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ORIGINAL RESEARCH ARTICLE

Phenolic composition and antioxidant activity assessment of southeastern and south Brazilian propolis

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Propolis is a resinous substance collected by honey bees *Apis mellifera* from several plant sources and used in the hive to seal the walls, to strengthen the ends of the honey comb or embalm dead invaders. The chemical specificity of propolis is directly determined by the variability of the plant origins and by geographical and climatic features of the collection site. The aim of this work was the quality assessment of 16 south and southeast Brazilian propolis samples through the identification and quantification of phenolic compounds using chromatographic and spectroscopic techniques such as HPLC and LC/DAD/ESI-MS^n. Generally, the samples presented a phenolic profile related to Brazilian green propolis with origin in *Baccharis* spp. leaves, where the caffeoylquinic acid derivatives as well as dihydrokaempferide and artepillin C were the main compounds. Moreover, DPPH$^•$ free radical-scavenging activity, reducing power and differential pulse voltammetry were applied to evaluate the antioxidant activity. Differential pulse voltammetry proved to be a rapid and easy tool for the quantification of the total electroactive species present in the samples. The results revealed a richer phenolic composition and higher bioactivity in Minas Gerais samples rather than the southern ones.

Composición fenólica y evaluación de la actividad antioxidante de los propóleos del sureste y sur de Brasil

El propóleos es una sustancia resinosa recogida por la abeja melífera *Apis mellifera* en varias fuentes vegetales que se usa en la colmena para sellar las paredes, reforzar los extremos de los cuadros de miel o embalsamar invasores muertos. La especificidad química del propóleos está directamente determinada por la variabilidad de los orígenes de las plantas y por las características geográficas y climáticas del lugar de recolección. El objetivo de este trabajo fue evaluar la calidad de dieciséis propóleos brasileños del sureste y el sur a través de la identificación y cuantificación de compuestos fenólicos utilizando técnicas cromatográficas y espectroscópicas como HPLC y LC / DAD / ESIMSn. En general, las muestras presentaron un perfil fenólico relacionado con el propóleos verde brasileño con origen en las hojas de *Baccharis* spp., donde los derivados del ácido cafeoilquínico, así como dihidrokaempferide y artepilina C fueron los principales compuestos. Por otra parte, la actividad de barrido de radicales libres DPPH$^•$, la reducción de la potencia y la voltametría de pulso diferencial se aplicaron para evaluar la actividad antioxidante. La voltametría de pulso diferencial demostró ser una herramienta rápida y fácil para la cuantificación de las especies electroactivas totales presentes en las muestras. Los resultados revelaron una composición fenólica más rica y una mayor bioactividad en muestras de Minas Gerais que en las del sur.

Keywords: propolis; HPLC; LC-MS; antioxidant activity; artepillin C; differential pulse voltammetry

Introduction

Propolis is the name of the resinous substance collected by honey bees (*Apis mellifera*) from various plant sources and used in the construction, repair and protection of their hives (Bankova, De Castro, & Marcucci, 2000). It is an important apicultural product widely used in folk medicine due to several pharmacological and nutritional applications, including anti-inflammatory, antifungal, antiviral properties and many other beneficial properties such as antiulcer, local anesthetic, immunostimulanting and cicatrizant (Salatino, Fernandes-Silva, Righi, & Salatino, 2011; Toreti, Sato, Pastore, & Park, 2013). Its effectiveness has been recognized in many fields and widely used in food and drinks to improve health and prevent diseases in the areas of dermatology, odontology, gynecological and cardiovascular problems, as well as in the prevention of diabetes (Banskota et al., 2001).

Propolis is a chemically complex product, mainly composed by beeswax, secreted by the bees and resin and volatiles, obtained from plants (Salatino, Teixeira, Negri, & Message, 2005). The special chemical properties of propolis are determined directly by the variability on the plant origin, which is linked with the geographical and climatic conditions of the site of collection (Bankova et al., 2000). In regions of temperate climate, where the poplar buds (*Populus* spp.) are the main source of resin,

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propolis has a composition rich in phenolic acids and their derivatives, flavonoids and their methylated and esterified derivatives (Falcão et al., 2010). In the tropics, where the poplars are not abundant, the bees seek other alternative floral sources for the production of resin. In commercial terms, green propolis, predominant in southeast Brazil, is the most important, with a composition rich in caffeoylquinic acids, prenylated phenylpropanoids, such as artepillin C and it is mainly produced with material obtained from the vegetative buds of *Baccharis dracunculifolia* (Salatino et al., 2011). Green propolis is hard and friable, made into powder by mechanical milling without difficult. It has a pleasant resinous odor and the color range varies from greenish-yellow to deep green (Salatino et al., 2005). Several other types of propolis were defined in respect to its plant source, chemical profile and geographical origin (Park, Alencar, & Aguiar, 2002a; Toreti et al., 2013). Northeast Brazilian propolis, with a composition rich in hyperibone A, has its origin in *Hyptis divaricata* buds and unexpanded leaves, while propolis mainly composed by chalcones, pterocarpans and isoflavonoids are originated from the resins of *Dalbergia ecastophyllum* (Silva, Rosalen, & Alencar, 2008). Propolis produced in the Brazilian Amazon contains predominantly polyprenylated benzophenones, which, most probably, are originated from *Clusia* spp. (Ishida, Negri, Salatino, & Bandeira, 2011).

Propolis from Brazil, has been the subject of many scientific studies due to its high biological activity, mainly anticancer, anti-HIV, antiinfluenza virus activities and as a immunosuppressant. Artepillin C, an important non-chromene prenylated cinnamic acid of green propolis, it's in the center of the majority of the pharmacological studies, which includes antimicrobial activity as well as toxicity to tumor cells (Toreti et al., 2013).

The demand for natural products such as propolis has increased in the society, and so the growing interest in propolis composition in association with the need of criteria for chemical standardization of the different propolis types makes the chromatographic and hyphenated techniques such as HPLC-DAD, LC-MS, LC-MS^n, GC-MS, powerful tools in the chemical profiling, enabling the identification and quantification of their bioactive constituents (Falcão et al., 2013a; Sforcin & Bankova, 2011).

In the present study, the propolis quality was evaluated through some physicochemical parameters, and the phenolic composition by spectrophotometry and chromatographic techniques such as HPLC and LC/DAD/ESI-MS^n from two Brazilian regions (south and southeast). The antioxidant activity of the phenolic extracts was also accessed through colorimetric assays as DPPH-based and iron(II) reduction capacity. Also, a new methodological approach, through electrochemical methods, was used for the rapid quantification of electroactive species (Falcão, Tomás, Freire, & Vilas-Boas, 2016).

