DETERMINATION OF QUALITY CHARACTERISTICS, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF PROPOLIS FROM SOUTHEASTERN MEXICO

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Abstract

The objective of this work was to investigate the variability of physicochemical parameters, phenolic compounds and *in vitro* antioxidant activity of propolis collected from different apiaries in southeastern Mexico. A high variability was found in the moisture content (1.96-8.26%), ash (0.66-5.50%) and sensory characteristics of raw propolis from southeastern Mexico, but the raw propolis samples met the requirements of the quality regulations. In the same way, most of the ethanolic extracts also complied with the quality regulations. Of all the extracts, PE2 obtained from Santa Cruz showed the highest values for dry extract, content of total phenolic compounds (TPC), total flavonoids (TF) and antioxidant activity (DPPH and ABTS). The content of the individual phenolic compounds varied according to the geographical location of the apiary, but the PE2 extract resulted in the highest pinocembrin and chrysin content. A positive correlation was obtained between TPC and TF with antioxidant activity. Propolis extracts were classified into two groups through principal component analysis (PCA). These results indicate that the apiary location in southeastern Mexico influenced the characteristics of propolis.

Keywords: antioxidant activity, phenolic compounds, propolis, quality

INTRODUCTION

Propolis is made from resinous material of various plant species that bees (*Apis mellifera* L.) collect and transport to the hive. It is processed to seal cracks and prevent the entrance of invaders and pathogenic microorganisms. Previous research has demonstrated that propolis possesses antibacterial, antifungal, antiviral, anti-inflammatory, and anti-tumor properties. Furthermore, the high antioxidant activity of propolis is attributed to the presence of phenolic compounds, especially the flavonoid group (Jerković et al., 2016). For these reasons, this natural product has gained scientific and commercial interest in the food and cosmetics

industries and as a popular alternative medicine. More than 300 chemical compounds have been reported in propolis made from different plant species and residues, including phenolic acids and their esters, flavonoids, terpenes, aromatic aldehydes, alcohols, fatty acids, stilbenes, amino acids, lignans and sugars (Trusheva et al., 2011; Piccinelli et al., 2013). The chemical composition of propolis varies qualitatively and quantitatively due to the diversity of plant resins from which it is made in addition to the various geographic and climatic characteristics of its place of harvest (Gardana et al., 2007; Reis et al., 2019; Xu et al., 2019). Thus, its chemical composition varies according to its country of origin (Europe, China, Argentina and USA) or region (as

in the case of Brazil).

However, few studies have investigated propolis samples collected from areas with particular territorial and climatic characteristics, such as those of the Yucatan Peninsula in southeastern Mexico. In this region, research on propolis has been mainly focused on studying the volatile constituents, triterpenoids, resorcinolic lipids and antimicrobial and antioxidant activity of samples collected from the same area (Pino et al., 2006; Boisard et al., 2015; Herrera-López et al., 2019). Few studies have aimed to evaluate the quality of propolis collected from different sites in southeastern Mexican or the variability in its content of phenolic compounds and antioxidant activity.

Thus, in the present study, the varied quality and composition of propolis collected from different apiaries in the Yucatan Peninsula was evaluated according to some of its

physicochemical characteristics, antioxidant activity and composition of phenolic compounds and flavonoids using spectrophotometry and high-performance liquid chromatography.

MATERIAL AND METHODS

Raw propolis samples

Raw propolis samples (850 g) of *A. mellifera* were collected in January-February 2019 from nine different apiaries (RP1-RP9) in southeastern Mexico (Fig. 1). This collection period coincided with the main flow of nectar and flowering season of *Viguiera dentata*, the main flower visited by bees. This region is the main honey-producing area in Mexico, corresponds with the karst region and has a sub-humid warm climate (Aw0) with rain in the summer. The main vegetation is low-medium deciduous and sub-deciduous forest, and the minimum and

Fig. 1. Map of the location of apiaries in the southeast region of Mexico.

