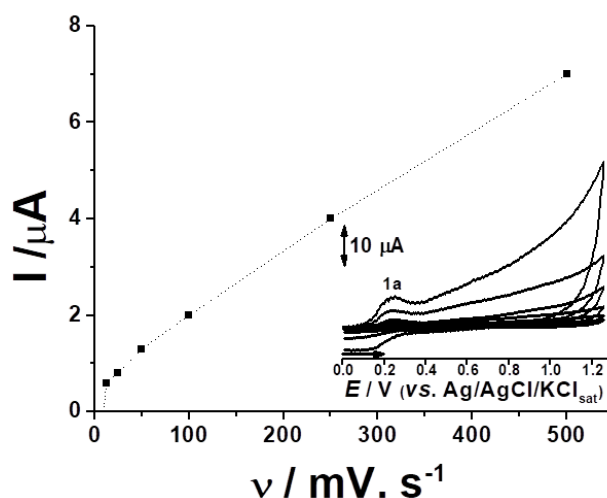
Voltammetric experiments were carried out in a potentiostat/galvanostat Autolab III<sup>®</sup> integrated to the GPES 4.9<sup>®</sup> software, Eco-Chemie, Utrecht, Netherlands. The measurements were performed in a 2.1 mL one-compartment/three-electrode system electrochemical cell consisting of glassy carbon electrode (GCE) 1.0 mm<sup>2</sup> area, a Pt wire and Ag/AgCl/KCl<sub>sat</sub> electrode (Lab solutions, São Paulo, Brazil), representing the working electrode, the counter electrode and the reference electrode, respectively. The experimental conditions for CV were: scan rate of 100 mVs<sup>-1</sup> and scan range from 0 to 1.25 V. The experimental conditions for SWV were: pulse amplitude 50 mV were frequency (f) 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate ( $v$ ) of 100 mVs<sup>-1</sup>. The experimental conditions for DPV were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs<sup>-1</sup>. The voltammetric assays were performed in 0.1 M phosphate buffer solution (PBS), at different pH (4.0, 5.5, 7.0, 8.5, 10.0). All experiments were performed in triplicates, DP voltammograms were background-subtracted and baseline-corrected to provide better data visualization, and all data was analyzed and treated with Origin 8<sup>®</sup> software.

## 3. RESULTS AND DISCUSSION

### 3.1. CV Results

Electrochemical assays such as voltammetry allows the detection of electroactive compounds, which in Ba case are mainly phenolic. Nevertheless, the extraction process was undergone with a

semi-polar solvent in order to enhance phenolic extraction. Although voltammetry is selective towards these chemicals, the complexity of vegetal samples associated to the similar structure of polyphenolic markers implies that, samples shall be treated as a pool of compounds and each exhibits singular redox properties [12-13-18]. Since hydroxyl moieties in polyphenols may undergo oxidation in similar fashion regardless of side chains, voltammetry can detect both glycoside bonded electroactive species and their aglycone. Therefore, sample acid hydrolysis or other pre-treatments are unneeded and assays can be carried out immediately after extract is prepared, thus contributing to agile analysis. In order to assess Ba redox dynamics, CV was conducted. The results can be seen in Figure 1.



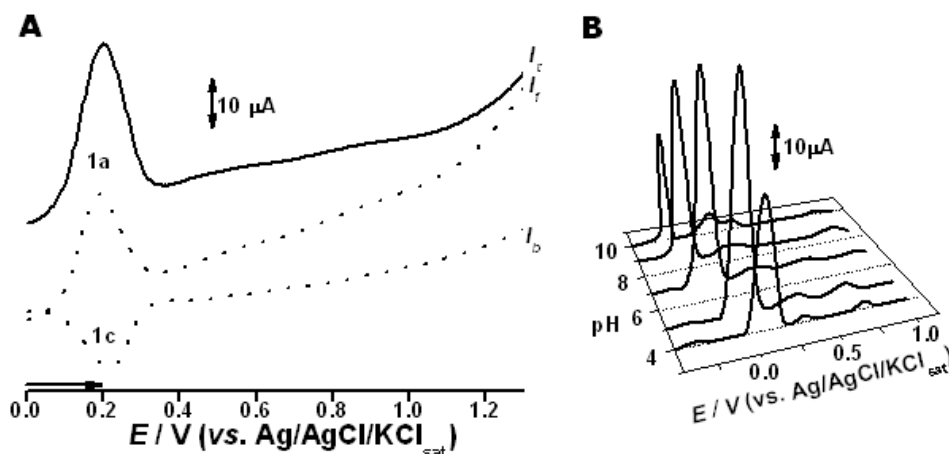
**Figure 1.** Plot of peak current ( $I$ ) vs scan rate ( $v$ ) obtained from first anodic process (1a), determination coefficient ( $r^2 = 0.987$ ). **Insert.** CV voltammograms of Ba at different scan rates (500, 250, 100, 50, 25, 12.5  $\text{mVs}^{-1}$ ) scan range from 0 to 1.25 V. All analysis carried out in 0.1 M PBS, pH 7.0 at GCE.