Materials and methods

Standards and reagents

Standard compounds such as chlorogenic acid, caffeic acid, ferulic acid, isoferulic acid, *p*-coumaric acid and galangin were acquired from Sigma Chemical Co (St Louis, MO, USA). Kaempferol was from Extrasynthese (Genay, France) and pinocembrin from Latoxan (Valence, France). Aluminium chloride, potassium acetate, sodium carbonate, ferric chloride, potassium ferrycianide, trichloroacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteau reagent were from Sigma Chemical Co (St Louis, MO, USA). Tetrabutylammonium perchlorate (TBAP), electrochemical grade, were purchased to Fluka (Sigma Chemical Co., St. Louis, MO, USA), and kept at 30 °C before use. Water was obtained in a Mili-Q purification system (TGI PWS, Houston, TX, USA). All other analytical grade reagents were obtained from Panreac (Barcelona, Spain). HPLC grade methanol, ethanol and acetonitrile were purchased from Fisher Scientific (Leics, UK).

Brazilian propolis samples

Sixteen propolis samples were collected in different parts of Brazil (south and southeast) for this work. Samples from Santa Catarina and Rio Grande do Sul were statistically analyzed together, due to the reduced number of samples and considering its closeness. Details of the sampling are shown in Table 1 and Figure 1.

Color index

The CIELAB system color parameters were recorded on a Minolta colorimeter CR-400 (Osaka, Japan), in raw propolis samples, according to the previously described method (Falcão, Freire, & Vilas-Boas, 2013b). A standard white plate was used to calibrated the colorimeter (L^* = 94.56, a^* = −0.31, b^* = 4.16, C^*ab = 4.18 and hab = 94.3). CIE 1931 2° observer and illuminant *C* were

Table 1. Sampling of Brazilian propolis.

Code	Collected year	Visual color	Brazilian state
MG1	2010	Green	Minas Gerais
MG2	2010	Brown	Minas Gerais
MG3	2010	Brown	Minas Gerais
MG4	2010	Brown	Minas Gerais
MG5	2010	Green	Minas Gerais
MG6	2011	Green	Minas Gerais
MG7	2011	Green	Minas Gerais
MG8	2011	Brown	Minas Gerais
MG9	2011	Green	Minas Gerais
P1	2011	Brown	Paraná
P2	2011	Brown	Paraná
P3	2011	Brown	Paraná
P4	2011	Brown	Paraná
SC1	2011	Brown	Santa Catarina
RGS1	2010	Brown	Rio Grande do Sul
RGS2	2011	Brown	Rio Grande do Sul

Figure 1. Propolis sampling locations. Minas Gerais (MG), Paraná (P), Santa Catarina (SC) and Rio Grande do Sul (RGS).

considered as references in the measurements. The average values of three measurements were used (triplicate).

Spectrophotometric determination of phenolic compounds

Phenolic compounds extraction

The phenolic extraction for propolis was performed according to previous work (Falcão et al., 2010). Briefly, 1 g of sample was mixed with 10 ml of 80% of ethanol/water and kept at 70 °C for 1 h. The mixture was filtered and the extraction was repeated under the same conditions. Finally, the resulting extracts were combined, concentrated and freeze-dried.

Total phenolic content

The determination of total phenolic content was performed according to the Folin-Ciocalteu methodology described previously (Falcão et al., 2013b). 0.5 ml of the ethanolic extract (0.5 mg/ml) was mixed with Folin–Ciocalteu's reagent (0.25 ml). After 3 min, 1 ml of a saturated $NaCO_3$ solution was added to the mixture and the volume was made up to 5 ml with distilled water. The solution was allowed to stand for 10 min at 70 °C and then cooled in the dark for 30 min. The absorbance was measured at 760 nm and a mixture of caffeic acid: galangin: pinocembrin (1:1:1) was used to obtained the calibration curve ($y = 4.4997x + 0.3956$; $R^2 = 0.998$). For each extract three replicates were performed.

Flavone and flavonol content

The flavone and flavonol content was estimated according to the method previously described (Falcão et al., 2013b). An aliquot (2 ml) of the ethanolic extract (0.5 mg/ml) was mixed with 0.2 ml of aluminum chloride solution (2% $AlCl_3$ in 5% acetic acid/methanol) and 5 ml of 5% acetic acid/methanol to adjust the final volume. After 30 min, in the dark at room temperature, the absorbance was measured at $\lambda = 415$ nm and galangin was used to estimate the standard curve ($y = 16.035x + 0.0028$; $R^2 = 0.998$). The results were given as mg of galangin equivalents per g of extract. Each extract was measured in triplicate.

Flavanone and dihydroflavonol content

The flavanones and dihydroflavonols content were measured using a previously described method (Falcão et al., 2013b). Briefly, 1 ml of the propolis ethanolic extract (10 mg/ml) was mixed with 2 ml of 2,4-dinitrophenylhidrazine (DNP) solution (1 g of DNP was dissolved in 2 ml of 96% sulphuric acid and methanol was added up to the final volume of 100 ml) and the mixture was allowed to stand for 50 min at 50 °C. The mixture, cooled to room temperature, was diluted to 10 ml in 10% KOH in methanol (w/v). The resulting solution (1 ml) was added to 10 ml of methanol and diluted to a final volume of 50 ml with methanol. The absorbance was measured at $\lambda = 495$ nm and pinocembrin was used for the estimation of the standard curve ($y = 0.1716x + 0.0075$; $R^2 = 0.996$). The results, made in triplicate, were given in equivalents of pinocembrin per g of extract.

LC/DAD/ESI-MS^n analysis of propolis

The LC/DAD/ESI-MS^n analyses were performed on a Finnigan Surveyor Plus HPLC instrument equipped with a diode-array detector and coupled to a mass detector. The chromatographic conditions used were described before (Falcão et al., 2013a). The mass spectrometer used was a Finnigan Surveyor LCQ XP MAX quadrupole ion trap mass spectrometer equipped with an ESI source, operating in the negative ion mode. Control and data acquisition was carried out with Xcalibur data system (Thermo Finnigan, San Jose, CA, USA). MS conditions were the same as described previously (Falcão et al., 2013a).

HPLC phenolic quantification

The phenolic profile of the propolis ethanolic extract was analyzed by reverse phase high-performance liquid chromatography (HPLC) based on previous work (Falcão et al., 2010). Analysis was performed by ultra-fast liquid chromatography (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PDA, using 280 nm as preferred wavelength. For the HPLC analysis the propolis ethanolic extracts

(10 mg) were diluted in 1 ml of 80% of ethanol. *p*-coumaric acid methyl ester, as the internal standard (IS), was added to all extracts. All samples were filtered through a 0.2 μm Nylon membrane (Whatman) and 10 μL of each solution was injected. Quantification was achieved using calibration curves for chlorogenic acid (0.003–0.8 mg/ml; $y = 2.58x-0.123$; $R^2 = 0.99$), caffeic acid (0.002–0.6 mg/ml; $y = 4.81x-0.086$; $R^2 = 0.99$), *p*-coumaric acid (0.002–0.6 mg/ml; $y = 6.85x-0.119$; $R^2 = 0.99$), ferulic acid (0.002–0.6 mg/ml; $y = 4.33x-0.072$; $R^2 = 0.99$), kaempferol (0.005–1.2 mg/ml; $y = 2.08x-0.084$; $R^2 = 0.99$). When the standard was not available, the compound quantification was expressed in equivalent of the structurally closest phenolic compound.

