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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

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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

electroactive species to which antioxidant activity is related, and their 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 × 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 the biosensor, 70 mg of graphite, and 250 μl of laccase from *Trametes versicolor* (27 mg ml^{-1}, Sigma–Aldrich, St. Louis, USA), dried at room temperature, were used. Then, 30 mg of mineral oil was added and mixed until a homogeneous paste was formed. The final PCP was constructed by adding the *Laccase* modified carbon paste into a support electrode, which consisted of 2 mm diameter and 0.5 mm depth cavity within the electrode support, namely a Teflon cylinder with a central hole overpassed by brass wire, acting as the electrical contact. As a control, the carbon paste electrode (CPE) was used without the enzyme laccase (Garcia et al., 2015). The results were expressed by means of rutin equivalents, μM/g of extract.

These assays were performed in 0.1 M PBS, pH 6, the optimum pH condition for PCP (Bara et al., 2008; Oliveira-Neto et al., 2017). The DPV conditions were: conditioning time of 30 s, pulse amplitude of 50 mV, pulse width 0.5 s, scan rate of 10 mV s^{-1} was used.

All electrochemical experiments were performed at room temperature (21 ± 1 °C) in triplicate ($n = 3$).

Antioxidant activity spectrometrical evaluation

Spectrometric antioxidant activity was evaluated using two traditional spectrophotometrical radical scavenging activity assays, namely the DPPH (Brand-Williams et al., 1995; Arteaga et al., 2012) and the ABTS methods (Re et al., 1999).

The DPPH assay was performed in accordance with established procedures (Brand-Williams et al., 1995; Oliveira-Neto et al., 2016). In summary, the blank control was composed of a 2.7 ml mixture of DPPH in ethanolic solution (0.1 mM) and 300 μl of ethanol, in which the final absorbance was approximately 0.7. The ethanol was used in order to adjust the baseline ($A = 0.000$). The amount of extract in μg/ml required to reduce the DPPH absorbance by 50% (EC_{50}) was calculated in order to evaluate the antioxidant capacity of each sample, whereas the percentage of discoloration express the free radical scavenging activity and is calculated with this equation:

$$\%\text{Discoloration} = \left[\frac{(\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{TEST}})}{\text{Abs}_{\text{DPPH}}}\right] \times 100$$

In which the DPPH absorbance corresponds to the initial DPPH solution, without addition of extract, and the test absorbance corresponds to DPPH with the evaluated extract. Absorbance at 517 nm was monitored with a UV-Vis spectrophotometer (Quimis Aparelhos Científicos, model Q-798U2VS). All samples were analyzed in a 1 cm length cuvette at room temperature and the tests were performed in triplicate.

The radical $ABTS^{\bullet+}$ was formed from the reaction of 5 ml of ABTS (7 mM) with 88 μl of potassium persulfate (140 mM), which were incubated in the absence of light for 16 h. Then, 2 ml of the prepared radical solution was diluted in ethanol to 150 ml, thus obtaining a solution with absorbance of approximately 0.700. To perform the analysis, a volume of 300 μl of the ethanol extract was added to test tube with 2.7 ml of ABTS radical. After that, tubes were covered with Parafilm® and kept in the dark for 20 min (Re et al., 1999). The absorbance was monitored at 734 nm with a UV-Vis spectrophotometer (Quimis Aparelhos Científicos, model Q-798U2VS) and all assays were performed in triplicate.

The percentage of inhibition expressed by the absorbance at 734 nm was calculated as:

$$\%\text{AA} = \left[\frac{(A_{\text{ABTS}} - A_{\text{TEST}})}{A_{\text{ABTS}}}\right] \times 100$$

where the %AA is the percentage of antioxidant activity, A_{ABTS} is the absorbance of the solution with the formed radical without the presence of sample. A_{TEST} is the absorbance observed in the presence of the radical and the analyte, and EC_{50} is the amount of extract in μg/ml of the tested samples required to decrease the initial concentration of ABST by 50%.

