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Antioxidant Study and Electroanalytical Investigation of Selected Herbal Samples Used in Folk Medicine

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The growing concern regarding oxidative-related cell damage has guided a myriad of investigations concerning the applicability of herbal mater to mop up reactive oxygen species generation. In this sense, the consumption of functional foods and the use of plants with antioxidant capacity are well understood in folk medicine. Thus, this work was intended to electroanalytically study tea and tincture preparations of selected herbs used in folk medicine in order to better understand their redox features. Henceforth, samples of *Cinnamomum zeylanicum* (CZB), *Pterodon emarginatus* (PER) (PES), *Cotyledon orbiculata* (COL), *Syzygium aromaticum* (SAF), *Commiphora myrrha* (CML), *Cyrtopodium puntactum* (CPR), *Uncaria tomentosa* (UTR) and *Brosimum gaudichaudii* (BGR) were also analyzed in colorimetric tests of radical scavenging activity ABTS and DPPH. Differential pulse voltammetry (DPV) measurements were performed using a potentiostat/galvanostat. A carbon paste electrode, Pt wire and the $Ag/AgCl/KCl_{sat}$ 3 M were used as working, counter and reference electrodes, respectively. The results evidenced that CZB, PER, SAF, CML and BGR presented better antioxidant features in a tincture form, probably due to better aglycone solubilization in hydroethanolic solutions, while CPR and UTR presented better antioxidant activity in aqueous preparations because of the polyphenolic components. The highest observed peak amongst all analyzed samples was that of SAF, attesting to its high content of electroactive compounds, while BGR, COL and PER showed good distribution of all anodic peak, being all correlated to phenolic metabolytes. Therefore, the antioxidant activity of these preparations changes heavily according to the employed extraction fluids, and this activity can be assessed either by electrochemical or colorimetric techniques, henceforth rendering reproducible results.

Keywords: Tea, Herbal Medicine, DPPH, ABTS, Voltammetry.

1. INTRODUCTION

Dietary intake of polyphenols is widely acknowledged to promote a plethora of health benefits, including the mitigation of inflammatory states as well as the contribution to overall homeostasis. Pharmacognostic reports evidence that the wide therapeutic applications of polyphenols from herbal origin may aid some allopathic treatments, therefore enhancing patient recovery. Nonetheless, polyphenols are broadly available in plant material, and the importance of these compounds in folk medicine raises concerns about the extraction methods therein employed [1-4].

Concerning polyphenol extraction from herbal material, aglycone form of polyphenols may have different solubility than the carbohydrate-coupled form. This property implicates in the importance of a standardized extraction process in order to render similar products concerning therapeutic applications. Albeit modern knowledge does take phytochemical markers solubility in consideration, traditional preparations in folk medicine were developed according to empirical data. This distinction leads to the manufacture of crude preparations whose reproducibility may be compromised [5-6].

In the context of folk medicine, teas and tinctures are widely used to extract phytocompounds. These preparations are regarded as the main therapeutic intervention in most third world countries, and their appeal usually resolves around the extraction of phenolic markers. Although their preparation does follow protocols, these are highly susceptible to bias such as temperature and volume alterations. Moreover, hence the main appeal of these compounds rely on antioxidant activity, the evaluation of these crude samples is noteworthy to support ethnopharmacological findings [7-13].

Regarding antioxidant activity evaluation, most pharmacognostic reports make use of colorimetric techniques. However, the analytical principle to which these methods rely upon may result in bias hence plant samples are nonetheless colored due to a plethora of distinct chromophores. In this context, tools such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis-3-thylbenzothiazoline-6-sulfonic acid (ABTS) may provide conflicting results according to the nature of the samples, and these features leads to the search for new techniques to assess antioxidant activity in vegetal material [14-16].

Amongst innovative methods to determine and characterize antioxidant activity, electrochemistry figures as the most versatile. Electroanalytical tools such as voltammetry provide information on redox kinetics, as well as sample thermodynamical features, which further corroborates findings concerning antioxidant power and quality. Due to these advantages, electroanalytcal assessment of plant material has been widely reported in recent literature [16-22].