Antioxidant activity

DPPH free radical-scavenging activity

The DPPH free radical-scavenging activity was estimated according to the previously described method (Falcão et al., 2013b**)**. The reaction was performed in a 96-wells microplate where different concentrations (2.5–40 μg/ml) of propolis extract solution (0.08 ml) was added to 0.292 ml of a 0.025 g/L DPPH solution in 80% ethanol/water, prepared daily. The bleaching of the DPPH radical was monitored at 515 nm using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc.). A standard solution of different phenolic compounds (caffeic acid: galangin: pinocembrin, 1:1:1) were also evaluated. The percentage of DPPH inhibition was calculated by the following equation:

$$\%\text{Inibition} = \left[\left(A_{\text{DPPH}^{\cdot}} - A_{\text{sample}}\right)/A_{\text{DPPH}^{\cdot}}\right] \times 100$$

The scavenging activity of propolis on the DPPH radical was expressed as EC_{50} (mg/ml) which was extrapolated from a % inhibition vs. extract concentration curve. All analyses were performed in triplicate.

Reducing power

For the reducing power estimation the method previously described (Falcão et al., 2013b) was followed. Briefly, 2.5 ml of the propolis ethanolic extract (10 to 200 μg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The final mixture was maintained during 20 min at 50 °C. After this time, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged during 10 min at 3000 rpm (Centurion K_2R series). The upper layer of the solution (2.5 ml) was removed and mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The reducing power was determined based on the absorbance at 700 nm and using a mixture of caffeic acid:-galangin: pinocembrin (1:1:1) to estimate the standard curve ($y = 5.4753x + 0.0153$; $R^2 = 0.993$). The assay was performed in triplicate and the results were expressed in g equivalents/g of propolis extract.

Differential pulse voltammetry

The electrochemical experiments were performed using an Autolab PGSTAT 302 potentiostat/galvanostat with a standard three electrode cell. The potentials were compared with an Ag/AgCl 3 M KCl reference electrode and measured using a glassy carbon with a 3 mm diameter as the working electrode. The electrical system was closed with a Pt wire as counter electrode, according with the previously described work (Falcão et al., 2016). Before each analysis, the surface of the working electrode was scratched on a polishing pad (Master-Tex -Beuhler) with an aqueous suspension of 0.3 μm alumina (Beuhler), and then rinsed with deionized water and sonicated, first in HCl 6 M during 1 min, and then in methanol.

The voltammetric study was performed in ethanol/Britton-Robinson buffer /TBAP (78:20:2) at pH 7, between −0.25 V and 1.5 V. The differential pulse voltammetry conditions used were 60 mV for the pulse amplitude and scan rate of 0.030 Vs^{-1}. The propolis extracts solutions were analysed at 1 mg/ml. Prior to each experiment, the background current was tested in the ethanol/buffer/electrolyte solution. The electrochemical response was recorded after immersion of the working electrode in freshly prepared solutions, to reduce adsorption phenomena into the electrode surface before scanning.

Differential pulse voltammograms were also reported for *p*-coumaric acid, using concentrations in the range 0.01–0.10 mg/ml. A calibration curve ($y = 31.253x + 4.468$; $R^2 = 0.9696$) was obtained for this standard and was used to calculate the electrochemical antioxidant power (EAP), which was expressed in equivalents of *p*-coumaric acid per gram of propolis extract (mg/ml of *p*-coumaric acid). The total electrochemical antioxidant power (TEAP) of a propolis sample corresponds to the sum of the current density of each electrochemical process value, calculated at peak maxima. Each experiment was run in triplicate and the results are presented as an average.

Statistics

The statistical analysis was performed using SPSS version 20 program. All values were considered using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. In each column, different superscript letters mean significance differences between sample groups.

Results and discussion

In the last years, many researches has been done on the chemical composition and biological activities of propolis, in special Brazilian propolis (Righi, Negri, & Salatino, 2013; Toreti et al., 2013), leading to the identification of over more than 300 chemical constituents in propolis

from different origins (Bankova et al., 2000). Although, many analytical methods have been used for the characterization of propolis constituents and its properties, there is still a need for the rapid propolis quality assessment, a key factor for its commercialization. Our proposal set the electrochemical methods as an innovative, fast, easy and low-cost tool for the evaluation of the redox profile and the quantification of the total antioxidant capacity in Brazilian propolis.

Physicochemical analysis

One of the first steps for the commercialization of propolis is its sensorial characteristics evaluation and within these, the propolis color, which can present different colors depending on the botanical origin and age (Salatino et al., 2005). The south and southeastern Brazilian propolis showed different colors depending on its provenance, Table 1. Through visual observation, the majority of the samples from southeast Brazil (Minas Gerais - MG) presented a greenish color, but four samples (MG2-4 and MG8) presented a brown color. This color was also observed for all the samples from the south regions (Paraná - P, Rio Grande do Sul - RGS, Santa Catarina-SC), Table 1. A rapid methodology, using the CIELAB color system (CIE, 1986), was previously developed for the determination of the propolis color index in Portuguese propolis (Falcão et al., 2013b). The CIELAB color system is composed by a component of lightness (L^*) which measures the intensity of light. The chromatic components are measured in two parameters, a^* which shifts from green (−a) to red (+a) and b^* represents blue (−b) to yellow (+b) (Falcão et al., 2013b), which are shown in Table 2, as well as the values for the color density C^*ab. Considering the chromatic components, the southeastern propolis, samples with a visual green color, such as MG1, MG5-7 and MG9, presented lower values of a^* than the samples with visual brown color, Table 2, confirming a greenish shift. The same statistical different was found for the b^* coordinate and the color density C^*ab, but in this case, with the brownish samples showing the lower values.