RP= raw propolis. RP1: Maxcanú (N 20° 34' 52.932", W 89° 59' 15.827"), RP2: Santa Cruz (N 20° 35' 6.324", W 89° 57' 39.563"), RP3: Hecelchakán (N 20° 10' 44.364", W 90° 7' 28.055"), RP4: Nunkiní (N 20° 23' 28.356", W 90° 8' 58.38"), RP5: Halachó (N 20° 28' 16.464", W 90° 4' 55.92"), RP6: Maxcanú (N 20° 35' 11.724", W 90° 0' 27.792"), RP7: Pomuch (N 20° 08' 16.00", W 90° 10' 28.0"), RP8: Calkiní (N 20° 22' 10.524", W 90° 3' 6.804"), RP9: Cuch Holoch (N 20° 26' 7.98", W 90° 5' 53.052").

maximum distance between the apiaries was 4 and 56 km, respectively.

The samples were collected by scraping the internal parts of the hive. The impurities were first removed, and the samples were then stored at -20°C in darkness in an inert atmosphere (N₂) to avoid material degradation. Before use, the raw propolis was broken into small pieces and ground with a coffee bean grinder (Hamilton Beach 80350).

Characterization of raw propolis

The moisture content was determined through gravimetry. Two grams of finely ground raw propolis were heated in a convection oven at 105°C for 5 h until a constant weight was reached. The ash content was determined through incineration. Two grams of finely ground raw propolis were heated to 550°C for 8 h and then desiccated until a constant weight was reached (Martínez et al., 2012).

The raw propolis was also sampled by a tasting panel of twenty individuals between the ages of 18 and 45 who had been selected through an interview. They performed discriminatory tests (triangular, duo-trio, basic flavors). The samples were evaluated using the check-all-that-apply (CATA) technique: Each panelist evaluated each sample, selecting the attributes they considered to be present in the samples. Each sample weighed 20 g and was coded with three random digits. They were randomly given to the panelists in a monadic sequential manner according to a Latin square experimental design (Ramón-Canul et al., 2020). The evaluated sensory attributes were color (dark greenish brown, reddish yellow or brown), aroma (resinous soft, resinous, odorless or resinous aromatic), taste (insipid, piquant or bitter), and consistency (malleable or rigid) (*NOM-003-SAG/GAN-2017*, 2017). The color was evaluated against a white light, the aroma nasally, the taste retronasally and the consistency by placing the sample between the fingers.

Preparation of propolis extracts

Powdered propolis (6 g) was extracted in 20 ml of ethanol (96%, v/v) during constant stirring

(100 rpm) for 12 days at 25.0±1.0°C in darkness. These extraction conditions had been established in preliminary studies; a higher total phenolic content (TPC) was recovered using this method. The extract was centrifuged (Changsha X-centrifuge, TGL-16M) at 2500 rpm for 10 min at 4°C, and the supernatant was filtered with Whatman™ no. 4 paper. The resulting ethanolic propolis extracts were stored at -20°C overnight and then filtered to remove waxes. They were then evaporated at reduced pressure to obtain the dry ethanolic extracts, and the percentage yield was determined based on the dry weight of the extracts and original weight of the raw propolis. The dry extracts were redissolved in ethanol (10 mL, 96%, v/v) and labeled as ethanolic extracts of propolis (PE1-PE9) and kept at 4°C in dark containers until analysis.