The tested samples presented very similar voltammetric profiles which implicated in only one being used to represent overall results. CV assessment allowed the visualization of one anodic peak ( $I_{pa}$ ) at anodic potentials ( $E_{pa}$ ) of approximately +0.21V (1a) (Figure 1). This peak represent oxidative processes occurrence and may be attributed to phenolic compounds, whose oxidative potentials are usually bellow to near +0.5V [14-15-18-21]. Moreover, peak currents increased proportionally to the applied scan rates ( $r^2 = 0.987$ ), a finding that suggests adsorption controlled electrochemical processes. Nevertheless, it also implicated that extra care should be taken prior analysis, as electrode fouling could be a concern. Therefore, the electrode was rigorously polished between assays, and blank tests were run in order to ensure reproducibility (Figure 1) [16-18-21]. The anodic peak seen can be also correlated to thermodynamic feasibility of reducing processes. Such finding implicate in the sample capacity to reduce endogenous antioxidants, which is nonetheless remarkable, as standard colorimetric tests concerning antioxidant activity are not capable of such assessment. Therefore, CV results corroborate to traditional uses of Ba hence the presence of highly oxidizable

phytochemicals whose antioxidant properties and synergic activities may exert therapeutic action [17-18-21]. In turn, Figure 1 displays that electrochemical processes are adsorption controlled. Thus, electropolymerized phenolic compounds may adsorb on electrode surface and hinder further electron transfer [16-17-18].

### 3.2. SWV and DPV Results

Since the anodic peak seen in CV is correlated to the presence of phenolic compounds, the electrochemical process concerning their redox dynamics may be associated to reversibility. Such reversible system relies on phenol/quinone shift, however they can only be henceforth attributed when anodic/cathodic processes occur at similar potentials [16-18]. Thus, SWV was employed hence its propriety to display both anodic and cathodic currents, which allows clear redox pairs visualization. Moreover, DPV was conducted to permit better faradaic current recording, thus providing higher resolution than overall voltammetric techniques. In this work, DPV was employed to study media pH influence on sample oxidative processes. Results are displayed in Figure 2.

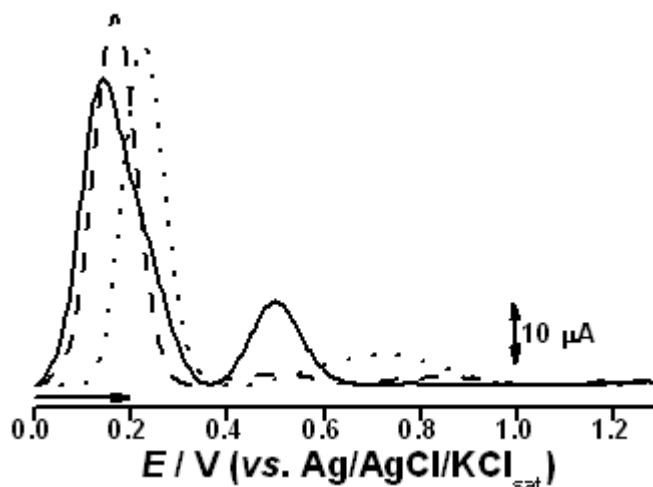


**Figure 2. A.** SW voltammograms of Ba. Pulse amplitude 50 mV, frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate ( $\nu$ ) of 100 mVs<sup>-1</sup>. Analysis carried out in 0.1 M PBS, pH 7.0. **B.** DP voltammogram of Ba samples at different pH (4.0, 5.5, 7.0, 8.5, 10.0). Pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs<sup>-1</sup>. Analysis carried out in 0.1 M PBS, at aforementioned pH at GCE.

Figure 2. A evidence that cathodic current presented a peak at similar potential to that of anodic current. Nevertheless, the peak amplitude ratio ( $I_{pa}/I_{pc}$ ) > 1.0 implies that the anodic processes are kinetically favored [14-16-18]. Since proton transfer plays a decisive role in redox dynamics, a pH study was undertaken. Therefore, DPV was employed hence its enhanced sensibility concerning faradaic current detection. The buffers used were at pH 4.0, 5.5, 7.0, 8.5 and 10.0 (Figure 2.B). Results followed an expected trend, where compounds suffer gradual deprotonation. Thus, higher pH leads to reduced values of peak potential [21]. The pH also influenced anodic peak amplitude, and the pH to which highest amplitude was recorded was henceforth used in further DPV assays.

### 3.3. Samples Comparison

In order to ensure the phenolic nature of the compounds responsible for the observed anodic peak, Ba samples voltammograms were compared to Gallic acid standard (Figure 3).



**Figure 3.** DP voltammogram of gallic acid standard (—); Ba sample 1 (■■■), Ba sample 2 (●●●). Pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs<sup>-1</sup>. Analysis carried out in 0.1 M PBS, pH 5.5 at GCE.

The DP voltammograms evidenced high similarity between gallic acid standard and samples (Figure 3). Hence the first anodic process concerning gallic acid is intimately linked to its phenolic moieties, the presence of this peak implicates in the presence of phenolic compounds in Ba samples. These findings are further corroborated by literature, which links this vegetal to high phenolic content. The results ensure Ba as a vegetal rich in antioxidants and also justify its medicinal appeal hence the therapeutic potential of phenolic compounds. Furthermore, the present study also sheds light in potential applications of electroanalytical tools such as voltammetry to assess antioxidant quality in vegetal material. Moreover, although it was not further investigated in this work, the clear peak definition obtained through voltammetric assessment may be a valuable tool to briefly analyze plant secondary metabolites, such as phenolic compounds, using minute solvent volumes [18-21].

### 4. CONCLUSION

This work investigated the antioxidant quality of Ba samples, and the findings indicated remarkable antioxidant activity quality, as well as one reversible redox system. The electrochemical processes implicate in electropolymerization which is further corroborated by sample adsorption dynamics. The small potentials to which anodic peak amplitude was recorded imply high antioxidant quality, which justify Ba relevance in therapeutics.

### 5. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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