One of the most important aspects of propolis quality can be based on the quantification of the main biologically constituents and, in general, a good propolis quality means a high phenolic content (according to the chemical type). The total phenols and flavonoids content in the propolis samples under study were assessed by spectrophotometric procedures after a hydro-alcoholic extraction. The phenolic content of the samples, Table 2, showed some variability within the same collection region, with values ranging from 2 to 31%, where the samples from Minas Gerais, particularly the green propolis samples (MG-G) showed the highest phenolic content, with an average value of 20%. This result suggest different floral contributions for the resin, which is in accordance with previously published results (Teixeira, Message, Negri, Salatino, & Stringheta, 2008). Melo, Matsuda, and Almeida-Muradian (2012) obtained similar values for total phenolic content in Brazilian propolis samples: 8–19% (Minas Gerais state); 10–13% (Parana state); 5–10% (Santa Catarina state) and 5–6% (Rio Grande do Sul state).

Table 2. Physicochemical analysis of Brazilian propolis (each value is the mean ± standard deviation, $n = 3$).

Samples	Color index				Total phenolics (%)	Flavones and flavonols (%)	Flavanones and Dihydroflavonols (%)
	L^*	a^*	b^*	C^*ab			
MG1	52 ± 1	3 ± 1	32 ± 1	32 ± 1	19 ± 1	3.2 ± 0.0	3.8 ± 0.0
MG5	46 ± 5	6 ± 1	27 ± 1	28 ± 1	11 ± 1	1.6 ± 0.1	2.8 ± 0.7
MG6	44 ± 0	3 ± 1	30 ± 0	30 ± 0	22 ± 2	2.2 ± 0.3	4.9 ± 0.1
MG7	43 ± 2	3 ± 0	28 ± 3	29 ± 3	17 ± 3	3.5 ± 0.2	5.2 ± 0.4
MG9	43 ± 1	4 ± 2	33 ± 0	33 ± 0	31 ± 0	1.2 ± 0.0	1.8 ± 0.3
X(MG − G) ± SD	46 ± 4[a]	4 ± 2[a]	30 ± 3[b]	30 ± 2[b]	20 ± 7[c]	2.3 ± 0.9[b]	4 ± 1[a]
MG2	39 ± 0	6 ± 1	21 ± 1	22 ± 1	19 ± 0	1.4 ± 0.1	5.0 ± 0.5
MG3	44 ± 1	6 ± 2	28 ± 1	29 ± 1	8 ± 1	1.4 ± 0.1	4.9 ± 0.6
MG4	45 ± 1	6 ± 1	28 ± 1	27 ± 1	11 ± 1	1.3 ± 0.1	3.8 ± 0.9
MG8	38 ± 1	4 ± 0	22 ± 1	22 ± 1	17 ± 0	2.6 ± 0.2	3.3 ± 0.9
X(MG − B) ± SD	42 ± 3[a]	6 ± 1[b]	26 ± 4[a]	25 ± 3[a]	14 ± 5[b]	1.7 ± 0.6[b]	4 ± 1[a]
P1	42 ± 2	7 ± 0	28 ± 1	29 ± 1	8 ± 0	1.1 ± 0.1	3.4 ± 0.0
P2	38 ± 4	6 ± 1	23 ± 4	26 ± 4	10 ± 0	0.9 ± 0.1	5.8 ± 0.9
P3	39 ± 1	4 ± 1	20 ± 1	20 ± 0	11 ± 0	1.3 ± 0.1	0.7 ± 0.9
P4	52 ± 3	5 ± 1	23 ± 2	24 ± 2	2 ± 0	0.3 ± 0.0	1.1 ± 0.8
X(P) ± SD	43 ± 6[a]	6 ± 1[b]	24 ± 4[a]	25 ± 4[a]	8 ± 4[a]	0.9 ± 0.4[a]	3 ± 2[a]
SC1	45 ± 0	5 ± 0	30 ± 0	31 ± 0	6 ± 0	0.1 ± 0.0	5.1 ± 0.0
RGS1	44 ± 1	6 ± 0	22 ± 1	23 ± 1	2 ± 0	0.7 ± 0.1	3.8 ± 0.4
RGS2	40 ± 2	6 ± 0	20 ± 1	21 ± 1	2 ± 0	0.0 ± 0.0	3.2 ± 0.3
X(SC/RGS) ± SD	43 ± 3[a]	6 ± 1[b]	24 ± 5[a]	25 ± 5[a]	3 ± 2[a]	0.3 ± 0.3[a]	4 ± 1[a]

Notes: X is the mean value for each group. Minas Gerais green propolis (MG-G); Minas Gerais brown propolis (MG-B); Paraná (P); Santa Catarina and Rio Grande do Sul (SC/RGS). In each column, different letters (a–c) mean significant differences between groups ($p < 0.05$).

Table 3. Characterization of the phenolic compounds from Brazilian propolis obtained by LC/DAD/ESI-MS^n.

Compound	RT (min)	λ_{max} (nm)	$[M-H]^-$ *m/z*	MS^n (% base peak)
5-*O*-Caffeoylquinic acid[a] (1)	9.6	325	353	191 (*66*), 179 (*100*), 135 (*14*)
Caffeic acid[a] (2)	13.7	322	179, $[M + 46]^-$: 225	
Dicaffeoylquinic acid[b,c] (3)	17.6	325	515	353
p-Coumaric acid[a] (4)	18.5	310	163, $[M + 46]^-$: 208	
Dicaffeoylquinic acid (isomer)[b,c] (5)	19.3	325	515	353
Ferulic acid[a] (6)	19.5	295sh, 322	193, $[M + 46]^-$: 238	
Isoferulic acid[a] (7)	20.2	298, 319	193, $[M + 46]^-$: 238	
Tricaffeoylquinic acid[b,c] (8)	29.5	325	677	515
Dihydrokaempferide[b,d] (9)	41.7	292	301	283 (*100*), 227 (*21*), 151 (*52*)
Drupanin[b,c] (10)	53.9	313	231	187
Capillartimisin A[b,d] (11)	55.4	310	315	285 (*65*), 271 (*100*), 241 (*55*)
Dihydroconiferyl *p*-coumarate[b,d] (12)	55.8	310	327	283
Kaempferide[b,e] (13)	63.8	265, 364	299	284 (*100*), 151 (*<1*)
Artepillin C[b,c] (14)	72.9	310	299	255

[a]Confirmed with standard.
[b]Confirmed with MS^n fragmentation.
[c]Confirmed with references: Gardana, Scaglianti, Pietta, and Simonetti (2007).
[d]Kumazawa et al. (2003).
[e]Falcão et al. (2013a).