Characterization of propolis extracts

The oxidation index (s) and solubility were determined in the lead acetate and sodium hydroxide of the ethanolic extracts with the procedures described by Tagliacollo & Orsi (2011). The TPC and TF contents, the Folin-Ciocalteu and aluminum chloride were determined with colorimetric methods, respectively, as described by Moo-Huchin et al. (2015). First, the ethanolic extract was diluted 20-fold to determine the TPC content. A calibration curve of standard solutions of gallic acid (100 to 1000 ppm) was used, and the linear regression equation was $Y=0.0008x + 0.0158$, with $R^2=0.998$. To determine the TF content, the ethanolic extract was diluted 100-fold. A calibration curve of standard solutions of quercetin (25 to 500 ppm) was used, and the linear regression equation was $Y=0.0014x + 0.0082$, with $R^2=0.997$. In both cases, the final results were calculated according to the weight of the dry extracts, the volume of the extracts, and the TPC and TF concentrations obtained from the calibration curves. The TPC was expressed as mg equivalents of gallic acid/g of dry propolis extract (mg GAE/g) and the TF content as mg equivalents of quercetin/g of dry propolis extract (mg QE/g). The DPPH antioxidant activity (mM Trolox/g of dry propolis extract) of the ethanolic extract

diluted 20-fold and the ABTS antioxidant activity (mM Trolox/g of dry propolis extract) of the ethanolic extract diluted 125-fold were determined according to the procedure described by Moo-Huchin et al. (2015). Trolox was used as a standard in both trials, and the absorbance of the samples was measured at 515 nm for DPPH ($Y=0.018x + 0.0062$, $R^2=0.999$) and at 734 nm for ABTS ($Y=0.0346x - 0.7895$, $R^2=0.997$). The final results were calculated according the weight of the dry extracts, the volume of the extracts, and the antioxidant activity obtained from the calibration curves.

The phenolic compounds in the propolis extracts were quantified through high-performance liquid chromatography (HPLC). The dry ethanolic extract of propolis (60 mg) was dissolved with HPLC-grade methanol (4 ml), centrifuged at 14,000 rpm for 5 min and filtered with a 0.45- μ m cellulose membrane filter (Millipore). It was then injected into an HPLC-1220 infinity system (Agilent Technologies, Palo Alto, CA, USA) equipped with a manual injector and UV-Vis detector. The chromatographic separation was performed according to the methodology described by Can-Cauich et al. (2017) using the same column, composition, mobile phase flow, wavelength and injection volume. To identify the compounds, the retention time was compared between samples and standards. The quantification of compounds was based on the calibration curves at six concentrations ranging from 20 to 200 ppm. The linearity of all compounds was satisfactory with R^2 values >0.995 . The results were expressed as mg of phenolic compound/100 g of dry propolis extract.

Statistical analysis

Data were expressed as the averages $\pm$ standard deviations of the two experiments performed in triplicate. The data were analyzed by a one-way ANOVA ($p \leq 0.05$), and the significant differences between the treatments were established by Tukey's range test in the Statgraphics Plus version 5.1 software (Statistical Graphics Corp, U.S.A). Pearson's correlation coefficients were calculated to evaluate the relationship between the studied variables. Lastly, a principal

component analysis (PCA) was carried out to characterize the propolis extracts.

RESULTS

Quality of raw propolis

The quality characteristics of the raw propolis samples collected from apiaries in southeastern Mexico varied significantly ($p \leq 0.05$), as shown in Tab. 1. The moisture content of the samples ranged from 1.96% (RP6) to 8.26% (RP8) and the ash content from 0.66% (RP5) to 5.50% (RP8), respectively. The moisture and ash content of RP8 (from Calkiní) was significantly higher ($p \leq 0.05$) than the other propolis samples. Based on the moisture values reported herein, samples RP3, RP4, and RP6 can be classified as having a low moisture level ($<5\%$); RP1, RP2, RP5, RP7, and RP9 an intermediate level (between 5% and 7%); and RP8 a high level ($>7\%$). Based on the ash values, samples RP5 and RP7 are classified as having a low amount of ash ($<2\%$); RP1, RP3, RP6, and RP9 an intermediate amount (between 2% and 4%); and RP2, RP4, and RP8 a high amount ($>4\%$).

