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Determination of Total Phenolic Compounds and Antioxidant Activity of Ethanolic Extracts of Propolis Using ATR–FT-IR Spectroscopy and Chemometrics

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Abstract
Fourier transform mid-infrared spectroscopy equipped with attenuated total reflectance (FT-IR–ATR) combined to partial least squares (PLS) regression was used for the quantification of total phenolic contents (TPCs) and antioxidant activities in 98 samples of ethanolic extract of propolis (EEP) from the southwest region of Paraná, Brazil. The Pearson's correlation coefficients were applied, and results ranged from 0.96 to 0.88 and showed higher correlation coefficients among TPC and ferric-reducing antioxidant power (FRAP) followed by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). Calibration was performed using a Savitzky-Golay filter (15 pt) and first derivative as well as standard normal variate (SNV) and mean center correction pretreatments. The determination coefficient in the calibration models ranged from 0.95 to 0.87. The range error ratio (RER) indicates the quality of estimation of the models and the results obtained were 10.0, 8.11, 16.8, and 8.99 for TPC, DPPH, ABTS, and FRAP, respectively. Thus, the results obtained for calibration and prediction parameters indicated that the models for DPPH, FRAP, and TPC have a low predictive capacity which complicates the data modeling. However, the ABTS model is validated and can be used for quantification of antioxidant activity of new extracts of propolis, being useful as an alternative to rapid analysis, reducing waste generation and cost, and indicating that the mid-infrared spectroscopy associated with PLS regression can be used to predict ABTS radical scavenger.

Keywords PLS · Propolis · Antioxidant activity · MIR · Predictive capacity

Introduction

Propolis is a resinous material produced by bees by mixing salivary enzymes (β-glucosidase), wax, pollen, and collected natural resins used to fill gaps and to seal parts of the hive (Silva-Carvalho et al. 2015; Daneshmand et al. 2015) and can be considered a nutraceutical and functional ingredient in food products (Salami et al. 2013; Pasupuleti et al. 2017). Almost all ancient civilizations used bee-derived products as resources in their medicine due to its pharmacological properties (Tiveron et al. 2016). Propolis has characteristics that are beneficial to human health, such as antioxidant (Oldoni et al. 2011; Calegari et al. 2017), anti-inflammatory (Luis-Villaroya et al. 2015), antifungal (Siqueira et al. 2015), antiviral, anticariogenic, and antibacterial properties (Bankova 2009). The chemical composition of propolis is very complex, varying according to the bee species, the seasonality, and the flora of the region. Because of the plant variability found in South America, the chemical composition of Brazilian propolis is completely different from the other parts of the world (Bankova 2009; Calegari et al. 2017; López et al. 2014).

In propolis, there are several bioactive compounds and it is possible to emphasize the presence of aromatic acids, esters, chalcones, phenolics, and terpenoids (Park et al. 2002; Soltani et al. 2017; Zabaiou et al. 2017; Al-Ghamdi et al. 2017). Among the phenolic compounds identified, the presence of chrysin, galangin, pinocembrin, pinostrobin, caffeic acid phenethyl ester (Vargas-Sánchez et al. 2015), caffeic acid, p-coumaric acid, acid benzoic (Schnitzler et al. 2010), and Artepilin C (Veiga et al. 2017) is common. Flavones,

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flavonols, flavanones, and dihydroflavonols are also present in samples of propolis, as reported by Popova et al. (2017). These bioactive compounds, mainly phenolic acids and flavonoids, act directly as collaborators of the pharmacological properties, contributing mainly to the antioxidant activity.

The compounds with antioxidant capacity are able to inhibit the oxidative stress caused by the presence of free radicals in the organism (Castro et al. 2014). Free radicals consist of reactive oxygen and nitrogen species generated by cellular metabolism, which are beneficial at moderate levels but at higher concentrations can damage tissues by oxidative stress caused by an imbalance between pro- and antioxidants, resulting in a loss of biological functions and homeostasis issues (Vasconcelos et al. 2007; Barbosa et al. 2010). The human body has several mechanisms to prevent oxidative stress by the neutralization of free radicals by antioxidant compounds, which are produced naturally in the body or supplied externally through food or supplements (Pham-Huy et al. 2008).