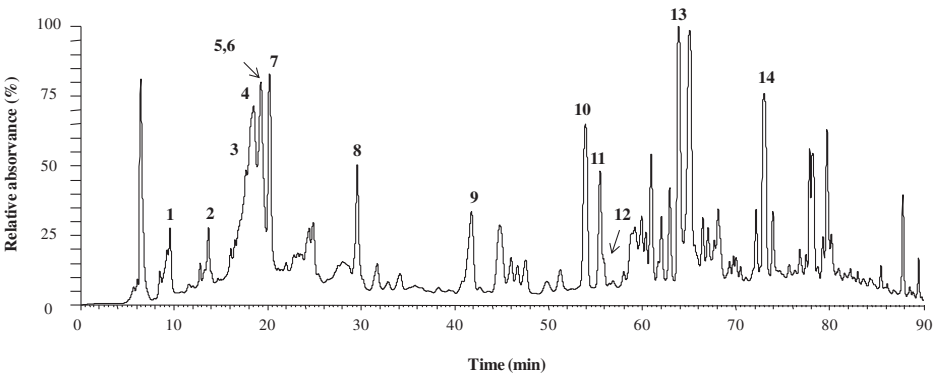

Figure 2. Chromatographic profile of the sample MG1 obtained at 280 nm for phenolic propolis extract by LC-MS.
Notes: (1) 5-O-caffeoylquinic acid (1); (2) caffeic acid; (3) dicaffeoylquinic acid; (4) *p*-coumaric acid; (5) dicaffeoylquinic acid (isomer); (6) ferulic acid; (7) isoferulic acid; (8) tricaffeoylquinic acid; (9) dihydrokaempferide; (10) drupanin; (11) capillartimisin A; (12) dihydroconiferyl *p*-coumarate; (13) kaempferide; (14) artepillin C.

Concerning the flavonoid content, Table 2, the values were low, with flavones/flavonols and flavanones/dihydroflavonols ranging from 0.1–3.5% to 0.6–5.8%, respectively. The results obtained in the present work are in agreement with those obtained for the São Paulo state propolis, with low values of flavonoids (Tagliacollo & Orsi, 2011), which is in accordance with the described composition of green propolis were the major compounds are phenolic acids derivatives (Salatino et al., 2011).

LC/DAD/ESI-MS^n analysis of Brazilian propolis

The complexity of propolis composition is correlated to the different floral sources visited by bees, being the chemical composition the best indicator for the floral origin associated.

The LC–DAD–ESI–MS^n study of the 16 south and southeast Brazilian propolis samples allowed the elucidation of phenolic compounds by comparison of their chromatographic behaviour, UV spectra and MS information with reference compounds, Table 3. When standards were not available, the structural information was confirmed with UV data combined with MS fragmentation patterns previously reported in the literature. This study was carried out using LC-MS in the negative ion mode because of its higher sensitivity in the analysis of the different polyphenol classes (Falcão et al., 2013a). The chromatographic profile is shown in Figure 2.

Fourteen phenolic compounds were identified which included simple phenolic acids like caffeic acid (2; *m/z* 179), *p*-coumaric acid (4, *m/z* 163), ferulic acid (6, *m/z* 193), isoferulic acid (7, *m/z* 193), phenolic acid esters like 5-*O*-caffeoylquinic acid (1; *m/z* 353), two isomers of dicaffeoylquinic acid (3,5; *m/z* 515), tricaffeoylquinic acid (8, *m/z* 677) and dihydroconiferyl *p*-coumarate (12, *m/z* 327), terpenic phenolic acids like drupanin (10, *m/z* 301), capillartemisin A (11, *m/z* 315) and artepillin C

(14, *m/z* 299), Figure 2. Also, two kaempferol derivatives, dihydrokaempferide (10, *m/z* 301) and kaempferide (13, *m/z* 299) were identified. The phenolic profile found in most of the samples is in agreement with the green propolis type whose origin are the vegetative apices of *Baccharis* sp., mainly *B. dracunculifolia* (Kumazawa et al., 2003; Park, Paredes-Guzman, Aguiar, Alencar, & Fujiwara, 2004), which occurs in mountain regions of the southern part of the state of Minas Gerais to the northern part of the state of Paraná. Samples from Santa Catarina and Rio Grande do Sul do not fit in the same profile.

HPLC phenolic quantification

The chemical diversity of south and southeastern Brazilian propolis was also evaluated through the phenolic quantification of the main compounds in the samples using HPLC/DAD. The *p*-coumaric acid methyl ester was chosen as internal standard, considering the detector response and the retention time.

The samples presented a similar composition, although significant differences were found in the concentrations between regions, Figure 3. Analysing the main phenolic classes, the simple phenolic, especially the phenolic esters, emerge as the main compounds, being the flavonoids a minor class in the samples, which is in accordance with green propolis type (Salatino et al., 2005). Minas Gerais samples, particularly the green samples (MG-G), showed the highest content in the total phenolics and total flavonoids, while the most southern propolis from Santa Catarina and Rio Grande do Sul where very poor in this respect.

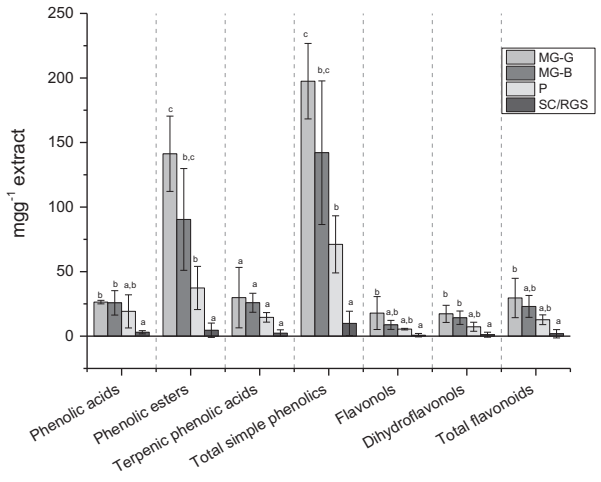

Figure 3. Composition for the main classes of phenolic compound present in Brazilian propolis samples from: Minas Gerais green propolis (MG-G), Minas Gerais brown propolis (MG-B), Paraná (P), Santa Catarina/Rio Grande do Sul (SC/RGS). Each value is the mean ± standard deviation, $n = 3$. In each column, different letters (a–c) mean significant differences between regions ($p < 0.05$). The individual values are available in the supplementary material.

In the present study, the two isomers of dicaffeoylquinic acid and artepillin C were the most abundant compounds in the samples, which was in accordance to the previously reported for samples of the same area (Midorikawa et al., 2001). The majority of the samples had the same profile, presenting all the compounds, with exception of samples SC from Santa Catarina, only containing *p*-coumaric acid and ferulic acid and RGS2 from Rio Grande do Sul containing *p*-coumaric acid, ferulic acid, tricaffeoylquinic acid, drupanin and capillartemisin A in low levels, Table 4. This different phenolic composition can be due to the contribution of other floral species rather than *Baccharis* spp. as was previously reported by Park, Alencar, Scamparini, and Aguiar (2002b) for propolis from south Brazil. Papers dealing with HPLC phenolic quantification of other compounds rather than artepillin C are not usual, due to the commercial importance of the artepillin C content in Brazilian samples (Midorikawa et al., 2001; Park et al., 2004; Sousa et al., 2007).