Total phenols spectrometrical determination

The spectrophotometrical method of Folin-Ciocalteu (FC) was employed to determine phenolic content, this method is based in the formation of a blue complex which can be analyzed at a wavelength of 765 nm. The total amount of phenols from each extract was obtained by a standard curve prepared with GA. Each 50 μl aliquot of 1% or 10% concentration sample were placed in a test tube containing 1 ml of deionized water and 250 μl of the FC reagent. After 5 min, it was added 750 μl of a 20% solution (Na_2CO_3) and 2950 μl of deionized water. The mixture was incubated in the absence of light for 60 min and then the absorbance was measured using the blank solution as reference. Quantification of phenolic compounds in the extracts was performed in triplicate and expressed by means of GA equivalents (GAE mg/g extract) (Olgun et al., 2014; Oliveira-Neto et al., 2017).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Comparisons among groups were made using ANOVA. Post hoc comparisons were performed using Tukey's comparison test. The significance level considered was 0.05.

Principal components analysis (PCA) is a multivariate statistical methods, it is widely used for data preprocessing and dimension reduction. It was selected Spearman coefficient, which is based on the ranks of the observations and not on their value, this coefficient is adapted to ordinal data as phenolic compounds quantification values (Brett et al., 2010; Oliveira-Neto et al., 2017). In addition, the software XlStat 2013.1.01 was used for computational purposes.

Results and discussion

The electrochemical characterization of each dried extract was performed using 1% stock solutions, which was added in an electrochemical cell reaching the final concentration of 1.25 mg/ml. The first step was to check the voltammetric profile in order to establish qualitative features for further identification assays. Therefore, the anodic and cathodic peaks were evaluated first by CV and SWV. GCE's surface was polished with alumina solution between each assay, and blanks were conducted in order to ensure reproducibility.

Cyclic and square wave voltammetric profile

The chosen samples of dried herbal extracts presented very distinct profiles. In fact, even CV shape was very distinguishable in most cases (Fig. 1). Though CV scans obtained from *Ca* and *Ah* were apparently similar, their current magnitude, peak potential, and reversible character were quite distinct. Moreover, such differences are clearly evident in their SW voltammograms (Fig. 1). Therefore, CV and SWV could be a useful, quick and low cost tool to accomplish preliminary sample identification. Nevertheless, sample's voltammetric fingerprints must be established in order to conduct a proper authenticity assay, being therefore the reported results only prospective uses for electroanalytical techniques as authenticity evaluation methods.

Moreover, the main electrochemical parameters, namely the peak potentials (E_p) and peak currents (I_p) can be provided by CV