Generally, the methods for determining the chemistry and biological activities of propolis involve conventional techniques such as UV, TLC, GC, and HPLC (Silva et al. 2007; Alves and Kubota 2013; Castro et al. 2014; Morlock et al. 2014; Rufatto et al. 2017), which are very useful in the identification and quantification of various chemical compounds in propolis (Wu et al. 2008). For the analysis of the total phenolic content (TPC), the Folin–Ciocalteu colorimetric method has been used in different types of samples. This method is based on a chemical reduction of a reagent, a mixture of tungsten and molybdenum oxides yielding a bluish compound that is measured at 740 nm (Singleton et al. 1999). For antioxidant capacity determination, indirect measurements such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays have been used because of their capacity to scavenge species of radicals and reduce Fe^{3+} to Fe^{2+} with the production of species that absorb at 517, 734, and 595 nm, respectively (Jurd and Geissman 1956; Brand-Williams et al. 1995; Re et al. 1999). These methods become infeasible if numerous samples are required to be analyzed. As a result, the development of a faster and simpler analytical method for high-throughput screening of propolis material is necessary.

As an alternative to colorimetric methods, spectroscopic fingerprint coupled with multivariate regression analysis has been proposed herein. The vibrational spectroscopy Fourier transform mid-infrared spectroscopy (FT-MIR) is a technique that expresses a unique "fingerprint" for a complex system of different samples. When FT-IR is equipped with an attenuated total reflectance (ATR) device, it enables the acquisition of spectra of both liquid and solid samples in which a light is totally internally reflected and the sample interacts with the evanescent wave. Because of the small light, penetration depth is ideal for highly absorbing samples, such as aqueous solutions, and for surfaces and thin film measurements (Wilson and Tapp 1999; Grdadolnik 2002). This spectroscopic technique presents some advantages, such as versatility, low cost, efficiency, and speed, with minimal or no sample preparation and without the use of expensive reagents, making it an attractive alternative for traditional analytical methods (Rodriguez-Saona and Allendorf 2011).

Partial least squares (PLS) regression enables correlation between two matrices. PLS is useful when the number of predictors (i.e., spectral peaks) is much higher than the number of samples in the dataset. Studies related the use of FT-NIR for quantification of compounds in propolis (Cai et al. 2012; Xu et al. 2013; González-Martín et al. 2017; Revilla et al. 2017) but there are few studies (Wu et al. 2008) on propolis using FT-IR–ATR and PLS, and the studies on Brazilian propolis that show a complex chemical composition are still limited and scarce (Picoli et al. 2016; Tiveron et al. 2016). Taking this into account, the aim of this study was to apply and evaluate PLS and FT-IR–ATR spectroscopy to quantify the total phenolic composition and antioxidant activity in ethanolic extract of propolis (EEP) produced by *Apis mellifera* honey bees in the south of Brazil.

Materials and Methods

Chemical Reagents

The reagents 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8 -tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu, and ethanol 99% were obtained from Sigma Co. (St. Louis, MO).

Samples

The samples of propolis (98 samples) were directly collected by beekeepers in south of Brazil, Parana state. The samples were collected mostly with a mesh and by using the scraping technique from different beekeepers. All samples were cleaned, crushed with N_2, homogenized, and stored at (− 6 °C) until used in the laboratory. The ethanolic extracts of propolis (EEP) were prepared as described by Oldoni et al. (2015) . Twenty five milliliters of ethanol/water (80:20 v/v^{-1}) was added to 2 g aliquot of sample, and extraction was subsequently carried out in a water bath at 70 °C for 45 min. The hydroalcoholic extract was filtered through Whatman grade No.4 filter paper, and the liquid filtrated was transferred to a volumetric flask.

Reference Analysis

Total Phenolic Content

The total phenolic content (TPC) present in EEP was determined by the Folin–Ciocalteu colorimetric method (Singleton et al. 1999). In a test tube, 0.5 mL of EEP (3.2 g L^{-1}) and 2.5 mL of Folin–Ciocalteu (10 g L^{-1}) reagent were added. After 5 min, 2.0 mL of a $NaCO_3$ (40 g L^{-1}) solution was added. The solutions were incubated at room temperature in the dark for 2 h, and subsequently, the absorbance was measured using a spectrophotometer (model UV–VIS Lambda 25, Perkin Elmer) at 740 nm. The gallic acid was used as a standard reference in concentrations ranging from 5 to 100 mg L^{-1}, and the results were expressed in mg gallic acid equivalent (GAE) g^{-1} dry weight (DW). All measurements were performed in triplicate.