Artepillin C has been established as a marker compound for the Brazilian green propolis due to its unique and characteristic presence in these samples and because it is known to be produced by its plant source, *Baccharis* spp. (Park et al., 2004; Salatino et al., 2011). Analyzing the results, propolis from southeast Brazil, showed high levels of artepillin C, in special the Minas Gerais samples with values ranging from 3 to 51 mg/g of extract, Table 4. Paraná propolis presented a similar phenolic profile but with a lower artepillin C content, with an average value of 8 mg/g of extract. In the southern propolis SC from Santa Catarina and RGS2 from Rio Grande do Sul state, artepillin C was not detected, which can be related to the possible absence of *Baccharis* spp. in the neighborhood of the hives. Artepillin C content was converted to % (Table S1, available in the supplementary material in the on-line version) to allow the comparison with the results reported previously. Matsuda and Almeida-Muradian (2008) analyzed the content of artepillin C in several samples of Brazilian propolis. The results presented a range of 6–10% in Minas Gerais state; 3–5% in Paraná state; 0.2–0.6% in Santa Catarina state and 0.1–0.2% in Rio Grande do Sul state. These values were different than the ones found in the present work which can be due to the different extraction conditions and to the fact that the latter were quantified with a calibration curve of *p*-coumaric acid. Despite the differences, the concentration pattern were the same, with the southeastern propolis presenting higher artepillin C values rather than the southern samples.

The composition variability registered between propolis samples both from distant and nearby locations are due to the possible contribution from other resin plants sources in the propolis manufacturing rather than *Baccharis* spp., which can be in less or higher extension. Righi et al. (2013) reported a correlation between the different shades of green in the propolis color and the

Table 4. Phenolic content in south and southeast Brazilian propolis samples in mg/g of extract (each value is the mean ± standard deviation, $n = 3$).

Samples	CafQA	CafA	DicafQA	*p*CoumA	DicafQA	FerA	iFerA	TricafQA	DiHKaemp	Drup	CaprtmA	Kaemp	ArtpC
MG1	15.3 ± 0.5	4.9 ± 0.0	55.0 ± 0.8	16.4 ± 0.1	71.5 ± 1.2	4.0 ± 0.0	2.5 ± 0.4	23.6 ± 0.8	23.6 ± 0.0	8.3 ± 0.1	3.8 ± 0.4	18.9 ± 0.2	27.7 ± 0.2
MG5	11.1 ± 0.1	3.6 ± 0.0	37.1 ± 0.0	10.7 ± 0.1	49.6 ± 0.2	10.1 ± 0.0	2.8 ± 0.0	17.7 ± 0.0	13.0 ± 0.2	5.1 ± 0.0	2.9 ± 0.0	6.4 ± 0.9	6.6 ± 0.0
MG6	15.3 ± 0.1	2.6 ± 0.0	43.2 ± 0.1	18.8 ± 0.0	59.7 ± 0.1	3.3 ± 0.0	2.0 ± 0.0	15.4 ± 0.0	15.1 ± 0.0	4.8 ± 0.0	3.0 ± 0.2	7.5 ± 0.7	12.8 ± 0.0
MG7	8.7 ± 1.4	2.5 ± 0.0	38.0 ± 0.1	16.5 ± 0.1	50.6 ± 0.0	3.1 ± 0.1	2.3 ± 0.0	16.6 ± 0.0	24.9 ± 0.6	9.6 ± 0.2	5.4 ± 0.0	24.1 ± 0.2	51.1 ± 0.0
MG9	13.4 ± 0.1	2.7 ± 0.0	61.8 ± 1.3	17.3 ± 0.0	80.6 ± 0.7	3.6 ± 0.1	2.3 ± 0.2	22.1 ± 0.1	9.8 ± 0.2	3.1 ± 0.0	2.1 ± 0.0	4.7 ± 0.1	3.1 ± 0.0
X(MG-G) ± SD	13 ± 3^{c}	3.3 ± 0.9^{c}	47 ± 10^{c}	16 ± 3^{b}	62 ± 12^{c}	5 ± 3^{b}	2.4 ± 0.3^{c}	19 ± 3^{d}	17 ± 6^{b}	6 ± $3^{b,c}$	3 ± $1^{b,c}$	12 ± 8^{b}	20 ± 18^{b}
MG2	5.9 ± 0.0	2.8 ± 0.0	32.7 ± 0.3	17.3 ± 0.1	47.6 ± 0.1	2.8 ± 0.0	2.3 ± 0.1	14.7 ± 0.0	13.2 ± 0.0	10.2 ± 0.1	3.8 ± 0.1	6.4 ± 0.1	11.1 ± 0.0
MG3	6.1 ± 0.6	2.5 ± 0.0	13.7 ± 0.2	6.4 ± 0.0	17.6 ± 0.1	3.1 ± 0.0	2.0 ± 0.4	9.4 ± 0.0	11.6 ± 0.2	6.0 ± 0.1	2.6 ± 0.0	9.5 ± 0.5	10.0 ± 0.1
MG4	8.8 ± 0.0	2.7 ± 0.0	23.7 ± 0.1	14.4 ± 0.0	29.5 ± 0.6	7.9 ± 0.0	1.9 ± 0.0	12.3 ± 0.0	10.4 ± 0.0	5.9 ± 0.0	4.1 ± 0.0	5.7 ± 0.0	13.9 ± 0.0
MG8	12.6 ± 0.8	3.7 ± 0.0	47.4 ± 0.1	27.2 ± 0.1	59.8 ± 0.3	3.8 ± 0.2	2.5 ± 0.0	19.7 ± 0.0	21.9 ± 0.0	11.8 ± 0.1	4.0 ± 0.0	13.4 ± 0.0	20.3 ± 0.0
X(MG-B) ± SD	8 ± $3^{b,c}$	2.9 ± $0.5^{b,c}$	29 ± 13^{b}	16 ± 8^{b}	39 ± 17^{b}	4 ± 2^{b}	2.2 ± 0.3^{c}	14 ± 4^{c}	13 ± 7^{b}	8 ± 3^{c}	4 ± 1^{c}	9 ± $3^{a,b}$	14 ± $4^{a,b}$
P1	3.5 ± 0.3	1.7 ± 0.2	6.1 ± 0.2	3.9 ± 0.2	8.6 ± 0.8	2.7 ± 0.0	0.9 ± 0.0	5.0 ± 0.0	3.8 ± 0.2	4.1 ± 0.1	1.4 ± 0.0	4.7 ± 0.0	6.7 ± 0.0
P2	3.2 ± 0.2	1.3 ± 0.0	6.7 ± 0.1	2.7 ± 0.0	8.8 ± 0.2	2.0 ± 0.1	0.9 ± 0.0	4.4 ± 0.0	5.3 ± 0.0	3.4 ± 0.0	1.5 ± 0.0	6.1 ± 0.4	6.1 ± 0.0
P3	13.8 ± 0.2	2.7 ± 0.0	10.8 ± 0.2	9.2 ± 0.0	21.2 ± 0.8	5.4 ± 0.0	2.1 ± 0.3	9.9 ± 0.0	11.7 ± 0.0	4.5 ± 0.0	4.2 ± 0.0	5.8 ± 0.1	10.3 ± 0.0
P4	6.0 ± 0.0	2.8 ± 0.0	14.1 ± 0.0	4.1 ± 0.0	18.1 ± 0.0	4.7 ± 0.0	2.0 ± 0.0	9.1 ± 0.0	8.3 ± 0.0	4.8 ± 0.0	2.6 ± 0.0	5.2 ± 0.3	8.8 ± 0.0
X(P) ± SD	7 ± $4^{a,b}$	2.1 ± $0.7^{a,b}$	9 ± 3^{a}	5 ± 3^{a}	14 ± 6^{a}	4 ± 1^{a}	1.5 ± 0.6^{b}	7 ± 3^{b}	7 ± 3^{a}	4 ± 1^{b}	2 ± 1^{b}	5 ± $1^{a,b}$	8 ± $2^{a,b}$
SC1	ND	ND	ND	1.1 ± 0.0	ND	1.0 ± 0.0	ND	ND	ND	ND	ND	ND	ND
RGS1	2.5 ± 0.0	1.1 ± 0.2	2.8 ± 0.0	1.0 ± 0.1	2.4 ± 0.0	1.7 ± 0.0	0.8 ± 0.0	3.1 ± 0.0	3.4 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	2.3 ± 0.4	2.6 ± 0.0
RGS2	ND	ND	ND	1.0 ± 0.1	ND	1.4 ± 0.0	ND	3.0 ± 0.0	ND	0.9 ± 0.0	0.9 ± 0.0	ND	ND
X(SC/RGS) ± SD	3 ± 0^{a}	1.1 ± 0.2^{a}	3 ± 0^{a}	1 ± 0^{a}	2 ± 0^{a}	1 ± 0^{a}	0.8 ± 0^{a}	3 ± 0^{a}	3 ± 0^{a}	2 ± 1^{a}	1 ± 0^{a}	2 ± 0^{a}	3 ± 0^{a}