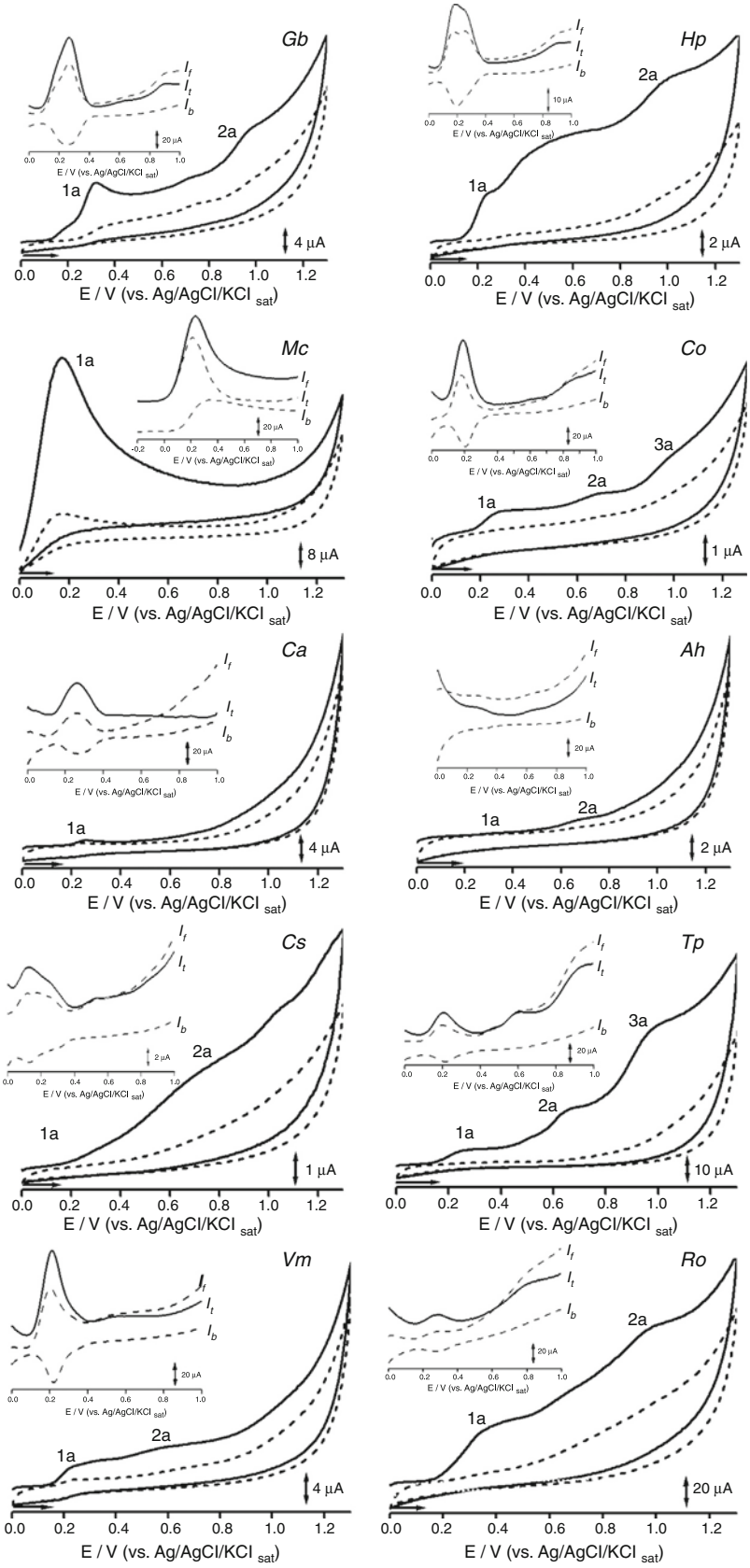

Fig. 1. Successive CV scans obtained for 1.25 mg/ml herbal extract solutions at GCE in 0.1 M PBS, pH 6.0. 1st scan (···), 2nd scan (—). Insert: SW voltammograms obtained under the same conditions, I_t – total current (···), I_f – forward current (—), I_b – backward current (—).

Table 2
Peak potential values from CV and SWV assays obtained for different herbal samples and related electroactive phytochemicals to which the observed redox processes could be attributed (RP): I, II and III.

Herbal samples	IE_{p1a}/E_{p1c} (V)	IIE_{p2a} (V)	IIIE_{p3a} (V)	Electroactive phytochemicals^{RP}	Ref.
Gb	0.32/0.26	–	0.98	My^{I,II}, Qu^{I,II}, Ru^{I,II}, La^{I}, Me^{I}, Ap^{I,II}, Lu^{I}, Ep^{I}, Ct^{I}, Ge^{I}, Gi^{II}, Is^{II}, Ka^{I}	Beck and Stengel (2016), Tang et al. (2001)
Ro	0.37/0.30	–	0.97	Caa^{I}, Ca^{I}, Ra^{III}, Ro^{I}, Mca^{I}, Roa^{I}	Amaral et al. (2018), Gil et al. (2013), Vallverdú-Queralt et al. (2014)
Vm	0.23/0.22	0.56	–	My^{I,II}, Is^{II}, Qu^{I,II}	Yan et al. (2002)
Hp	0.22/0.20	–	0.99	Bi^{II}, Am^{II} Ru^{I,II}, Hy^{I,II}, Iq^{I,II}, Qi^{I,II}, Qu^{I,II}	Heydarian et al. (2017), Liu et al. (2000), Karakashov et al. (2015)
Tp	0.26/0.22	0.65	1.0	Fo^{III}, Ba^{I,II}, Ge^{II}, Da^{III}	Sachse (1984)
Mc	0.18/0.12	–	–	Ru^{I,II}, Ct^{1a}	Krishnaiah et al. (2015), Levand and Larson (2009)
Cs	0.28/0.15	0.67	–	Ct^{I,II}, Egc^{I,II}, Egg^{I}, Ecg^{I}, Ga^{I}	Gonbad et al. (2015)
Co	0.27/0.20	0.67	0.97	Ru$^{1a/2a}$, Hy^{1a}, Vi^{II,III}, Cha^{I}, Cfa^{I}	Kostić et al. (2012)
Ca	0.28/0.25	–	–	Cha^{I}, Ka^{I,II}	Alqahtani et al. (2015)
Ah	0.32/0.31	0.68	–	Qu^{I,II}, Ep^{I,II}, Ka^{I,II}, Pc^{I,II}, Ae^{II}, Es$^{Epa>1.2\,V}$	Vašková et al. (2015), Otajagić et al. (2012), Oszmiański et al. (2014)