Antioxidant Activity Using 2,2-Diphenyl-1-Picrylhydrazyl Hydrate-Free Radical Scavenging Method

The antioxidant activity was performed through the DPPH method described by Brand-Williams et al. (1995); the technique is based on the reaction of EEP 0.5 mL (1.6 g L^{-1}), 3 mL of ethanol P.A., and 0.3 mL of DPPH radical solution (0.5 mmol L^{-1}) in ethanol P.A. After 45 min in the dark, the absorbance was measured in a spectrophotometer (UV–VIS model lambda 25, Perkin Elmer) at 517 nm. The calibration curve was constructed using Trolox as a standard in concentrations that ranged from 15 to 200 μmol L^{-1}. The results were expressed in μmol of Trolox g^{-1} DW. All measurements were performed in triplicate.

Antioxidant Activity Using 2,2′-Azino-Bis (3-Ethylbenzothiazoline-6-Sulphonic Acid) $ABTS^{\cdot+}$ Method

The determination of antioxidant activity was performed by the ABTS method according to Re et al. (1999). The ABTS radical was prepared from the reaction of 5 mL of ABTS solution (7.0 mmol L^{-1}) with 88 μL of potassium persulfate (140 mmol L^{-1}), incubated at 25 °C in the dark for 16 h. The radical was diluted in ethanol and resulted in an absorbance value of 0.700 ± 0.200 at 734 nm. Under dark conditions, a solution of reaction was prepared with 30 μL EEP (16 g L^{-1}) and 3 mL of the radical solution. The calibration curve was constructed using Trolox as a standard in concentrations that ranged from 100 to 2000 μmol L^{-1}. The results were expressed in μmol of Trolox g^{-1} DW. All measurements were performed in triplicate.

Antioxidant Activity Using the FRAP Method

Proposed by Benzie and Strain (1996), the FRAP reagent was prepared from the mixture of 25 mL of acetate buffer solution 300 mmol L^{-1} at pH 3.6, 2.5 mL of TPTZ solution 10 mmol L^{-1}, and 2.5 mL of an aqueous ferric chloride solution 20 mmol L^{-1}. The method consisted of a mixture of 100 μL of the EEP (1.6 g L^{-1}) with 3 mL of the FRAP reagent; the mixture was incubated in a water bath (37 °C) in the dark for 30 min and then the absorbance was measured in a spectrophotometer (model UV–VIS lambda 25, Perkin Elmer) at 595 nm. The calibration curve was constructed using ferrous sulfate as a standard in concentrations ranging from 200 to 2000 μmol L^{-1}. The results were expressed in μmol of $FeSO_4$ g^{-1} DW. All measurements were performed in triplicate.

Spectra Acquisition

The extracts were analyzed in a Fourier transform mid-infrared spectrometer (FT-IR) Frontier from Perkin Elmer. All MIR spectra were acquired between 4000 and 400 cm^{-1} using an optical resolution of 8 cm^{-1} and 32 accumulations. For the signal horizontal ATR, the index of refraction (η) of diamond was 2.4 and leads to a depth of penetration (Dp) of 1.66 μm, with data interval 1 cm^{-1} and scan speed 0.2 cm/s. Approximately 0.05 ml of sample was placed onto the ATR crystal, and after each analysis, the ATR crystal was washed with deionized water and dried with non-abrasive wipe.

Regional Spectral Data Preprocessing

In order to transform the data into a form suitable for analysis, data are often pre-treated, avoiding baseline drift, light scattering, and other factors during the process of sampling. In commonly used spectral pretreatment methods, first derivative (1D) can be used to correct the baseline drift and spectral rotation in the background of this system, Savitzky-Golay (SG) smoothing can filter out the high-frequency noise in the spectral data and effectively preserve the authenticity of the original signal, and standard normal variate (SNV) is a filter based on individual observation and is less sensitive to peculiarities in the raw data, mainly used for the scattering correction of spectra (Tan et al. 2018). This study adopted the SNV, 1D, SG smoothing, and their combination methods to preprocess the original spectral data.

Data Analysis

All samples were subjected to multivariate analysis by PLS. PLS-toolbox 5.8 of Matlab version 7.8.0.347 (MathWorks. Inc. USA) was used for the chemometric analysis. The 98 spectra were divided into two datasets: calibration set (69 samples) and test set (29 samples). In this work, the developed

models were evaluated through root mean square error of cross-validation (RMSECV), root mean square error of prediction (RMSEP), ratio of performance to deviation (RPD), range error ratio (RER), and coefficient of determination (R^2).