The raw propolis samples also had heterogeneous sensory characteristics. In regard to appearance, RP1 and RP3 had irregular shiny pieces. RP2 had irregular pieces with little brightness, and RP8 was grainy. The remaining RP4, RP5, RP6, RP7 and RP9 had opaque, irregular pieces. In regard to aroma, the samples RP1 and RP8 had a mild resinous aroma, RP2, RP4 and RP6 a resinous aroma, and RP7 a aromatic resinous aroma. The others- RP3, RP5, and RP9 were odorless. In regard to color, RP1, RP2, RP3, RP7 and RP9, slightly over half (55.5%) of the samples, had a dark greenish brown color, RP4 and RP5 (22.2%) a reddish yellow color, and RP6 and RP8, 22.2%, a brown color. In regard to taste, RP4 and RP7 had a piquant taste, whereas RP5 and RP9 were bitter. The rest of the samples were characterized by a lack of taste (insipid). Finally, most samples had a malleable consistency (RP1, RP3, RP5, RP6, RP7, and RP9) rather than rigid (RP2, RP4, and RP8).

Table 1.

Moisture content, ash and sensory characteristics of raw propolis

Samples	Moisture (%)	Ash (%)	Appearance	Aroma	Color	Taste	Consistency
RP1	6.63±0.11 ^e	3.73±0.11 ^e	Bright irregular pieces	Resinous soft	Dark greenish brown	Insipid	Malleable
RP2	6.30±0.00 ^e	4.06±0.11 ^f	Low brightness irregular pieces	Resinous	Dark greenish brown	Insipid	Rigid
RP3	4.63±0.11 ^c	2.26±0.11 ^c	Bright irregular pieces	Odorless	Dark greenish brown	Insipid	Malleable
RP4	3.90±0.08 ^b	4.03±0.05 ^f	Opaque irregular pieces	Resinous	Reddish yellow	Piquant	Rigid
RP5	6.66±0.05 ^e	0.66±0.05 ^a	Opaque irregular pieces	Odorless	Reddish yellow	Bitter	Malleable
RP6	1.96±0.05 ^a	2.86±0.05 ^d	Opaque irregular pieces	Resinous	Brown	Insipid	Malleable
RP7	6.53±0.30 ^e	1.83±0.05 ^b	Opaque irregular pieces	Resinous aromatic	Dark greenish brown	Piquant	Malleable
RP8	8.26±0.28 ^f	5.50±0.17 ^g	Powder or Granules	Resinous soft	Brown	Insipid	Rigid
RP9	5.50±0.17 ^d	2.03±0.05 ^{bc}	Opaque irregular pieces	Odorless	Dark greenish brown	Bitter	Malleable

Different letters within a column denote significant differences according to Tukey test ($n = 6$, $p \leq 0.05$). Values are means $\pm$ standard deviation.

Quality of propolis extracts

The chemical quality characteristics of the propolis ethanolic extracts varied significantly ($p \leq 0.05$), as shown in Tab. 2. The amount of dry extract (soluble solids extracted in ethanol)

varied widely from 2.30% (PE9) to 11.52% (PE2). The TPC and TF values ranged from 4.17 (PE5) to 97.02 mg GAE/g (PE2) and from 1.79 (PE3) to 42.68 mg QE/g (PE2), respectively.

Table 2.