Legends: My, myricetin; Qu, quercetin; Ru, rutin; La, laricitrin; Me, mearnsetin; Ap, apigenin; Lu, luteolin; Ep, epicatechin; Ct, catechin; Ge, genistein; Gi, ginkgetin; Is, isorhamnetin; Ka, kaempferol; Caa, carnosic acid; Ca, carnosol; Ra, rosmadial; Ro, rosmanol; Mca, methyl carnosate; Roa, rosmarinic acid; Bi, biapigenin; Am, amenthoflavon; Hy, hyperoside; Iq, isoquercitrin; Qi, quercitrin; Fo, Formononetin; Ba, biochanin A; Da, daidzein; Egc, epigallocatechin; Egg, epigallocatechin-gallate; Ecg, epicatechingallate; Gaa, gallic acid; Vi, vitexin; Cha, chlorogenic acid; Cfa, caffeic acid; Pc, Procyanidin; Ae, aesculin; Es, escin.
RP, redox processes: (I) 1a/1c; (II) 2a; and (III) 3a, related to the anodic peaks in the columns on the left (Gil and Couto, 2013).

and SWV assays (Fig. 1), thus bringing some insights about the expected antioxidant activity of each crude sample.

Some features suggesting high antioxidant activity include the presence of electroactive species which undergo electrochemical oxidation at anodic peak potentials (E_{pa}) bellow 0.5 V in mild acid pH. On the other hand, the higher the anodic peak current (I_{pa}) is, higher may be the concentration of related species and/or kinetic in which the electron transfer would occur in a reduction reaction. Also, since the regenerating ability may improve the antioxidant function, the reversibility of a redox process is a remarkable behavior. For instance, the presence of polyphenols presenting a catechol moiety, *i.e.* rosmarinic acid in Ro, quercetin in Hp, rutin in Gb, gallic acid in Cs, catechin in Mb, caffeic acid in Co, and epicatechin in Ah combines the reversibility of catechol/quinone system with higher reducing activity associated to inherently low peak potentials, thus providing high antioxidant power (Gil and Couto, 2013).

Table 2 presents E_p values collected from Fig. 1, and some possible polyphenols which they may be associated to.

Therefore the voltammetric profile and peak potentials pattern, have dual function: being useful as a potential fingerprint to aid herbal extracts identification, to predict the antioxidant power and to provide qualitative and quantitative insights about the polyphenol content.

Antioxidant activity quality

Antioxidants exert their reducing activity through electron transfer mechanisms, in other words, they undergo oxidation whereas protected species are reduced or kept unchanged from oxidizing agents. Hence, the anodic peak parameters have been used to get an EI in which E_{pa} and the thermodynamic parameter express the reducing power, while I_{pa} and the kinetic and quantitative parameter express the swiftness of electron transfer donation and the amount of antioxidant content. These parameters: E_{pa} and I_{pa} are quantitatively better acquired from DPV assays (Fig. 2).

Therefore, the resulting EI was compared with traditional radical scavenging assays in order to establish correlations and access antioxidant power of the herbal products, herein studied (Table 3).