The RMSEP value can be obtained from Eq. (1):

$$\mathrm{RMSEP} = \sqrt{\sum_{i=1}^{I_p}\left(\frac{\left(yi-\tilde{y}i\right)^2}{I_p}\right.} \quad (1)$$

where $\tilde{y}_i$ is the predicted value for set sample i, y_i the measured value for predicted sample I, and I_p is the number of observations in the prediction set (Viegas et al. 2016).

The RMSECV was calculated as follows:

$$\mathrm{RMSECV} = \sqrt{\frac{1}{I_C-1}\sum_{i=1}^{I_c}(\hat{y}_i-y_i)^2} \quad (2)$$

where $\hat{y}_i$ is the predicted value of the ith observation, y_i the measured value of ith observation, and Ic is the number of observation in the calibration set (Viegas et al. 2016).

The coefficient of determination (R^2) between the experimental and predicted values was calculated using Eq. (3), where n is the number of observations in the calibration and prediction sets; $\hat{y}_i$, y_i are the predicted and measured values of sample i in the calibration and prediction sets, and $\overline{y}$ the mean of the reference measurement results for all samples in the calibration and prediction sets (Viegas et al. 2016).

$$R^2 = 1-\frac{\sum_{i=1}^{n}(\hat{y}_i-y_i)^2}{\sum_{i=1}^{n}\left(y_i-\overline{y}\right)^2} \quad (3)$$

The range error ratio (RER) can be determined using E q. (4):

$$\mathrm{RER} = \frac{(\mathrm{ymax}-\mathrm{ymin})}{\mathrm{RMSEV}} \quad (4)$$

being ($y_{max} - y_{min}$) the calibration range, and RMSEV the validation error (RMSECV or RMSEP). RER values above 10 are indicative of models with good estimate (Páscoa et al. 2013). The RPD is the relationship between the standard deviation of values measured by the conventional method and the standard validation error. For RPD values above 2, the calibration equations are considered good (Dal Zotto et al. 2008). In order to avoid overfitting in models, the choice of the number of PLS latent variables was based on the minimization of the error, calculated by means leave-one-out cross-validation (Laghi et al. 2011; Martins and Ferreira 2013).

Results and Discussion

The results obtained by the referenced methods for the total phenolic content, the antioxidant capacity (determined by FRAP, ABTS, and DPPH) of the propolis analyzed are shown in Table 1. The samples were divided into two datasets, that of calibration (69 samples) and that of external validation (29) and Table 1 shows the mean, minimum, maximum, standard deviations, and coefficient of variation for both groups. The observed range for total phenolic content (5.3 to 50.4 mg EAG g^{-1}) and antioxidant activity evaluated by the DPPH method (11.68 at 275.2 μmol Trolox g^{-1}) were similar to those reported by Tiveron et al. (2016) and Picoli et al. (2016) for propolis produced in the south of Brazil. With regard to antioxidant activity determined by the ABTS method, the results showed a wide range of scavenging capacity from 19.0 to 1077 μmol Trolox g^{-1}, and the values obtained by the FRAP method ranged from 66.74 to 1164 μmol $FeSO_4$ g^{-1}.

Currently, there is a great variety of in vitro assays because it is unrealistic to expect a single assay to be able to determine the total antioxidant activity in the screening of numerous samples. It is well known that each assay has particularities, especially with respect to the radicals and reagents involved, so ideally, the choice should cover a mix of methods based in hydrogen atom transfer (HAT) and/or single electron transfer (SET), encompassing different antioxidant mechanisms.