Therefore, this work was intended to electroanalytically study tea and tincture preparations of selected herbs used in folk medicine in order to better understand their redox features. Henceforth, the following samples were used: *Cinnamomum zeylanicum* (Cinnamon; bark) (CZB), *Pterodon emarginatus* (Sucupira; root and seed) (PER) (PES), *Cotyledon orbiculata* (Round-Leafed Navel-Wort; leaf) (COL), *Syzygium aromaticum* (Carnation; flower bud) (SAF), *Commiphora myrrha* (Myrrh; leaf) (CML), *Cyrtopodium puntactum* (Rabo-de-tatu; root) (CPR), *Uncaria tomentosa* (Unha-de-gato; root) (UTR) and *Brosimum gaudichaudii* (Mama-cadela; root) (BGR). Moreover, radical scavenging tests with spectrophotometry were also assayed to compare results.

2. EXPERIMENTAL

2.1. Materials and Reagents

The medicinal plants: *Cinnamomum zeylanicum* (Cinnamon; bark) (CZB), *Pterodon emarginatus* (Sucupira; rhizome and seed) (PER) (PES), *Cotyledon orbiculata* (Round-Leafed Navel-Wort; leaf) (COL), *Syzygium aromaticum* (Carnation; flower bud) (SAF), *Commiphora myrrha* (Myrrh; leaf) (CML), *Cyrtopodium puntactum* (Rabo-de-tatu; rhizome) (CPR), *Uncaria tomentosa* (Unha-de-gato; root) (UTR) and *Brosimum gaudichaudii* (Mama-cadela; root) (BGR) were purchased from the local central market (Goiânia – GO, Brazil).

Ethanol (99.8% purity), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-thylbenzothiazoline-6-sulfonic acid (ABTS) reagents, gallic acid (99.9% purity) and resveratrol (99.8% purity) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 $\mu S\ cm^{-1}$) (Millipore S. A., Molsheim, France).

2.2. Sample Preparation

All parts of the medicinal plants used in this study were pulverized prior to the experiments. Solutions were prepared with intent of simulating the popular use of medicinal plant teas and tinctures of the samples.

In order to prepare the tea samples, 5 g of the pulverized plant part were transferred into a Becker. Thereafter, 40 mL of purified water was transferred. The mixture went through decoction at 80 °C for 15 minutes in a heating mantle. The resulting solution was transferred into a volumetric flask and the volume was completed to 50 mL with purified water. In order to prepare the tincture samples, 5 g of the pulverized plant part was transferred into a Becker and then, 50 mL of hydroethanolic solution (2:3; ethanol:water) was transferred. The resulting solution was left to stand for 3 days.

2.3. Radical Scavenging Assays

Absorbance measurements were recorded with a spectrometer Q798U2VS (Quimis Aparelhos Científicos, São Paulo, Brazil). All samples were analyzed in a glassy cell of 1 cm length at room temperature (25 ± 1 °C).

2.4. DPPH

The radical scavenging activity assays were performed using DPPH reagent, in accordance with established procedures [23]. Briefly, the blank control was composed by a mixture of 2.7 mL DPPH ethanolic solution (0.1 mM) and 0.3 mL of ethanol, in which the final absorbance at = 517 nm was of c.a. A = 0.7. The ethanol was used in order to adjust the baseline (A = 0.000). The sample test was performed with the addition of 0.3 mL of sample in 2.7 mL of DPPH, the reaction was kept at room

temperature, 25 ± 1 °C, by 5 min before measurement. Gallic acid (GA) and resveratrol (RE) were used as markers for total phenols and flavonoids, respectively. Calibration curves were made for both these markers with DPPH and the antioxidant activity was given in terms of mg of GA/RE per g of dry extract.

2.5. ABTS

The radical scavenging activity assays were executed using the stable ABTS reagent, in accordance with established procedures [23]. ABTS radical was prepared by mixing 5 mL of the ABTS solution (7 mmol.L^{-1}) with 88 mL of potassium persulfate (140 mmol.L^{-1}). The reaction was kept at room temperature in the dark during 16 h. The initial control solution was prepared by diluting the radical with ethanol until the absorbance reached values close to 0.70 at 734 nm. Briefly, the blank control was composed by a mixture of 2.7 mL ABTS ethanolic solution (0.1 mM) and 0.3 mL of ethanol, in which the final absorbance at = 734 nm was of c.a. A = 0.7. The ethanol was used in order to adjust the baseline (A = 0.000). The sample was performed with the addition of 0.3 mL of sample in 2.7 mL of ABTS, the reaction was kept at room temperature, 25 ± 1 °C, by 5 min before measurement. GA and RE were used as markers for total phenols and flavonoids, respectively. Calibration curves were made for both these markers with ABTS and the antioxidant activity was given in terms of mg of GA/RE per g of dry extract.

