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Original Article

Antioxidant activity evaluation of dried herbal extracts: an electroanalytical approach

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ABSTRACT

The prevention of chronic and degenerative diseases, is a health concern deeply associated with oxidative stress. Such progressive phenomena can be avoided through exogenous antioxidant intake, which set up a reductant cascade, mopping up damaging free radicals. Medicinal herbs are commonly associated with high antioxidant potential, and hence their health benefits. The commerce of dried herbal extracts movements a big portion of developing countries economy. The determination of medicinal herbs the antioxidant activity capacity is of utmost importance. The assessment of antioxidant activity in phytotherapics is mostly achieved by spectrophotometric assays, however colored substances can produce interferences that do not occur in electroanalytical methods. Therefore, the aim of this paper is to compare spectrophotometric and voltammetric techniques to evaluate antioxidant activity in herbal drugs such as: *Ginkgo biloba* L., *Camellia sinensis* (L.) Kuntze, Theaceae; *Hypericum perforatum* L., Hypericaceae; *Aesculus hippocastanum* L., Sapindaceae; *Rosmarinus officinalis* L., Lamiaceae; *Morinda citrifolia* L., Rubiaceae; *Centella asiatica* (L.) Urb., Apiaceae; *Trifolium pratense* L., Fabaceae; *Crataegus oxyacantha* L., Rosaceae; and *Vaccinium macrocarpon* Aiton, Ericaceae.

The spectrophotometric methods employed were DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and the Folin-Ciocalteu assays. The electroanalytical method used was voltammetry and it was developed a *phenoloxidase* based biosensor. The redox behavior observed for each herbal sample resulted in distinguishable voltammetric profiles. The highest electrochemical indexes were found to *G. biloba* and *H. perforatum*, corroborating to traditional spectrophotometric methods. Thus, the electroanalysis of herbal drugs, may be a promising tool for antioxidant potential assessment.

Introduction

The epidemiological correlation between high natural antioxidants intake and the prevention of degenerative diseases can be attributed to these compounds free radical scavenging proprieties (Ruiz et al., 2008). The exogenous antioxidants intake is therefore a way to balance free radical related oxidative injuries, and hence regulate redox homeostasis (Arts et al., 2003; Pisoschi et al., 2015).

Among the main natural antioxidant sources, vegetables, nutraceuticals and herbal medicines are the most known. In developing countries, primary healthcare relies mainly on herbal medicines and their commerce is considered prolific (Calixto, 2000; Braz et al., 2012; Oliveira-Neto et al., 2017; Macêdo et al., 2017).

Nutraceutical and herbal medicine quality is usually correlated to their antioxidant power, which can be determined by spectrophotometric or electrochemical methods (Chevion et al., 2000; Huang and Prior, 2005; Reis et al., 2009).

Spectrophotometric methods with similar analytical principles such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′azinobis (3-ethylbenzothiazoline-6-sulfonate) evaluate antioxidant reducing power according to electrons and/or protons transfer between electroactive species (Zegarac et al., 2010; Arteaga et al., 2012). Concerning herbal medicines, the results of these assays can be influenced by phytocompounds which exhibit antioxidant behavior. Therein, phenols are particular electroactive species to which antioxidant activity is related, and their

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Table 1
Standardized dried extracts listed according to their phytochemical marker employed for standardization and supplier.

Extracts	Standardized in	Supplier
Hypericum perforatum	0.3% hypericin	A
Ginkgo biloba	24% flavonoid glycosides	B
Trifolium pretense	8% isoflavones	B
Rosmarinus officinalis	Dried non standardized powder	B
Vaccinium macrocarpon	25% proanthocyanidins	C
Morinda citrifolia	Dried non standardized powder	D
Crataegus oxyacantha	0.4–1% proanthocyanidins	B
Camellia sinensis	30% catechin and 50% polyphenols	C
Centella asiatica	4–6.5% saponins	B
Aesculos hippocastanum	1–4% aescin	E

determination is often advised when the sample's therapeutical applications are concerned (Reis et al., 2009; Oliveira-Neto et al., 2017).

Although spectrophotometrical methods provide valuable information on oxidant activity, electrochemical methods can provide further information on stability and redox processes reversibility. Electrochemical data on antioxidant activity is therefore noteworthy, since it provides vast and reproducible information about electrodynamic processes through a simple, low cost, and quick execution (Reis et al., 2009; Zegarac et al., 2010; Escarpa, 2012).

Electrochemical methods also allow the use of modified electrodes, for example, *phenoloxidase* based biosensors therefore enabling selective determination of total phenol content (Escarpa, 2012; Garcia et al., 2015; Oliveira-Neto et al., 2016).

Phenoloxidase based biosensors are commonly designed for phenolic compounds determination and can be used to determine natural products antioxidant activity through biochemical oxidation followed by electrochemical reduction (Escarpa, 2012; Garcia et al., 2015; Macêdo et al., 2017).