Table 1 Reference chemical data of ethanolic extract of propolis samples in calibration and validation set

Model	Calibration (69 samples)					External validation (29 samples)				
	Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV
TPC[a]	5.294	50.41	18.68	11.09	59.36	5.430	49.75	18.15	11.38	62.69
DPPH[b]	11.68	275.2	49.57	46.67	94.14	13.67	228.9	58.11	46.50	80.02
ABTS[b]	19.03	875.4	133.5	143.7	107.6	24.00	1077	166.8	214.2	128.4
FRAP[c]	66.74	987.0	287.7	197.3	68.58	80.97	1164	325.7	249.1	76.51

SD standard deviation ($n = 3$), *CV* coefficient of variation, *EAG* equivalent to gallic acid

[a] mg EAG g^{-1}

[b] μmol Trolox g^{-1}

[c] μmol $FeSO_4$ g^{-1}

Table 2 Pearson's correlation coefficients between the antioxidant activities and total phenolic content (reference analysis) of propolis

	ABTS	DPPH	FRAP	TPC
ABTS	1			
DPPH	0.954*	1		
FRAP	0.9323*	0.934*	1	
TPC	0.882*	0.926*	0.963*	1

*Significant correlations at $p \leq 0.01$

Thus, to ensure a better evaluation of the antioxidant activity of extracts of propolis, we used a combination of assays which cover different antioxidant mechanisms and radicals: TPC and FRAP assays are based in SET mechanism and DPPH and ABTS assays are based in both SET and HAT mechanisms.

The Pearson's correlation coefficients (PCCs) among the studied antioxidant reference analysis are shown in Table 2, being a measure of the strength and direction of a linear relationship between two random variables (Zhou et al. 2016). In this paper, significant positive correlations ($p \leq 0.01$) were observed between all variables, PCC ranged from 0.96 to 0.88, and higher correlation coefficients were obtained between TPC and FRAP, followed by ABTS and DPPH. Revilla et al. (2017) reported correlation coefficients lower than those observed in this study.

FT-IR–ATR Spectroscopy

Figure 1 shows the IR spectra of the 98 ethanolic extracts of propolis obtained using Fourier transform middle infrared (FT-MIR) equipped with an attenuated total reflectance (ATR) device. In the range of 900–690 cm^{-1}, bands can be seen related to the stretching and bending vibrations of C–H from aromatic rings, related to phenolic compounds (Soares 2002; Sousa et al. 2007). The range of 3000–2800 cm^{-1} is the location of bands connected with asymmetric and symmetric stretching modes of C–H: ν_{as} (CH_3), ν_s (CH_3), ν_{as} (CH_2), and ν_s (CH_2), arising from methyl and methylene groups. Two intense bands between 1300 and 1000 cm^{-1} represent C–O stretching and C–OH bending originated from alcohols, ethers, esters, and carboxylic acids, which are functions present mainly in phenolic acids and flavonoids (de Cardoso et al. 2017; Cao et al. 2017) which are found in extracts of propolis (Soltani et al. 2017; Bankova 2005; Menezes 2005; Banskota et al. 2001; Chang-Bravo et al. 2014).

The vibration of the carbonyl group arising from the stretching vibration of the carbonyl group and C=C from the stretching of aromatic rings are located at 1680–1630 cm^{-1} and 1680–1600 cm^{-1}, respectively (Pavia et al. 2010). The same stretching was observed in extracts of red propolis Chang-Bravo et al. (2014). In the 3400 cm^{-1} region, a very intense band is present which represents the absorption of the OH functional group (Pavia et al. 2010), which represents alcohols. This band was already expected, since EEP is prepared with ethanol as described in samples in the "Materials and Methods" section.

Partial Least Square Regression Modeling

The calibration model was implemented with MIR spectra and reference analysis. The best prediction models were obtained using the Savitzky-Golay filter (15 pt) and first derivative as well as standard normal variate (SNV) and mean center preprocessing, and the statistical descriptors are shown in Table 3. The number of latent variables used for the construction of these models was determined from the cross-validation (CV) in order to avoid overfitting the data (Martins and Ferreira 2013).

For CV, the leave-one-out technique was applied; one sample was removed from the calibration model and the remaining samples were then used to predict the value corresponding