2.6. Electrochemical Assays

Differential pulse voltammetry (DPV) measurements were performed using a potentiostat/galvanostat PGSTAT® model 204 with FRA32M module (MetrohAutolab) integrated with NOVA 2.1® software. The measurements were performed in a 5.0 mL one-compartment electrochemical cell, with a three-electrode system consisting of a carbon paste electrode, a Pt wire and the Ag/AgCl/KCl_{sat} 3 M (both purchased from Lab solutions, São Paulo, Brazil), representing the working electrode, counter electrode and reference electrode, respectively. The experimental conditions for DPV assays were pulse width 0.5 s, pulse amplitude 50 mV and scan rate of 10 mV.s^{-1}. DPVs were baseline corrected and background subtracted to improve data visualization, and all data was treated in Origin 8® Software.

2.7. Electrochemical Index

To calculate the electrochemical index, the following equation was used:

$$EI = \frac{I_{pa1}}{E_{pa1}} + \frac{I_{pa2}}{E_{pa2}} + \cdots + \frac{I_{pan}}{E_{pan}}$$

Where peak current (Ipa) is a kinetic parameter, correlated with the quantity of electroactive species, and peak potential (Epa), is a thermodynamic parameter, related to the higher capacity to donate electrons. The EI, derived from the previous equation, is directly linked to the antioxidant activity [23].

2.8. Statistical analysis.

Principal components analysis was used in this work to correlate results. Henceforth, Spearman coefficient was selected and adapted to ordinal data. Moreover, the software XlStat 2013.1.01 was used for computational purposes.

3. RESULTS AND DISCUSSION

3.1. Gallic acid and resveratrol calibration curves

In order to quantify phenolic and flavonoid content in the tea and tincture (ethanol: water 2:3) samples, calibration curves were performed for GA and RE (Figure 1).