Since spectrophotometrical findings can be associated to those of electroanalytical methods in order to provide a better understanding of the sample's redox behavior, and moreover aid in identification assays through voltammetric fingerprint determination (Alothman et al., 2009; Reis et al., 2009; Oliveira-Neto et al., 2016). This research aims to: the antioxidant activity determination of commercial dried herbal extracts usually employed in folk medicine and the comparison between the results of spectrophotometrical and electroanalytical assays.

Materials and methods

Samples and reagents

Eight standardized dried extracts and two dried herbal extracts commonly used in traditional medicine were selected, namely: *Hypericum perforatum* L., Hypericaceae; *Ginkgo biloba* L., *Morinda citrifolia* L., Rubiaceae; *Camellia sinensis* (L.) Kuntze, Theaceae; *Centella asiatica* (L.) Urb., Apiaceae; *Crataegus oxyacantha* L., Rosaceae; *Rosmarinus officinalis* L., Lamiaceae; *Trifolium pratense* L., Fabaceae; *Vaccinium macrocarpon* Aiton, Ericaceae; and *Aesculus hippocastanum* L., Sapindaceae. All vegetal material was of pharmaceutical grade, and purchased from local pharmacies. The used extracts are listed in Table 1 according to their supplier and phytochemical marker employed for standardization.

The analytical solutions of such dried extracts solutions were coded as: Hp, Gb, Mc, Cs. Ca, Co, Ro, Tp, Vm, Ah, respectively.

All electrolyte salts, solvents and reagents were of analytical grade. The phenolic antioxidants: gallic acid (GA), rutin, ethanol and DPPH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All electrolyte solutions were prepared with double distilled Milli-Q water (conductivity $\leq 0.1\ \mu S\ cm^{-1}$) 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.

Extract preparation

A suitable amount of each powdered herbal extract (60–80 mesh) was weighed (1 g) then solubilized in 10 ml of hydroalcoholic solution (70% ethanol) by sonication for 15 min, in order to reach 10% hydroalcoholic extract. The crude extracts were centrifuged at $112 \times g$. Then 1 ml (supernatant) of each sample was diluted with 9 ml of water to reach out to 1% hydroalcoholic extract. The aliquots of these solutions were used to compare the antioxidant power of each sample.

Electrochemical assays

Voltammetric experiments were carried out with a potentiostat/galvanostat Autolab III® integrated to the GPES 4.9® software, Eco-Chemie, Utrecht, The Netherlands. The measurements were performed in a 3 ml one-compartment electrochemical cell, with a three-electrode system consisting of a glassy carbon electrode (GCE) with 1 mm^2 of area or a carbon paste electrode chemically modified with laccase, a Pt wire and the $Ag/AgCl/KCl_{sat}$ (both purchased from Lab solutions, São Paulo, Brazil), representing the working electrode, the counter electrode and the reference electrode, respectively.

The experimental conditions for cyclic voltammetry (CV) were: scan rate of 100 $mV\ s^{-1}$ and scan range from 0 to 1.4 V. The experimental conditions for Square Wave Voltammetry (SWV) were: pulse amplitude 50 mV were frequency (f) 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate (υ) of 100 $mV\ s^{-1}$. The experimental conditions for differential Pulse Voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 $mV\ s^{-1}$ in 250 The voltammetric assays were performed in 0.1 M phosphate buffer solution (PBS), pH 6.0. The employed scan rate was selected in order to minimize adsorption of oxidized species on electrode surface, thus providing reproducible results.

The DP voltammograms were background-subtracted and baseline-corrected, and then all data was analyzed and treated with Origin 8® software.

Antioxidant activity electrochemical evaluation

The electrochemical index (EI) was initially proposed by Escarpa, it uses the anode peak potential (E_{pa}) and the anode peak current (I_{pa}), parameters that evaluate the ease that a species oxidizes and the amount of current generated during the process. Based on the fact that the lower the E_{pa} (thermodynamic parameter), the higher is the electron donor ability, and the higher the I_{pa} (kinetic parameter), the higher is the amount of electroactive species. The EI was calculated by the following equation:

$$EI = \left(\frac{I_{pa1}}{E_{pa1}}\right) + \left(\frac{I_{pa2}}{E_{pa2}}\right) + \cdots + \left(\frac{I_{pan}}{E_{pan}}\right)$$

In which E_{pa} and I_{pa} correspond to the values of each potential and each peak current that appears in the analysis by DPV (Bara et al., 2008; Escarpa, 2012; Macêdo et al., 2017).

Phenolic content electrochemical determination

The phenolic content was evaluated by using a *polyphenoloxidase* carbon paste (PCP) based biosensor. For the preparation of

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