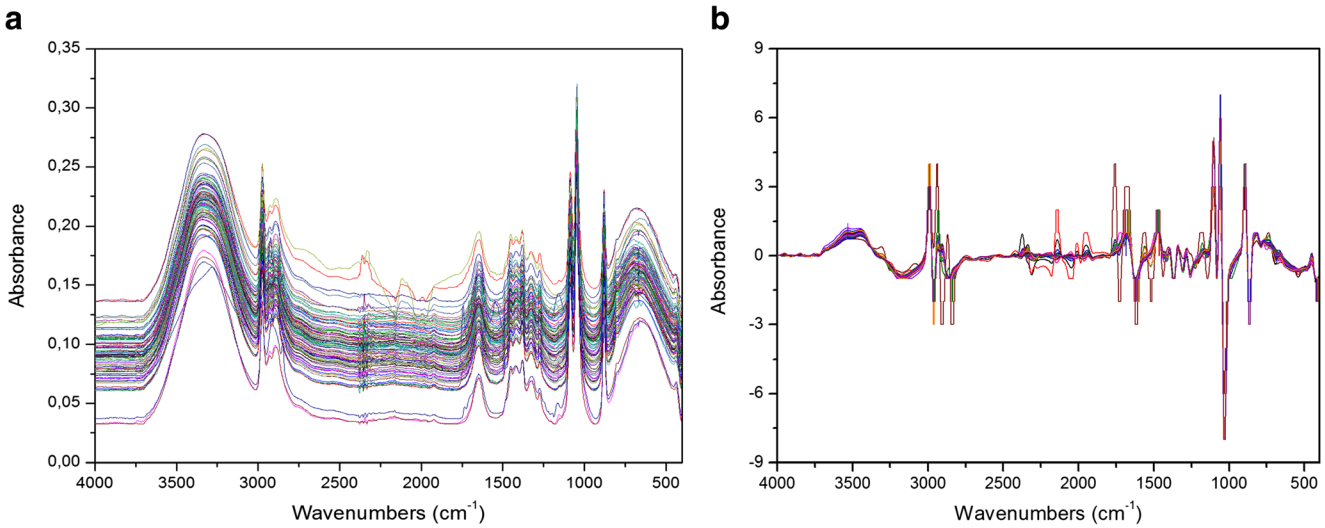

Fig. 1 MIR spectra of ethanolic extracts of propolis in the range 4000–400 cm^{-1}. **a** Spectra without preprocessing. **b** First derivative + SNV preprocessing

Table 3 Statistical descriptors of calibration and validation by MIR

	Number of samples	Mean	SD	Est. Min	Est. Max	LV	RMSEC	R^2_{cal}	RMSEP	R^2_{pred}	RPD
TPC[a]	68	18.7	10.54	3.36	50.3	11	3.41	0.90	9.15	0.71	1.21
DPPH[b]	68	49.6	44.0	2.94	223	12	15.4	0.89	24.86	0.72	1.88
ABTS[b]	69	133	140.3	4.65	874	12	31.1	0.95	63.60	0.74	2.26
FRAP[c]	67	287.9	199.7	30.1	1032	10	80.3	0.87	128.57	0.59	1.53

SD standard deviation, *LV* latent variables, *RMSEC* root mean square error of calibration, *RMSEP* root mean square error of prediction, R^2 determination coefficient, *Est. Min and Est. Max* minimum and maximum value estimated by the model developed

[a] mg GAE g^{-1}

[b] μmol Trolox g^{-1}

[c] μmol $FeSO_4$ g^{-1}

to the removed sample. This process was repeated until all calibration samples were removed once (Silva et al. 2014). Results of CV showed higher RMSECV values than RMSEC obtained in calibration. The equations developed for the determinations of TPC, DPPH, ABTS, and FRAP showed high values for the R^2 coefficient (0.95–0.87), indicating that the results of the calibration adjustment were good for these parameters.

The predictive capacity of the models was verified by means of external prediction. Figure 2 shows the correlation of the values obtained in the laboratory (reference value) with regard to those predicted by the FT-IR for TPC and antioxidant activity. The values of R^2 closer to 1 indicate a higher probability that the FT-IR-predicted value (y-axis) is related to the reference analysis (x-axis) (Silva et al. 2014). As shown in Table 3, the best model was obtained for ABTS, in which the calibration and validation parameters were the best, with a R^2_{cal} of 0.95, lowest RMSEP, highest RER (16.8), and R^2_{pred} (0.74) and RPD above 2, suggesting a good practical utility (Fagan et al. 2007; Dal Zotto et al. 2008; Kapper et al. 2012).

The determination coefficients and RPD obtained for DPPH, FRAP, and TPC were lower than 0.72 and 2, respectively, suggesting a low correlation of these parameters with the calibration models, indicating a low predictive capacity. The estimated range and high RMSEP values obtained (Table 3) also support these observations. As suggested by Silva et al. (2014), the low prediction obtained for antioxidant activity models can be attributed to the fact that the range of data references used in this study was not representative for the respective models.