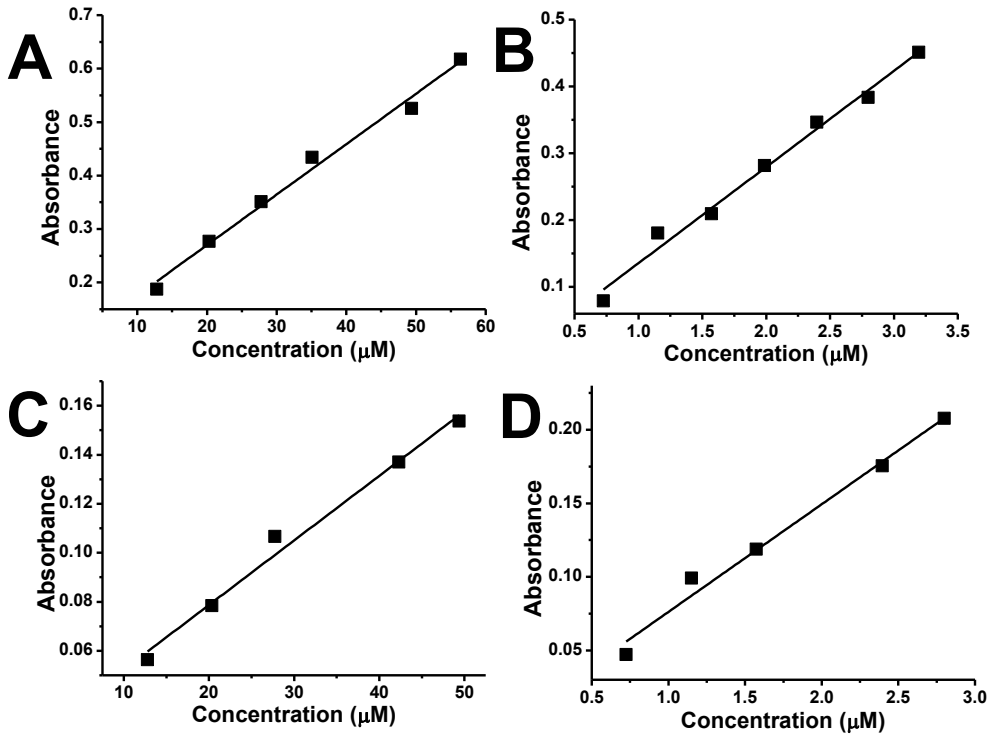

Figure 1. Calibration curves of resveratrol (A) and gallic acid (B) with ABTS radical and calibration curves for resveratrol (C) and gallic acid (D) with DPPH radical (r = 0,99).

A similar linear range was chosen in both assays and linear correlation was verified in all curves (r = 0.99). The linear regression equations were: y = 0.0094x + 0.0815, y = 0.1439x + 0.0084, y = 0.0026x + 0.026 and y = 0.0732x + 0.0029 for RE-ABTS, GA-ABTS, RE-DPPH and GA-DPPH, respectively.

3.2. Phenolic and Flavonoid Content Determination

With the calibration curves performed for both markers, in ABTS and DPPH assays, the concentration of GA and RE in the samples was found. Moreover, the equivalent of each marker was calculated and results were given as equivalent mg per g of dry extract, for the tea and tincture samples, as shown in Figures 2 and 3, respectively.