The analytical principle of DPPH and ABTS radical scavenging assays is based on the conversion of former radical to the reduced form, which is observed by the discoloration effect measured spectrometrically at suitable wavelength (Zegarac et al., 2010; Oliveira-Neto et al., 2016). Thus, the lower is the amount of added sample to decrease the color intensity to half its original value, higher is the antioxidant content. Meanwhile, the correlation between both methods is direct, however indirectly proportional to EI values (Brand-Williams et al., 1995; Escarpa, 2012).

Table 3
Results antioxidant activity evaluation by DPPH and ABTS methods.

Samples	DPPH, EC_{50} ± SD (mg/ml)	ABTS, EC_{50} (μg/ml)	EI ± SD (μA/V)
Gb	0.56 ± 0.02	0.50 ± 0.03	24.66 ± 0.08
Ro	0.57 ± 0.03	0.76 ± 0.02	7.54 ± 0.03
Vm	0.61 ± 0.02	0.51 ± 0.01	7.58 ± 0.04
Hp	0.61 ± 0.02	0.50 ± 0.02	19.29 ± 0.23
Tp	0.72 ± 0.02	0.57 ± 0.01	7.89 ± 0.07
Mc	1.02 ± 0.01	1.01 ± 0.07	7.74 ± 0.11
Cs	1.93 ± 0.03	10.70 ± 1.87	0.68 ± 0.05
Co	2.39 ± 0.18	1.71 ± 0.09	3.76 ± 0.03
Ca	7.61 ± 0.06	11.90 ± 0.19	4.69 ± 0.08
Ah	29.0 ± 2.04	81.34 ± 7.56	0.35 ± 0.10

Since, the maximum wavelength of each spectrometric method is distinct, colored substances may produce different effect on the deviation of expected correlations (Bara et al., 2008; Reis et al., 2009; Macêdo et al., 2017). For instance, the highest EC_{50} values for DPPH and ABTS assays were achieved for Gb, whereas Ro was the second of DPPH list, but the fourth in ABTS.

Also not only polyphenols, but any reducing compound, even non-electroactive species, will contribute for the overall antioxidant power of the sample, therefore reducing sugars and polysaccharides may influence the results of antioxidant tests in plant material (Escarpa, 2012; Oliveira-Neto et al., 2017; Heydarian et al., 2017). Such, assumption would lead to lower EI values in comparison to spectrometric assays. On the other hand, a chemical redox reaction between oxidants and reductants depend on steric diffusion factors, what could explain the higher EI classification of Hp, Mc and Tp in comparison to those obtained from spectrometric assays (Tables 2 and 3).

Total phenol content

In order to evaluate the specific contribution of phenolic compounds to antioxidant activity, FC method was used. Nevertheless, it is well know that besides phenolic compounds, proteins and other complexants can bind to FC reagent, thus interfering in the results (Oliveira-Neto et al., 2016; Heydarian et al., 2017). Hence, more specific methods are mandatory, and the use of biosensors have been successfully used (Escarpa, 2012; Garcia et al., 2015).