Chemical quality of propolis extracts

Propolis extract	Dry extract (%)	Solubility in Pb	Solubility in NaOH	Oxidation index (s)	TPC (mg GAE/g)	TF (mg QE/g)	DPPH mM Trolox/g	ABTS mM Trolox/g
PE1	7.85±0.00 ^d	+	+	16.16±0.55 ^c	33.66±0.48 ^e	11.93±0.00 ^e	0.61±0.01 ^b	2.29±0.13 ^c
PE2	11.52±0.04 ^h	+	+	6.00±0.00 ^a	97.02±5.40 ^g	42.68±1.79 ^g	2.71±0.01 ^d	4.64±0.30 ^g
PE3	9.62±0.00 ^f	+	+	12.03±0.55 ^b	28.28±1.55 ^d	1.79±0.00 ^a	1.60±0.03 ^b	2.50±0.00 ^{cd}
PE4	6.02±0.03 ^c	+	+	30.66±0.45 ^e	51.90±1.78 ^f	3.35±0.10 ^{ab}	0.66±0.04 ^b	2.88±0.04 ^{ef}
PE5	11.24±0.14 ^g	+	+	43.06±0.75 ^f	4.17±0.00 ^a	2.62±0.04 ^{ab}	0.20±0.01 ^a	1.35±0.10 ^b
PE6	6.05±0.18 ^c	+	+	17.56±0.89 ^{cd}	14.43±0.00 ^b	5.56±0.12 ^c	0.65±0.00 ^b	2.50±0.02 ^{cd}
PE7	8.67±0.01 ^e	+	+	42.56±0.75 ^f	19.32±0.52 ^{bc}	3.88±0.05 ^{bc}	0.26±0.00 ^a	0.92±0.00 ^a
PE8	5.19±0.00 ^b	+	+	13.36±0.63 ^b	23.31±0.04 ^{cd}	10.15±0.09 ^d	0.25±0.00 ^a	3.10±0.08 ^f
PE9	2.30±0.00 ^a	+	+	18.46±0.65 ^d	27.42±0.08 ^d	23.03±0.41 ^f	1.55±0.08 ^c	2.68±0.10 ^{de}

Different letters within a column denote significant differences according to Tukey test ($n = 6$, $p \leq 0.05$). Values are means $\pm$ standard deviation. The symbol (+) means positive result. PE1: propolis extracts from Maxcanú; PE2: propolis extracts from Santa Cruz; PE3: propolis extracts from Hecelchakán; PE4: propolis extracts from Nunkiní; PE5: propolis extracts from Halachó; PE6: propolis extracts from Maxcanú; PE7: propolis extracts from Pomuch; PE8: propolis extracts from Calkiní; PE9: propolis extracts from Cuch-holoch.

Table 3.

Pearson's correlation between different parameters

Parameters	ABTS	DPPH	TF	TPC
DPPH	0.78*	-		
TF	0.76	0.94	-	
TPC	0.83	0.84	0.78	-
Oxidation index	-0.81	-0.57	-0.57	-0.52

* All correlations are significant with a value of $p \leq 0.05$

All of the propolis extracts passed the lead acetate and sodium hydroxide solubility test. In the calculation of the oxidation index, the time required for the violet color of the oxidizing agent (potassium permanganate) to disappear ranged from 6.0 s (PE2) to 43.06 s (PE5). In regard to *in vitro* antioxidant activity, the DPPH values ranged from 0.20 (PE5) to 2.71 mM Trolox/g (PE2), and the ABTS values ranged from 0.92 (PE7) to 4.64 mM Trolox/g (PE2). The antioxidant activity of the extracts quantified with the use of the ABTS assay was higher than with the use of the DPPH assay. A comparison of the extracts showed that PE2 (from Santa Cruz) had a significantly higher ($p \leq 0.05$) dry extract

yield, TPC, TF, and DPPH and ABTS activity and a lower value ($p \leq 0.05$) on the oxidation index. Furthermore, the correlation between *in vitro* antioxidant activity (DPPH and ABTS) and TPC, TF, and the oxidation index was also analyzed (Tab. 3). The antioxidant measures, DPPH and ABTS, had a strong positive relationship ($r=0.78$, $p \leq 0.05$). The antioxidant activity, DPPH and ABTS, had a strong linear relationship with TF ($r=0.94$ and $r=0.76$, respectively, $p \leq 0.05$) and TPC ($r=0.84$ and $r=0.83$, respectively, $p \leq 0.05$) whereas a negative relationship with the oxidation index ($r=-0.57$ and $r=-0.81$, respectively, $p \leq 0.05$). Furthermore, TF ($r=0.78$, $p \leq 0.05$) was positively associated with TPC, and the TPC

Fig. 2. A typical chromatogram of the phenolic compounds detected in extracts PE1 (A), PE2 (B) and PE7 (C).