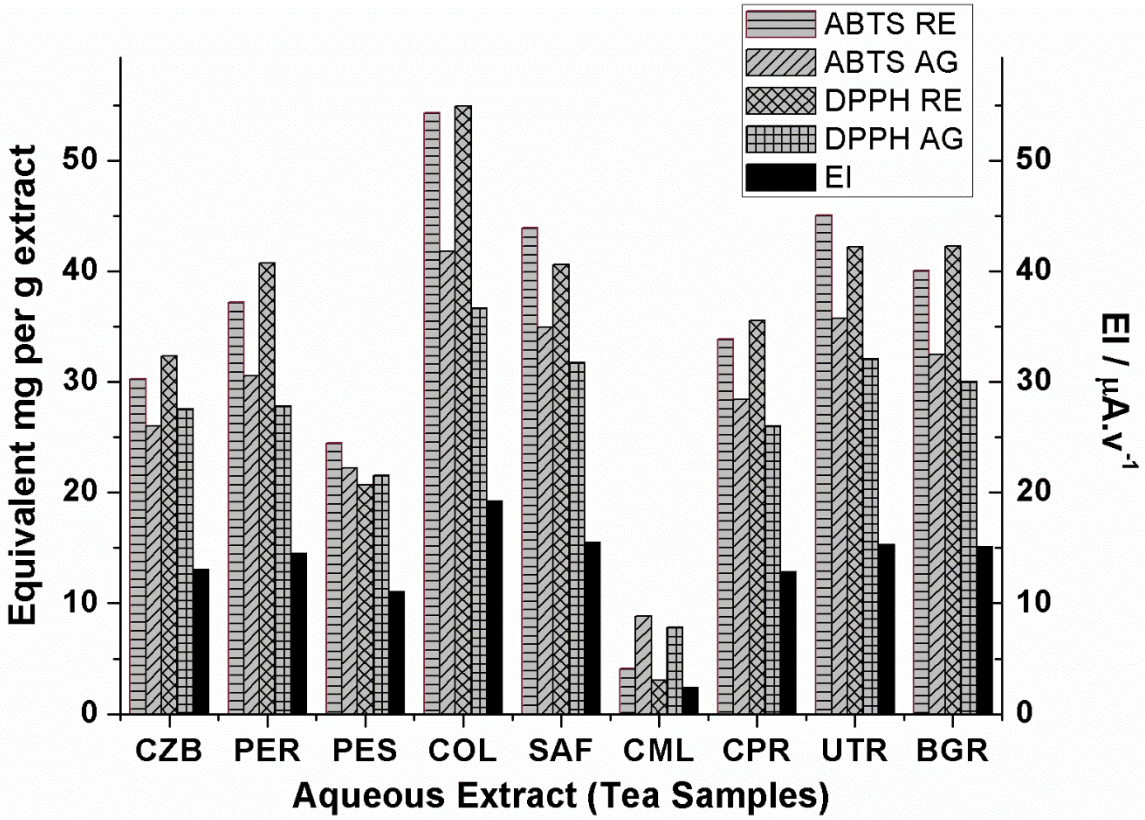

Figure 2. Values of equivalent mg of resveratrol (RE), gallic acid (GA) per gram of dry extract (left axis) and Electrochemical Index (EI) (right axis), for the respective medicinal plants in the tea samples (aqueous extraction).

Results evidence that the solvent used in the extraction deeply influence antioxidant activity in the final preparation. Literature reports that water does extract lesser compounds than hydroethanolic extraction due to differences in polarity, henceforth samples CZB, PER, SAF, CML and BGR presented better antioxidant features when their tincture form is concerned. It is well stated that aglycones do solubilize better in hydroethanolic solutions, which may explain results herein shown (Figure 2 and 3) [5-6].

The variability between herbs is nonetheless in accordance to literature, as leaves and barks are rich in phenylpropanoid metabolytes such as flavonoids and tannins, whereas roots may have lignin and terpenoids. Since some polyphenolic phytopolymers found in roots are soluble in water, root samples such as CPR and UTR presented better antioxidant activity in preparations, which used water as extraction solvent (Figure 2 and 3). Although this trend is known in literature, some varieties such as BGR do present high furanocoumarin concentration in roots. This metabolite is highly photo-active, which turns electrochemistry a good alternative for its analysis [7-8].