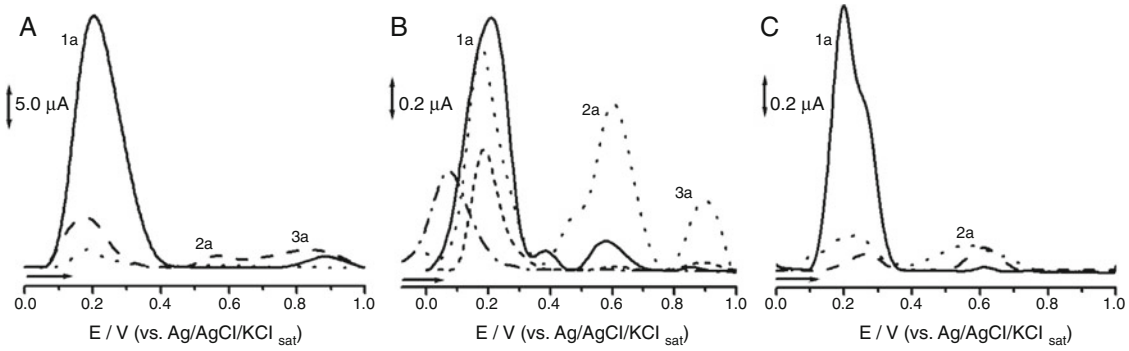

Fig. 2. DP voltammograms obtained from the 1.25 mg/ml solutions of the extracts: (A) *Hp* (┄), *Gb* (—) and *Vm* (●●●); (B) *Ro* (┄), *Tp* (●●●), *Co* (—) and *Mc* (-●-●-); (C) DP voltammograms obtained from the 25.0 mg/ml solutions of the ethanolic extracts: *Ca* (┄), *Ah* (—) and *Cs* (●●●). All in 0.1 M PBS, pH 6.0 at GCE.

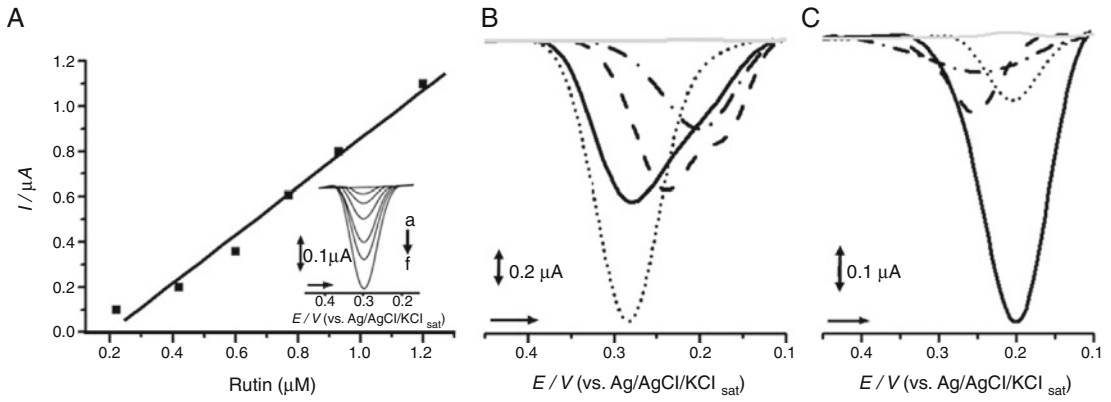

Fig. 3. (A) Calibration curve obtained for rutin after increasing concentrations 0.2–1.5 µM (a → f) at PCP biosensor; DP voltammograms obtained for: (B) CPE-Lcc in the analysis of *Gb* (●●●); *Hp* (┄); *Ro* (—); *Co* (-●-●-) and (C) *Vm* (┄); *Tp* (●●●); *Ca* (—); blanks (—), all in 0.1 M PBS pH 6.0.

Table 4
Results of total phenols analysis obtained from calibration curves expressed as GaE and RuE for the plant extracts.

Samples	GAEµM ± SD	RuEµM ± SD
Hp	84.0 ± 0.5	6.2 ± 2.2
Gb	63.3 ± 0.4	11.9 ± 5.5
Tp	54.9 ± 0.7	1.2 ± 0.2
Ro	39.6 ± 0.3	5.6 ± 0.6
Vm	34.1 ± 0.1	3.0 ± 0.9
Mc	10.1 ± 1.8	1.1 ± 0.9
Co	9.1 ± 0.2	3.4 ± 0.1
Cs	3.5 ± 0.6	nd
Ca	2.4 ± 0.2	1.2 ± 0.3
Ah	1.2 ± 0.1	nd

FC method is usually expressed by means of gallic acid equivalents (GAE) (Oliveira-Neto et al., 2016; Macêdo et al., 2017). A standard concentration curve for GA from 14.7 to 88.2 µM was constructed, and the resulting linear equation ($r = 0.991$) was used to obtain GAE for each herbal extract.

In turn, a calibration curve for rutin using PCP was performed from 0.2 to 1.5 µM (Fig. 3A) and the linear equation ($r = 0.9910$) provided the rutin equivalent (RuE) for each herbal extract (Fig. 3).