Table 4.

Content of individual phenolic compounds (mg/100 g of dry propolis extract)

Samples	1	2	3	4	5	6	7	8	9
PE1	122.32±4.56	229.13±6.79	235.87±4.87	72.99±0.82	72.26±3.39	62.40±0.82	18.56±0.98	23.09±2.03	55.55±1.50
PE2	41.63±0.30	106.86±1.28	215.24±1.31	62.48±1.37	53.90±0.20	172.36±5.62	3.10±0.19	153.75±0.00	388.26±25.46
PE3	22.17±0.46	145.51±2.31	207.09±4.66	38.53±1.07	55.19±0.47	12.57±0.62	3.23±0.05	28.72±0.00	22.05±0.41
PE4	51.07±2.13	140.60±0.98	nd	4.04±0.12	74.35±3.42	nd	nd	5.62±0.00	13.61±0.33
PE5	10.30±0.03	nd	47.28±1.06	10.30±0.45	54.28±0.47	7.23±0.19	nd	2.35±0.00	20.98±0.17
PE6	215.01±8.64	48.62±1.55		0.88±0.12	79.45±0.54	8.86±0.11	2.26±0.07	nd	2.15±0.05
PE7	24.64±0.40	nd	273.28±1.73	9.42±0.29	80.60±3.11	62.21±0.00	nd	44.74±0.00	53.02±2.29
PE8	31.12±0.68	nd	61.89±3.24	16.59±0.07	40.91±0.21	26.05±0.85	4.19±0.07	38.03±0.00	60.36±1.92
PE9	nd	91.36±0.37	nd	50.51±2.21	76.80±1.42	19.66±0.19	16.97±0.56	22.46±0.00	29.95±1.03

1: Gallic acid, 2: Chlorogenic acid, 3: Catechin, 4: Vanillin, 5: Ellagic acid, 6: Sinapic acid, 7: Ferulic acid, 8: Pinocembrin and 9: Chrysin. Different letters within a column denote significant differences according to Tukey test ($n = 6$, $p \leq 0.05$). Values are means $\pm$ standard deviation. PE= propolis extracts. PE1: Maxcanú; PE2: Santa Cruz; PE3: Hecelchakán; PE4: Nunkiní; PE5: Halachó; PE6: Maxcanú; PE7: Pomuch; PE8: Calkiní; PE9: Cuch Holoch. nd: not detected.

Fig. 3. PC1 vs. PC2 scatter plot; A) distinction between the samples (scores); (B) based on chemical quality (loadings).

and TF contents were negatively correlated with the oxidation index ($r=-0.52$ and $r=-0.57$, $p \leq 0.05$, respectively).

The content of individual phenolic compounds in the propolis extracts varied widely, as shown in Tab. 4. Nine phenolic compounds were identified and quantified in the propolis extracts, including three hydroxycinnamic acids (ferulic, sinapic, and chlorogenic acid), one flavone (chrysin), one flavanol (catechin), three hydroxybenzoic acids (gallic and ellagic acid and vanillin) and one flavanone (pinocembrin) (Fig. 2). In particular, gallic acid and catechin were the most abundant phenolic compounds in PE6. Chlorogenic acid and vanillin were the most abundant phenolic compounds in PE1, and PE1, PE4, PE6, PE7 and PE9 were rich in ellagic acid. PE1 (18.56 mg/100 g) and PE9 (16.97 mg/100 g) were compared and stood out for their high ferulic acid content. Also, sinapic acid, pinocembrin and chrysin were the predominant compounds in the PE2 extract with the highest TPC, TF, and antioxidant activity.