Another important parameter that impacts the quality of PLS models is the presence of outliers, which are as important as the determination of principal components employed in the

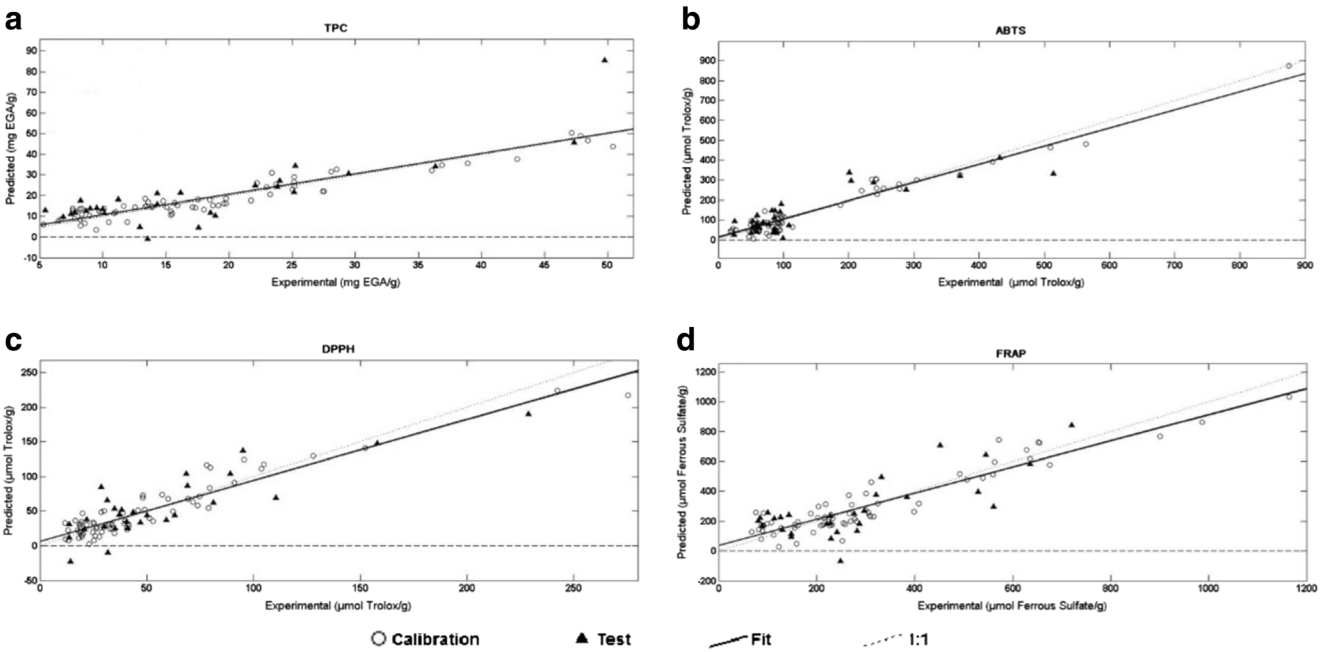

Fig. 2 Correlation between predicted and experimental **a** TPC, **b** ABTS, **c** DPPH, and **d** FRAP by the PLS