The highest GAE and RuE values were obtained for Gb and Hp, whereas the lowest was found for Ca, Ah and Cs, the last ones below the quantification limit of the biosensor (Table 4).

Moreover, the overall findings for TPC were in agreement with those obtained for antioxidant activity (Tables 3 and 4). Meanwhile, some few distortions are related to the different principles among the methods, herein used (Tables 3 and 4).

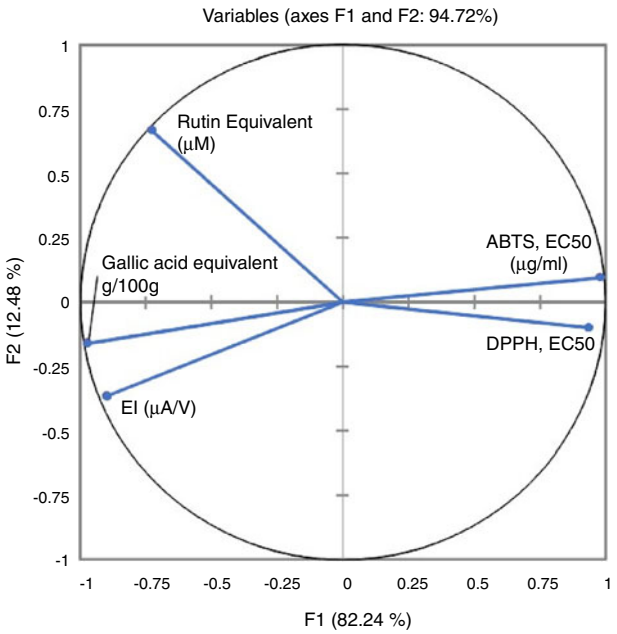

Fig. 4. PCA – raw dataset with correlations between methods.

In order, to check the statistical correlations between the employed methods, principal components analysis (PCA) was performed.

Table 5
Correlations between variables and factors:.

Variables	DPPH, EC_{50} (μg/ml)	ABTS, EC_{50} (μg/ml)	EI (μA/V)	GAE (μM)	RuE (μM)
DPPH, EC_{50} (μg/ml)	1	0.8994	−0.7477	−0.8815	−0.6930
ABTS, EC_{50} (μg/ml)	0.8994	1	−0.8997	−0.9605	−0.6444
EI (μA/V)	−0.7477	−0.8997	1	0.9152	0.4424
GAE (μM)	−0.8815	−0.9605	0.9152	1	0.6000
RuE (μM)	−0.6930	−0.6444	0.4424	0.6000	1

Multivariate statistics – principal components analysis

PCA results (Fig. 4) shows the similarities and differences between the different methods. By one hand, it shows a strong correlation between voltammetric assays and EI direct correlation between DPPH and ABTS, and inverse correlation between regarding EI, as was related by Oliveira-Neto et al. (2017). In the other hand, it can be shown the non-linear correlation of biosensor method and total phenol assays. PCA F1–F2 explains 94.72% variability of the studied variables, so the multivariate statistics model corroborates to the employment of electroanalytical methods in order to study antioxidant activity in plant material. The correlation between each methods is presented in Table 5.

In this research, the highest antioxidant activity was found in Gb and Hp extracts. It was also possible to observe and compare results obtained by spectrophotometrical and electroanalytical methods. The divergences found may be related to intrinsic factors regarding each methodology. Nonetheless, electroanalytical methods proved to be less prone to colorimetric interferences, therefore indicating that techniques such as voltammetry may be reliable in antioxidant activity evaluation. Moreover is noteworthy to mention the possible application of CV in plant material fingerprinting, as results suggested it could be an easy and low cost tool to aid and complement herbal products authenticity assays.

Authors' contributions

KCSL (PhD student) contributed in running the laboratory work and drafted the paper. LFG contributed to developed a phenoloxidase based biosensor. GSL contributed in analysis of the data. DVT, EKGM and MFC contributed spectrometric analysis. MLR and WTPS contributed to critical reading of the manuscript. ESG supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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