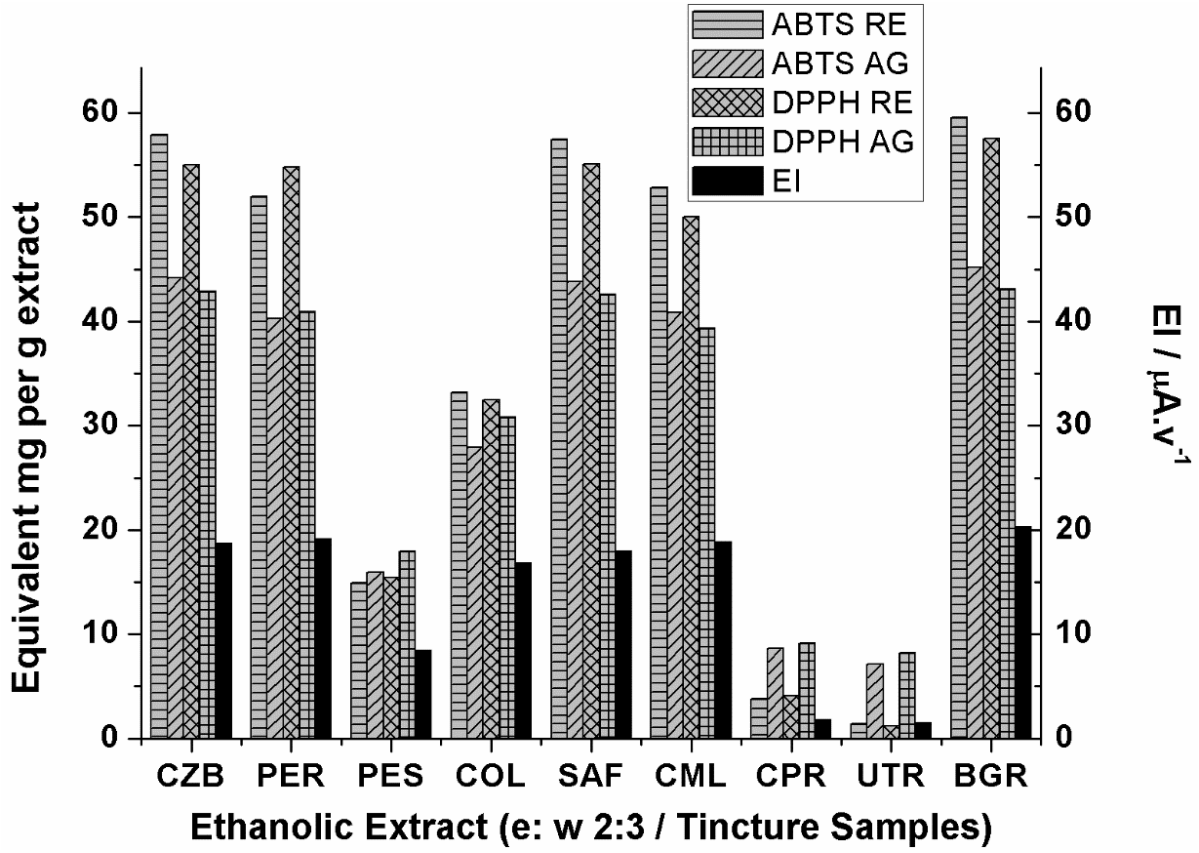

Figure 3. Values of equivalent mg of resveratrol (RE), gallic acid (GA) per gram of dry extract (left axis) and Electrochemical Index (EI) (right axis), for the respective medicinal plants in the tincture samples (ethanol:water 2:3) (hydroethanolic extraction).

3.3. Electrochemical assays

Different pulse voltammetries were performed for the respective tea/tincture form with highest contents of GA/RE in DPPH/ABTS (Figure 4).

Findings evidenced that each sample possesses fairly distinct electrochemical profiles. However, an anodic peak at c.a. E_a = 0.3 V was seen in all samples. The anodic peaks seen at 0.3 V < E_a < 1.0 V bear striking similarity to the behavior of polyphenolic compounds, which corroborates to the results herein exhibited, since polyphenols are nonetheless extracted by polar solvents [24-25].