PCA

In the principal component analysis, two main components with eigenvalues greater than

1 were extracted. Fig. 3 (A and B) shows the two-way loadings and score plots. The first two components explained 72.6% of the total variability of the data. The first component was positively correlated with TPC, TF, DPPH, sinapic acid, pinocembrin and chrysin. The second component was positively correlated with ferulic acid and negatively correlated with pinocembrin, chrysin and the oxidation index (Fig. 3B). Fig. 3A shows the classification of the propolis extracts in two groups: Group 1 contains PE2 and group 2 contains the rest of the extracts. The single extract in group 1 was separated for its high TF, TPC, pinocembrin, sinapic acid, chrysin contents and high antioxidant activity (DPPH and ABTS). The samples in group 2 were rich in ferulic acid but had a low pinocembrin and chrysin content and high values on the oxidation index.

DISCUSSION

Quality of raw propolis

The moisture content of the raw propolis samples is within the limits established by the Argentine standard (maximum 10%) (IRAM-INTA 15935-1, 2008). It is important to control the moisture in raw propolis since a high moisture

content creates optimal conditions for the growth of fungi and or possibly lead to fermentation during storage. The moisture values obtained herein are similar to those reported for brown, green, and red propolis from different regions in Brazil (Machado et al., 2016).

The ash content of propolis (except RP8) is within the limits established by Brazilian legislation (maximum 5%) (*TRPIQ*, 2001) and is comparable to that obtained by Machado et al. (2016) for Brazilian propolis. This quality parameter is important because it indicates the existence of mechanical impurities including wood, soil, plant remains, insects and dead bees.

In regard to the sensory characteristics, most of the samples presented irregular opaque pieces. According to Vilorio et al. (2012), the brightness of raw propolis may be related to the phyto-geography or external oxidation. These authors also indicate that raw propolis obtained through the scraping method can contain irregular and opaque pieces, as found herein. Based on the present results, it can also be inferred that the raw propolis with the highest ash content is the most rigid. In addition, the samples showed high variability in the aroma, color, taste, and consistency, similar to raw propolis samples from Colombia and Brazil (Vilorio et al., 2012; Machado et al., 2016). The lack of aroma in RP3, RP5 and RP9 could result from their low content of essential oils.

The high variability in the moisture and ash content and sensory characteristics are attributed to the apiaries' geographical location, type of propolis, collection period, handling of hives, and surrounding vegetation (much of which is endemic). Given the high variation in the quality and sensory characteristics, future studies should apply palynological methods to determine the specific flora visited by bees to collect materials (e.g., resin) to make propolis.

Quality of propolis extract

Around one-fifth (22.2%) of the ethanolic propolis extracts complied with the Brazilian and Argentine standards for minimum dry extract content (11 and 10 g of dry extract/100 mL of ethanolic extract, respectively) (*TRPIQ*,

2001; *IRAM-INTA 15935-1*, 2008). The low dry extract content of PE9 can be attributed to the type of plant species near the hive, which may not have a high amount of resin, in addition to the collection season or improper handling by beekeepers during harvest (Vilorio et al., 2012). The dry extract content herein was comparable to that reported for extracts of propolis collected in several localities in Brazil (Tagliacollo & Orsi, 2011). The extracts passed the lead acetate and sodium hydroxide solubility test, complying with the Brazilian and Argentine standards established for propolis extracts (*TRPIQ*, 2001; *IRAM-INTA 15935-1*, 2008). In regard to the oxidation index, the Brazilian and Mexican standards suggest a maximum reaction time of 22 s (*TRPIQ*, 2001; *NOM-003-SAG/GAN-2017*, 2017). Herein, 66.66% of the extracts passed this test, similar to Brazilian propolis (Tagliacollo & Orsi, 2011).

Minimum values for the TPC (0.5% or 5 mg/g) and TF (0.25% or 2.5 mg/g) of propolis extracts were established by Brazilian legislation. Most of the extracts herein met these requirements, except for PE5 and PE3 with respect to TPC and TF, respectively. However, the TPC and TF reported herein were lower than those reported by Xu et al. (2019) for propolis extracts from China and the United States. These differences can mainly be attributed to the influence and diversity of the botanical origin of resin, which differs among each region of the world (Palomino et al., 2009).