Notes: CafQA = 5-*O*-Caffeoylquinic acid; CafA = Caffeic acid; DicafQA = Dicaffeoylquinic acid; *p*CoumA = *p*-Coumaric acid; DicafQA = Dicaffeoylquinic acid; FerA = Ferulic acid;iFerA = Isoferulic acid; TricafQA = Tricaffeoylquinic acid; DiHKamp = Dihydrokaempferide; Drup = Drupanin; CaprtmA = Capillartimisin A; Kaemp = Kaempferide; ArtpC = Artipillin C: ND – Not detected. *X* is the mean value for each group. Minas Gerais green propolis (MG-G); Minas Gerais brown propolis (MG-B); Paraná (P); Santa Catarina and Rio Grande do Sul (SC/RGS). In each column, different letters (a–d) mean significant differences between groups ($p < 0.05$).

Table 5. Evaluation of antioxidant activity of Brazilian propolis using DPPH, reducing power and electrochemical antioxidant power (obtained by differential pulse voltammetry in EtOH) methods (each value is the mean ± standard deviation, $n = 3$).

Sample	EC_{50} DPPH[a]	Reducing Power[b]	EAP[c]			TEAP[d]
			0.2 V	0.4 V	0.8 V	
MG1	0.04 ± 0.01	0.27 ± 0.07		0.31 ± 0.01		0.31 ± 0.01
MG5	0.03 ± 0.01	0.46 ± 0.00	0.01 ± 0.01	0.15 ± 0.01		0.17 ± 0.01
MG6	0.02 ± 0.00	0.58 ± 0.02		0.44 ± 0.01		0.30 ± 0.04
MG7	0.01 ± 0.00	0.55 ± 0.00	0.04 ± 0.02	0.34 ± 0.02		0.38 ± 0.03
MG9	0.04 ± 0.00	0.47 ± 0.04		0.49 ± 0.02	0.11 ± 0.04	0.60 ± 0.05
X(MG-G) ± SD	0.03 ± 0.01[a]	0.5 ± 0.1[b]				0.4 ± 0.1[b]
MG2	0.03 ± 0.00	0.68 ± 0.02		0.22 ± 0.01	0.12 ± 0.01	0.34 ± 0.02
MG3	0.04 ± 0.01	0.35 ± 0.01		0.12 ± 0.03		0.12 ± 0.03
MG4	0.04 ± 0.02	0.39 ± 0.01	0.06 ± 0.02	0.20 ± 0.02	0.06 ± 0.00	0.29 ± 0.06
MG8	0.02 ± 0.00	0.56 ± 0.01		0.30 ± 0.02		0.30 ± 0.02
X(MG-B) ± SD	0.04 ± 0.02[a,b]	0.5 ± 0.1[b]				0.3 ± 0.1[b]
P1	0.03 ± 0.00	0.31 ± 0.02	0.01 ± 0.00	0.07 ± 0.01		0.08 ± 0.01
P2	0.05 ± 0.01	0.26 ± 0.01	0.01 ± 0.01	0.07 ± 0.05		0.07 ± 0.05
P3	0.03 ± 0.00	0.50 ± 0.01	0.02 ± 0.00	0.09 ± 0.01		0.11 ± 0.01
P4	0.11 ± 0.01	0.08 ± 0.01	0.01 ± 0.01	0.06 ± 0.01		0.07 ± 0.01
X(P) ± SD	0.06 ± 0.03[b,c]	0.3 ± 0.2[a]				0.1 ± 0.0[a]
SC1	0.06 ± 0.01	0.25 ± 0.01		0.09 ± 0.01		0.09 ± 0.01
RGS1	0.04 ± 0.00	0.22 ± 0.01		0.11 ± 0.01		0.11 ± 0.01
RGS2	0.07 ± 0.00	0.09 ± 0.00		0.06 ± 0.05		0.06 ± 0.05
X(SC/RGS) ± SD	0.06 ± 0.01[c]	0.2 ± 0.1[a]				0.1 ± 0.0[a]

Notes: *X* is the mean value of each group. Minas Gerais green propolis (MG-G); Minas Gerais brown propolis (MG-B); Paraná (P); Santa Catarina and Rio Grande do Sul (SC/RGS). In each column, different letters (a–c) mean significant differences between groups ($p < 0.05$).
[a]mg/ml.
[b]g/g extract.
[c]Electrochemical antioxidant power.
[d]Total electrochemical antioxidant power, at pH 7, expressed in mg/ml of *p*-coumaric acid at the different potentials presented by the propolis and plant sources extracts.

quantities of terpenoids and phenolic compounds, being the greenish richer in the later ones. The same profile was found in the present study, setting a geographical decay from the green propolis specifications as we move from southeast to south regions of Brazil.