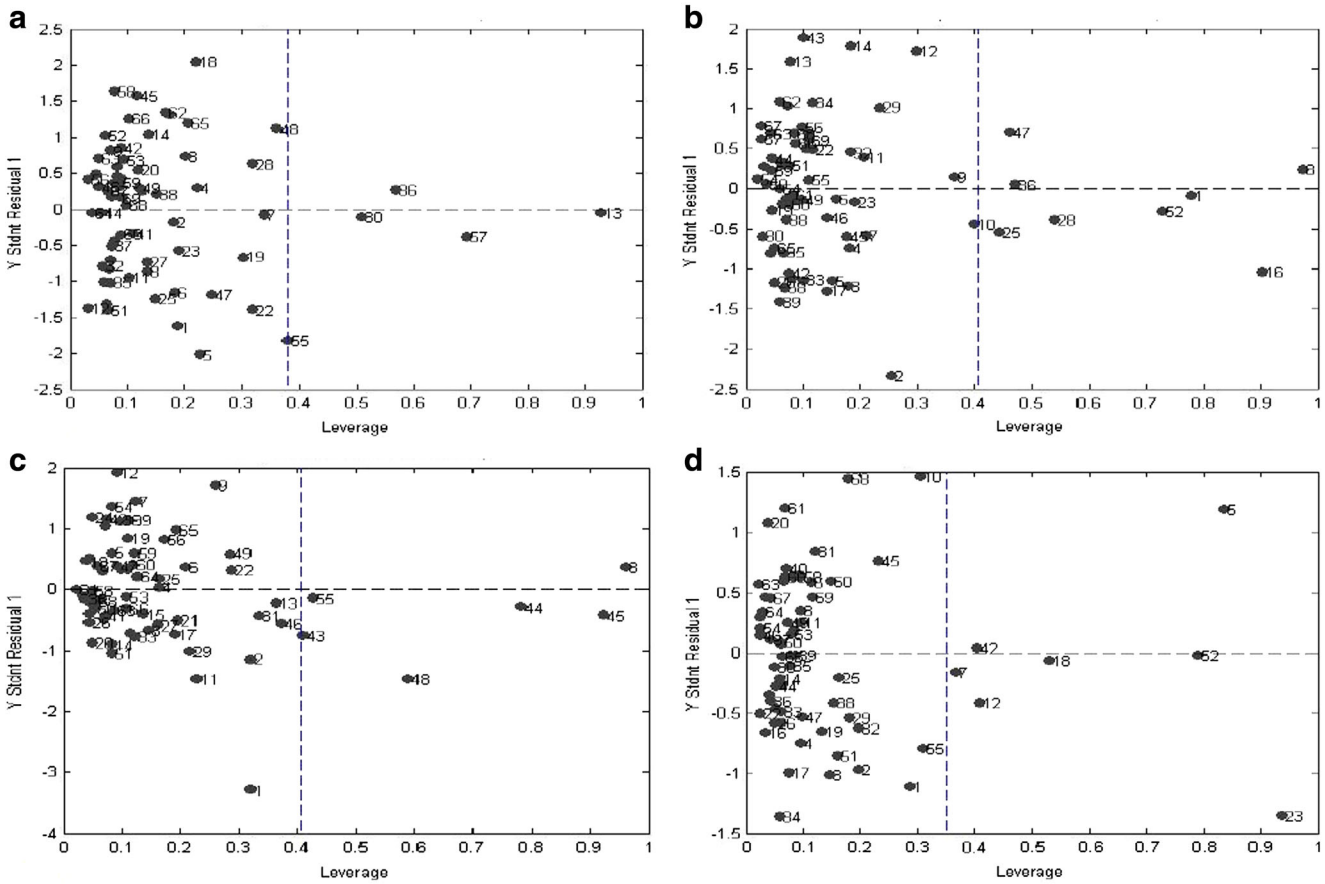

Fig. 3 Leverage vs residuals graphics **a** ABTS, **b** TPC, **c** DPPH, and **d** FRAP

model. The quality of the calibration set should ensure that samples produce a homogenous set by removing atypical values from the dataset. In this study, the leverage vs residuals graph (Fig. 3) was used for detection of outliers. The studentized residuals as a function of leverage for the ABTS, DPPH, FRAP, and TPC datasets showed that there were no influential values for the ABTS, FRAP, and TPC responses, because the studentized residuals values are between − 2.5 and + 2.5. The same case no. is verified for the DPPH response, because there exists a value (sample 1) considered influent close by − 3.5 for the studentized residuals.

In general, some samples may present values with some influence in ours PLS models. Our models were constructed to detect wide range values of ABTS, DPPH, FRAP, and TPC. However, some models were prejudiced to present extremes values.

Conclusions

Our primary objective in this work was to develop, for the first time, a reliability method using FT-IR–ATR spectroscopy for the determination of antioxidant activity of ethanolic extract of propolis. The results obtained for calibration and prediction parameters indicated that the ABTS model is validated and can be used for quantification of antioxidant activity of new extracts of propolis. The models for DPPH, FRAP, and TPC indicated a low predictive capacity which complicates the data modeling. However, for application purposes, further investigation is required to develop a mathematical model to control and predict the optimization parameters of the antioxidant activity. Green extraction techniques are a useful alternative to rapid analysis, reducing waste generation and cost.

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Compliance with Ethical Standards

Conflict of Interest C. da Silva declares that she has no conflict of interest. A. Prasniewski declares that she has no conflict of interest. M. A. Calegari declares that he has no conflit of interest. V. A. de Lima declares that he has no conflict of interest. T. L. C. Oldoni declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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