The antioxidant activity found herein was higher than that reported for propolis extracts from Colombia and Tunisia according to both the ABTS and DPPH assays (Palomino et al., 2009; Gargouri et al., 2019). These results confirm the potential of propolis from southeastern Mexico for use in the pharmaceutical and food industries. However, the antioxidant activity of the propolis extracts was higher according to the ABTS assay than the DPPH assay. There are several possible explanations for this phenomenon (Cerretani & Bendini, 2010; Gulcin, 2020):

A) The ABTS assay is known to be less selective than the DPPH assay in reacting with

donors of hydrogen atoms because it is reduced by OH-aromatic groups (unlike DPPH) that do not contribute significantly to antioxidant activity;

B) Phenolic compounds react differently with free radicals in the water phase (ABTS assay) and organic phase (DPPH assay);

C) Small molecules may be able to better access the active center of ABTS radicals and

D) ABTS radicals are more reactive than DPPH radicals.

Despite these differences, a high correlation between the DPPH and ABTS assays is expected and observed herein, since DPPH and ABTS radicals can be neutralized by both single-electron transfer (SET) and hydrogen-atom transfer (HAT) (Bankova et al., 2019; Gulcin, 2020).

Overall, the correlation of TPC and TF with DPPH and ABTS antioxidant activity suggests that the phenolic compounds contribute to the antioxidant activity of the propolis extracts. However, this is presumably due to not only flavonoids but also non-flavonoid phenolics. In addition, the negative correlation of the oxidation index with TPC, TF, DPPH and ABTS indicates, that the higher the content of TPC and TF and the higher the antioxidant activity of the extracts the smaller the amount of time required for the violet color of potassium permanganate to disappear.

The extracts analyzed herein showed differences in their phenolic composition. However, these differences seem to be more quantitative than qualitative in nature, as many compounds are shared among the extracts, albeit in different concentrations. The concentration of individual phenolic compounds in propolis extracts is relevant because it is indicative of antioxidant activity. Such data constitute a starting point for the use of propolis or its components in functional products in the food and non-food industries.

However, one extract that stood out for its high antioxidant activity was the extract PE2. Its high antioxidant activity can be preliminarily attributed to its high content of sinapic acid, pinocembrin, and chrysin. This latter sample was collected in the rural locality of Santa Cruz,

where the population is small (<200 inhabitants) and the vegetation abundant, unlike the other localities. This may partially explain why this extract had a higher content of phenolic compounds and antioxidant activity and was grouped alone in the PCA.

The presence of ferulic acid, pinocembrin and chrysin in the propolis documented herein is also common in propolis extracts from Tunisia and Poland (Gargouri et al., 2019; Woźniak et al., 2019). These compounds can be found in popular-type propolis from the countries of Europe, Asia, North America and continental Australia (Ristivojević et al., 2015). Meanwhile, gallic acid, ellagic acid, chlorogenic acid and vanillin were reported previously in propolis extracts from Argentina and Croatia (Chaillou & Nazareno, 2009; Jerković et al., 2016).

In conclusion, the majority of propolis sampled herein from the Yucatan Peninsula in southeastern Mexico met the quality criteria stipulated in the regulations. The chemical composition and antioxidant activity varied greatly among different propolis and depends on the geographical location and diversity of flora surrounding the apiary that bees use as a source of resin. Unlike the propolis characteristic of temperate zones in tropical regions such as southeastern Mexico, bees visit floral resources unique to the region including *Viguiera dentata*, *Gymnopodium floribundum*, *Mimosa bahamensis*, and *Piscidia piscipula* (Moguel-Ordoñez et al., 2005), likely giving the propolis a unique composition and set of properties. Overall, the analyzed propolis extracts have a significant amount of phenolic compounds and antioxidant activity and, therefore, can potentially be used as a functional food or in health products and the food industry.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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