Antioxidant activity

The bioactivity of southeast and south Brazilian propolis was evaluated through $DPPH^{\bullet}$ free radical-scavenging activity, reducing power and a novel approach using electrochemical techniques which were a rapid and easy tool for the TEAP assessment.

The model system of scavenging DPPH free radical is a simple method for evaluating the antioxidant activity of compounds, where the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine (Magalhães, Segundo, Reis, & Lima, 2000). Table 5 shows EC_{50} values for the different samples, which is the amount of antioxidant necessary to decrease by 50% the initial $DPPH^{\bullet}$ concentration and thus, the lower EC_{50} values, the higher is the antiradical capacity, which is related to the hydrogen-donating ability of the antioxidants present in the samples. The values ranged from 0.01 mg/ml for the sample MG7 from Minas Gerais to 0.11 mg/ml for sample P4 from Paraná, Table 5. Generally, Minas Gerais green samples showed the highest EC_{50} values while the southern samples, in special sample P4 from Paraná, Santa Catarina and Rio Grande do Sul propolis, presented a lower capacity to capture free radicals, Table 5. This result is in accordance with the HPLC phenolic quantification, were these samples presented the lowest content.

The reducing power assay measures the ability of antioxidants to reduce Fe(III) to Fe(II). The antioxidant activity is evaluated by measuring the absorbance of the blue colored ferrous complex formed at 700 nm (Oyaizu, 1986). The results varied widely according to the samples origin with values ranging from 0.08 g/g for the sample P4 from Paraná and 0.68 g/g for sample MG2 from Minas Gerais, Table 5. Generally, Minas Gerais samples (both green and brown samples) presented higher activities with an average value of 0.5 g/g of extract and the lower values were observed for the southern samples. This pattern, where the southern samples showed the lowest values, is in agreement with the other parameters analyzed in the present work.

Electrochemical properties of pure compounds, foods, and biological samples may be used for the evaluation of their reducing/antioxidant capacity, since the electric oxidation potential has conceptually relation with the expected antioxidant capacity (Magalhães et al., 2000). Among the electrochemical methods, the differential

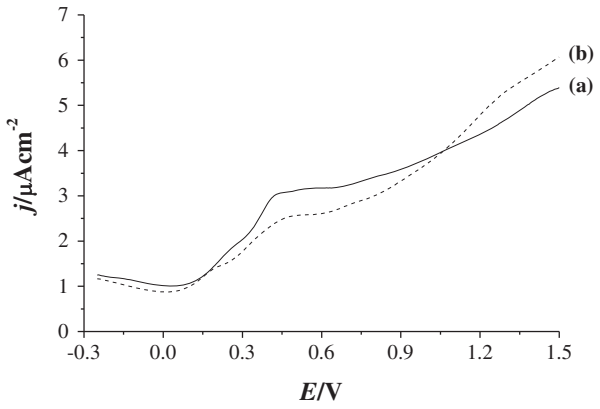

Figure 4. Differential pulse voltammograms of 1 mg/ml propolis extract (a) MG5, (b) P4 in EtOH/ Britton-Robinson buffer /TBAP (78:28:2) solutions at pH 7 obtained with a glassy carbon electrode between −0.25 and 1.5 V, with 0.06 V pulse amplitude at 0.03 V/s.

pulse voltammetry (DPV) technique has been used for the evaluation and quantification of the overall electrochemical antioxidant power (Barros et al., 2008; Falcão et al., 2016). This electrochemical method was applied as an effective tool for the quantification of the total electroactive species of Brazilian propolis from different sources. Representative propolis voltammograms are showed in Figure 4. Several oxidation processes were present in the samples, which varied accordingly to the propolis origin. Generally, all samples showed a common oxidation process at 0.4 V. Two additional anodic peaks were present, one at 0.2 V, observed in some samples from Minas Gerais and in all samples from Paraná and other at 0.8 V, observed only in three samples from Minas Gerais, Table 5. These additional peaks, and the fact that they are not found in all green propolis samples, means that also for this propolis there are additional floral sources rather than *Baccharis* spp. The southern propolis from Santa Catarina and Rio Grande do Sul were the geographical origins with poorest electrochemical performance, with only one oxidation process, at 0.4 V. Table 5 presents the quantitative results, expressed in terms of *p*-coumaric acid equivalents, for all the resolved oxidation peaks obtained with DPV for the Brazilian propolis samples under this study. The electrochemical antioxidant power (EAP) calculated for each oxidation process, reflects the existence of easily oxidized species (low oxidation potential) and their amount, Table 5. To account for the contributions of all species, we expressed the sum of *p*-coumaric acid equivalents as total electrochemical antioxidant power, TEAP. In general, MG samples exhibit the highest "antioxidant power" with an average value of 0.3–0.4 mg/ml of *p*-coumaric acid equivalents in agreement with the results from DPPH and reducing power, Table 5. The TEAP values for the southern samples P, SC and RGS were very similar and significantly lower, as found for the other antioxidant assays as well as for the phenolic composition, suggesting a poor composition in easily oxidized species.

Conclusions

This work reported the quality assessment of sixteen Brazilian south and southeast propolis, through the determination of physicochemical parameters and the phenolic composition through spectrophotometry and chromatographic techniques such as HPLC and LC/DAD/ESI-MS^n. Overall, the samples presented typical *Baccharis* spp. propolis characteristics with a phenolic profile, with fourteen compounds identified, rich in dicaffeoyilquinic acid isomers (*m*/*z* 515), dihydrokaempferide (*m*/*z* 301) and artepillin C (*m*/*z* 299), nevertheless there is evidence of other floral contributions as we move towards south. The southern samples under analysis from Paraná, Santa Catarina and Rio Grande do Sul presented a brownish color, poorer phenolic composition and lower bioactivity. The brownish color was also notice in some samples from Minas Gerais, with impact in its phenolic content. The absence of some compounds on the phenolic profile of the southern samples points to other floral sources rather than *Baccharis* spp.

All samples proved to have antioxidant properties, being Minas Gerais propolis more bioactive than the southern samples. Through the quantification of the total electroactive species, it was possible to attribute the highest "antioxidant power" to the Minas Gerais samples. Finally, differential pulse voltammetry, with similar results to the reducing power, proved to be a powerful tool for the rapid quantification of the antioxidant activity in propolis, and therefore it could be explored as a quicker and low cost alternative to the spectrophotometric methods.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary material

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