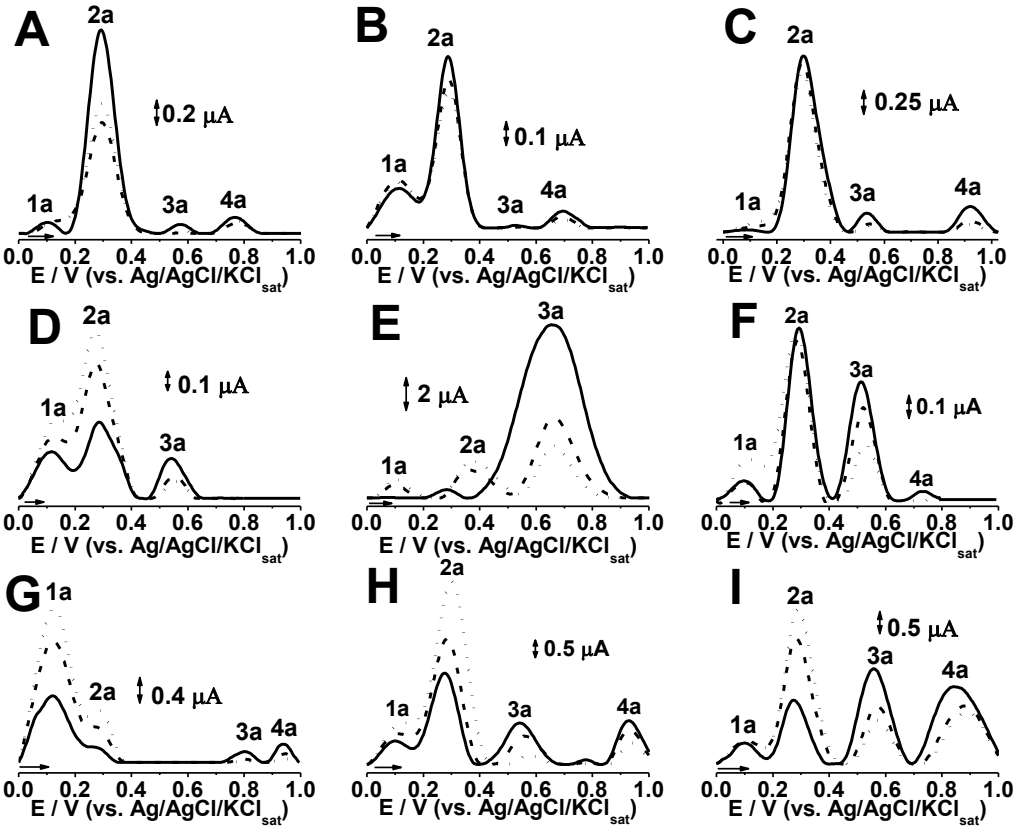

Figure 4. Differential pulse voltammetries, with sequential scanning (Scan 1 ——; Scan 2 – – –; Scan 3 • • •), performed in the medicinal plant extracts with higher antioxidant activity (in ABTS/DPPH assay) A: CZB (Tincture), B: CML (Tincture), C: PES (Tea), D: UTR (Tea), E: SAF (Tincture), F: BGR (Tincture), G: CPR (Tea), H: COL (Tea) e I: PER (Tincture).

CZB (Cinnamon) tincture showed anodic peaks c.a. E_a = 0.5 V, c.a. E_a = 0.8 and a small peak c.a. E_a = 0.1 V, which is in consonance to literature data concerning the electrochemical study of CZB used as foodstuff [26]. PES and PER (*Pterodon emarginatus*) likely possess phenolic compounds and flavonoids, as seen in their electrochemical behavior and supported by their therapeutic effects [27]. Their oxidation processes appeared to be quite similar, differing only in peak current, as expected for the unequal distribution of compounds within the plant parts, such as seed and rhizome [16].

CZB and PES electrochemical profile showed remarkable resemblance (Figure 4A, C), likely due to their similar composition, regarding antioxidant species, while CML, UTR, and CPR (Figure 4B, D, G) presented peaks c.a. E_a = 0.1 V with higher current values, which suggests higher concentrations. SAF (*Syzygium aromaticum*) presented the highest observed peak amongst all analyzed samples, attesting to its high content of electroactive compounds (Figure 4E), while BGR, COL and PER showed good distribution of all anodic peak, being all correlated to phenolic metabolytes (Figure 4F, H, I) [16-18].

There was no significant difference between tea and tincture samples, regarding the presence of electroactive compounds. This suggests that the wide variety of species in the samples may have partial solubility in both aqueous and organic solvents.

3.5. Statistical Analysis

In order to verify the agreement between the radical scavenging assays (DPPH and ABTS), a correlation matrix was calculated through principal component analysis, as displayed in Table 1.

Table 1. Correlation matrix calculated through Principal Components Analysis.

	Tea (Aqueous Extract)					**Tincture (e: w / 2:3 Hydroethanolic extract)**					
	ABTS RE	**ABTS GA**	**DPPH RE**	**DPPH GA**	**EI**		**ABTS RE**	**ABTS GA**	**DPPH RE**	**DPPH GA**	**EI**
ABTS RE	1	1	0,9835	0,9856	0.9569	**ABTS RE**	1	1	0,9974	0,9963	0.9648
ABTS GA	1	1	0,9835	0,9855	0.9569	**ABTS GA**	1	1	0,9974	0,9963	0.9645
DPPH RE	0,9835	0,9835	1	0,9762	0.9749	**DPPH RE**	0,9974	0,9974	1	0,9968	0.9859
DPPH GA	0,9856	0,9855	0,9762	1	0.9666	**DPPH GA**	0,9963	0,9963	0,9968	1	0.9799
EI	0.9569	0.9569	0.9749	0.9666	**1**	**EI**	0.9648	0.9645	0.9859	0.9799	1

GA and RE equivalent values correlated extremely well in both tea and tincture samples ($r > 0.98$). The correlation between both scavenging assays was excellent. Moreover, the EI derived from the DP voltammograms showed excellent correlation with both markers in each scavenging assay, thus, despite being methodologies that derive from different analytical signals, their inferences about antioxidant activities of phenolic compounds are in consonance. Correlation matrixes are excellent tools in the evaluation of the concordance and relationship of variables. The results herein shown demonstrate that the inferences derived from the equivalents with both scavenging methods and the EI reach the same conclusion.

4. CONCLUSIONS

This work evidences that solvent plays a decisive role in the extraction of electroactive compounds present in the selected herbs used in folk medicine. Moreover, the antioxidant activity of these preparations changes heavily according to the employed extraction fluids, and this activity can be assessed either by electrochemical or colorimetric techniques, henceforth rendering reproducible results.

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